Varicella-Zoster Virus

Published in association with the VZV Research Foundation, this is a comprehensive account of the biology and clinical features of the varicella-zoster virus. The successful development of a vaccine reflects intense research interest in this virus over recent years, and this book surveys current knowledge of the molecular biology, pathogenesis and clinical features of VZV as the causative agent of chickenpox and zoster (shingles).

Topics covered include viral replication, latency, immune mechanisms, epidemiology and disease manifestations, and complications of varicella and zoster. There is detailed information on live attenuated varicella vaccine, treatment strategies and the management of postherpetic pain in zoster patients. As the most authoritative review and guide to the virus and its diseases, this book will appeal to a wide range of clinicians and investigators, including pediatricians, geriatricians, neurologists, dermatologists and infectious diseases specialists as well as virologists interested in the herpes viruses.

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The VZV Research Foundation was founded by Richard T. Perkin in 1991 as a nonprofit organization dedicated to research and education on VZV. It has awarded numerous research grants, bestowed four Scientific Achievement Awards and sponsored four major international conferences and a public information campaign about the problems caused by VZV and current knowledge about varicella and zoster.

The editors and authors dedicate this volume to Richard T. Perkin, President of the VZV Research Foundation. His unfailing support of scientific research on VZV has been an inspiration to all of us and we are truly grateful for his many efforts on our behalf.

Varicella-Zoster Virus

Virology and Clinical Management

Edited by

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Preface

Major milestones in the understanding of varicella-zoster virus (VZV) occurred in the eighteenth century when varicella was recognized to be distinct from smallpox, and in the mid-twentieth century Weller and colleagues first succeeded in isolating the virus in cell culture. Nevertheless, as recently as 30 years ago only a handful of virologists were pursuing research on VZV, mainly because the diseases it caused were perceived as minor and it proved to be extremely difficult to propagate the virus in the laboratory. There were several achievements, however, that resulted in a veritable explosion in research on this pathogen, beginning in the early 1970s. First was development of a live attenuated varicella vaccine by Takahashi and colleagues. Other significant new interventions were introduced almost simultaneously, including passive immunization and antiviral therapy, along with the realization that diseases caused by VZV are not necessarily benign, especially in developed nations with aging populations and increasing numbers of immunocompromised patients. Next came the availability of molecular techniques, permitting bypass of propagation of the virus for its study, and allowing mutation of viral genes within the virus, leading to elucidation of the processes of VZV gene expression, latency, viral pathogenesis and immune responses. Finally the organization of the Varicella-Zoster Research Foundation by Richard Perkin led to improved communication between basic and clinical investigators studying VZV. In the brief period of about 10 years, this Foundation has hosted four international meetings on VZV, produced three volumes of Proceedings, presented four awards to premier senior scientists involved with VZV, and supported seven research fellowships for young investigators interested in VZV. Thirty years ago, there were about 100 publications on VZV annually; now there are ten times that many. At this time of great progress and success on our understanding of molecular events, natural history, prevention, and treatment of VZV, it seemed appropriate to develop a consensus of where this field stands today. Therefore this volume was conceived and executed, and the editors are extremely grateful for the efforts of all the outstanding contributors. Hopefully the

comprehensive chapters contributed by current international experts in the various aspects of VZV will inspire young scientists to continue to explore and develop this exciting field even further.

Ann M. Arvin
Anne A. Gershon
January 2000

Introduction

Ann M. Arvin and Anne Gershon

Throughout the years, there have been many scientists who have contributed to our knowledge of the varicella zoster virus and the prevention, diagnosis and management of VZV-related diseases. In recent years, four of them, including two Nobel Laureates, have had the distinction of receiving the VZV Research Foundation (VZVRF) Scientific Achievement Award, which recognizes lifetime achievements in VZV research. Established in 1991, the Foundation is the first and only nonprofit organization in the world dedicated to VZV research and education.

Thomas H. Weller, M.D.

Thomas H. Weller, M.D., professor emeritus of the Harvard School of Public Health, Boston, and a Nobel Laureate, was the recipient of the first VZVRF Scientific Achievement Award in 1993. He remarked that his VZVRF award was in a way more gratifying than his Nobel Prize.

According to Dr. Weller, "Although my work in isolating and growing the poliomyelitis virus in tissue cultures was the most significant contribution I have made to medical science in terms of global impact, I am most proud of my work with the varicella-zoster virus. It's something I planned to do and worked for years to do."

It was the work that Dr. Weller and his colleagues did on the poliomyelitis virus that garnered them the Nobel Prize in 1954, and led to the Sabin and Salk vaccines. Likewise, Dr. Weller's work on the varicella-zoster virus led to many important discoveries in VZV research, including Dr. Michiaki Takahashi's development of the varicella vaccine. In 1953, in addition to isolating VZV from cases of chickenpox and zoster, Dr. Weller was able to show that the same virus is responsible for both illnesses.

Dr. Weller's research – conducted alone or in collaboration – also helped pave the way for vaccines against mumps and rubella. He was co-discoverer of the rubella virus, the cause of German measles, and the cytomegalovirus, which he named. He was one of the first to grow the mumps virus in tissue culture.

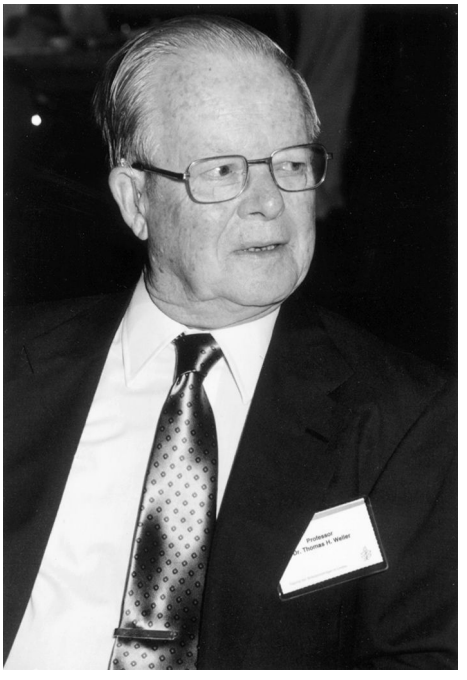

Figure I.1 Thomas H. Weller, M.D.

Figure I.2 Gertrude B. Elion, D.Sc.

Trained as a pediatrician, Dr. Weller is also a recognized expert in the field of tropical medicine and is a past president of the American Society of Tropical Medicine and Hygiene. In addition to the Nobel Prize, he is the recipient of many awards, including the George Ledlie Prize from Harvard University, the Bristol Award from the Infectious Diseases Society of America and the Walter Reed Medal from the American Society of Tropical Medicine and Hygiene. Dr. Weller graduated from Harvard Medical School, Boston, in 1940.

Gertrude B. Elion, D.Sc.

In 1995, the late Gertrude B. Elion, D.Sc., another Nobel Laureate, became the second recipient of the VZVRF Scientific Achievement Award. The award recognized Dr. Elion's "pioneering work in antiviral therapy," specifically, VZV research. Dr. Elion was instrumental in the development of antiviral therapy for herpes zoster.

Dr. Elion died in February of 1999 at the age of 81. In 1988 she and colleague George Hitchings, Ph.D., whom she worked with for 40 years, along with Sir James

Black, were awarded the Nobel Prize for their research leading to drugs for leukemia, gout, malaria, zoster and other diseases of the immune system, in addition to drugs that eventually made organ transplants possible. Their scientific collaboration also led to the development of AZT for AIDS.

In a February 22, 1999 Associated Press story that reported on her death, Dr. Elion was remembered as "blazing new trails as a woman scientist in what was then a man's world."

Dr. Elion was a scientist emeritus at Glaxo Wellcome Inc., where she had served as head of the Department of Experimental Therapy. She was the recipient of more than 35 honors and awards, including the National Medal of Science, the Medal of Honor from the American Cancer Society and election to the Institute of Medicine of the National Academy of Science.

She received her master's degree in chemistry from New York University in 1941 and held honorary doctorates from 20 universities.

Michiaki Takahashi, M.D., D.M.Sc.

In 1964, during his research fellowship at Baylor Medical College in Houston, Dr. Michiaki Takahashi's 3-year-old son was suffering from a severe case of varicella. He remembers asking himself, "What if varicella could be prevented by a vaccine?" Eight years later, Dr. Takahashi, the VZVRF's third Scientific Achievement Award winner, began development of a live varicella vaccine.

Dr. Takahashi's involvement with vaccines began at Osaka University's Institute for Microbial Diseases, where he worked on a measles vaccine. He later studied adenovirus and herpes simplex virus from the viewpoint of cellular transformation by these viruses. In conjunction with these studies, he collaborated on the development of a live mumps and rubella vaccine. He then commenced work on the varicella vaccine.

According to Dr. Takahashi, "The varicella-zoster virus was one of the most difficult viruses to study because of its poor cell-free virus yield and heat-labile property." Nevertheless, he overcame these difficulties and developed the varicella vaccine in 1974. Twenty-one years later, in March 1995, the US Food and Drug Administration approved the country's first varicella vaccine for use in children and adults who have not had varicella.

Dr. Takahashi received the VZVRF Award in 1997, calling it "the highest honor in my career." He currently is professor emeritus at Osaka University, Japan, and director of The Foundation for Microbial Diseases of Osaka. Dr. Takahashi earned his M.D. degree from Osaka University Medical School.

Figure I.3 Michiaki Takahashi, M.D., D.M.Sc.

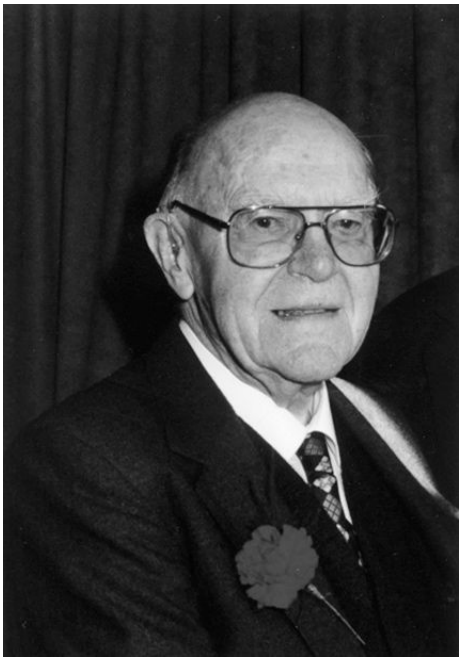

Figure I.4 Robert Edgar Hope-Simpson, O.B.E., F.R.C.P.

Robert Edgar Hope-Simpson, O.B.E., F.R.C.P.

The most recent recipient of the VZVRF Scientific Achievement Award is Robert Edgar Hope-Simpson, O.B.E., F.R.C.P., a British general practitioner who wrote, in 1965, a definitive paper on which future VZV research would be based. His 18-year study of zoster and chickenpox among his patients led him to the conclusion that zoster is due to reactivation of latent VZV. That same year, he also hypothesized that the increased incidence and severity of zoster in older people is the result of declining immunity.

With no formal training in research or epidemiology, Dr. Hope-Simpson used the 3400-patient base of his Cirencester practice to conduct his life's work of epidemiological field studies of infectious diseases. In total, he authored more than 80 papers on infectious disease and epidemiology, including his 26-year experience with postherpetic neuralgia.

Dr. Hope-Simpson was the first chairman and founding member of the College of General Practitioners. He is the recipient of many awards, including the Stewart Prize and the Ekke von Kuennsberg Prize from the Royal College of General Practitioners. Dr. Hope-Simpson was honored by Queen Elizabeth with an O.B.E. (Officer of the Order of the British Empire) for his services in Public Health Medicine. He graduated from St. Thomas Hospital, London, in 1932.

Figure I.5 Richard T. Perkin, Chairman and President, VZV Research Foundation.

Richard T. Perkin, Chairman and President, VZV Research Foundation

Richard T. Perkin founded the VZV Research Foundation in 1991, as a result of his then 82-year-old mother's struggle with zoster and postherpetic neuralgia. In an attempt to help his mother cope with her excruciating pain, he searched for information and assistance from several medical specialists. Mr. Perkin soon learned that while scientists had collected a great deal of knowledge on these afflictions, more research and education was needed to better understand and fight the virus that causes them. Yet there was no single organization championing this effort.

He soon began the work of forming the VZV Research Foundation, a nonprofit organization dedicated to research and education on VZV, and in October 1991, held the Foundation's first scientific and organizational meeting in Harriman, NY.

Since then, under his leadership, the Foundation has amassed more than 30 of the leading international scientists in VZV research to serve on its Scientific Advisory Board. The Foundation has awarded ten research grants (totaling more than $1 million by the year 2001), bestowed four Scientific Achievement Awards, and sponsored four major international conferences, CME programs, and a public information campaign including an 800 number (1–800–472–VIRUS), web site (www.vzvfoundation.org), newsletter (VZV FOCUS) and educational literature on chickenpox and zoster.

Mr. Perkin is chairman emeritus of The Perkin Fund, a family foundation that supports scientific research. He is also a trustee of The Juilliard School and the Wildlife Conservation Society, and a member of the Executive Committee of the Rockefeller University Council. He also serves on the Committee for Planetarium Policy at the American Museum of Natural History and the Advisory Board of New York Presbyterian Hospital. He formerly served on the Visiting Committee of Harvard University's Department of Astronomy.

Earlier in his career, Mr. Perkin was active in public media, including television documentary syndication. He is a graduate of Harvard College.

Part I

History

1

Historical perspective

Thomas H. Weller

Introduction

The development of our knowledge of the ubiquitous varicella-zoster virus has been fascinating, illustrating as it does the interplay of different scientific disciplines and the changing nature of the human host. Initially, clinicians differentiated varicella from variola. Then, epidemiologists provided evidence in support of the view that chickenpox and shingles had a common etiology, a thesis supported by pathologists who studied the lesions. Additional evidence of co-identity was provided on cultivation of the viruses in the laboratory. Yet proof of this fact awaited the application of molecular biological techniques.

Concurrently, and paradoxically in large part due to the advances of curative medicine, varicella lost its benign label as an ever-increasing number of high risk subjects in whom varicella might be lethal was recognized. Also concurrently in the developed countries the prevalence of zoster increased in parallel with the increasing longevity of the human population. As varicella emerged as a lethal disease, the need for therapeutic drugs and vaccines became obvious and the efforts of pharmacologists and immunologists yielded effective antiviral drugs and vaccines.

The differentiation of varicella from variola

Whereas zoster was recognized and described in medieval times, varicella was considered to be a mild form of smallpox until 1767 when Heberden read a paper entitled "On the Chickenpox" before the College of Physicians in London (Heberden, 1768). He indicated that chickenpox, then also called swinepox, was a mild disease, but said "yet it is of importance on account of the small-pox, with which it may otherwise be confounded, and so deceive the persons, who may have had it, into a false security, which may prevent them either from keeping out of the way of small-pox or from being inoculated". He described the evolution of the pox and listed criteria by which the cutaneous lesions of the two diseases could be distinguished.

In spite of Heberden's description of varicella, the possible relationship of the

disease to smallpox continued to be considered for many years. In 1892, Osler wrote "there can be no question that varicella is an affection quite distinct from variola and without at present any relation whatsoever to it". He described a case "documenting that an attack of one does not confer immunity from an attack of the other" (Osler, 1892). Yet Tyzzer in 1904 found it necessary to explore the possible relationship and experimentally elminated variola as a causative agent in his study of a varicella outbreak.

Origin of nomenclature

The origin of the term chickenpox is not clear. One opinion (Lerman, !981) credits Richard Morton with the first use of the word in the literature when in 1694 he described chickenpox as a mild form of smallpox. In his text of 1886 Fagge attributed the term to the phrase "chickpease" derived from the French "chiche" and Latin "cicer" (Fagge, 1886). Lerman notes that the surface texture and cream color of one kind of chickpea is similar to the early pustular chickenpox vesicle.

Christie (1969) offered an alternative explanation of the derivation, noting that in old English the term "cicen" refers to a barnyard fowl. A third suggested derivation is that the term may be derived from the old-English word "gican" meaning to itch (Englund & Balfour, 1989).

The origin of the term varicella likewise has variable interpretations. Taylor-Robinson & Caunt (1972) state that the term 'is an irregular diminutive of variola (smallpox) from the Latin "varius", various or mottled'. Another author, in an early pediatrics textbook, indicated that the term, which was introduced by Vogel in 1764, is a derivative of "varus", a pimple (Jennings, 1890).

Juel-Jensen & MacCallum (1972) summarized the terms commonly used for varicella. *French:* Varicelle; *Scandinavian:* Skaalkopper, Skoldkopper, Vandkopper, Vattenkopper; *German:* Windpocken, Wasserpocken, Spitzblattern. Varizellen; *Italian:* Varicella, Vaiuolo acquaiuolon; *Spanish:* Varicela, Viruelas locas.

The derivation of the terminology relating to zoster is less obscure. Christie (1969) noted that nomenclature relating to the segmental nature of the lesions in zoster derives from the classical Greek, where a warrior used a zoster – a belt-like binding – to secure his armor. The term shingles derives from the medieval Latin word "cingulus", a girdle.

Nature of the varicella-zoster agent

That varicella is caused by an infectious agent was demonstrated in 1875 by Steiner, who transmitted the disease to children by inoculation of vesicle fluid samples from patients with chickenpox (Steiner, 1875). However, the nature of the agent

remained unknown. Thus when Tyzzer in 1904 initiated his studies on an epidemic of varicella in Bilibid prison in the Philippines, since some physicians still maintained that the disease was a mild form of smallpox, his first task was to rule out smallpox. He noted that most of his patients with varicella either bore the scars of a past attack of smallpox or else had smallpox vaccination scars. He wrote "If the two diseases are identical as asserted by Hebra, it is difficult to explain why the severe form as seen in variola vera, as well as the oft-repeated vaccinations, should not protect against so slight a form as varicella". Further, aware that the agent of variola would produce lesions in monkeys and on the corneas of rabbits, Tyzzer inoculated monkeys and the corneas of rabbits with both clear vesicle fluid and crusts from lesions of his cases. He concluded "the negative character of these inoculations indicates clearly that the disease is distinct from smallpox" (Tyzzer, 1906).

Tyzzer noted that whereas varicella was considered to be a childhood disease, in the Philippines he was dealing with an epidemic in adults. This observation, the first report of the now well-recognized occurrence of chickenpox in adults in tropical climates, might be explained by "race, climate, and confinement in a crowded prison".

Tyzzer took serial biopsies of the cutaneous lesions of 11 cases of varicella. His eosin–methylene blue stained sections still retain their color. He published camera lucida drawings and photomicrographs of typical cellular changes. These he summarized as "the initial change consists in the appearance of peculiar eosin-staining inclusions within the nuclei and cytoplasm of epithelial and various other cells. Direct division of nuclei without subsequent division of the cytoplasm is associated with these inclusions. Cells undergoing these changes often attain relatively enormous dimensions . . .". (Figure 1.1 is a photomicrograph of a slide prepared by Tyzzer and Figure 1.2 depicts one of his camera lucida drawings).

Based on his studies, Tyzzer recommended that the differential diagnosis of cases of varicella and of smallpox could be made rapidly by microscopic examination of the cutaneous lesions. He wrote "The contents of early clear vesicles . . . may be examined under the microscope. The presence of large multinucleated cells is consistent with varicella and against smallpox. This test seems quite reliable and may be applied at the bedside". Thus, the procedure now referred to as the Tzanck test was described in 1906.

In 1921, Ernest Goodpasture studied the enlarged cells of cytomegalic inclusion disease and noted similarities with the histopathology of varicella as described by Tyzzer (Goodpasture & Talbot, 1921). Then Goodpasture initiated a series of animal experiments that demonstrated that intranuclear inclusions were a characteristic of an infection with herpes virus (Goodpasture & Teague, 1923). By analogy it was assumed that varicella was caused by a virus. Rivers, in 1926,

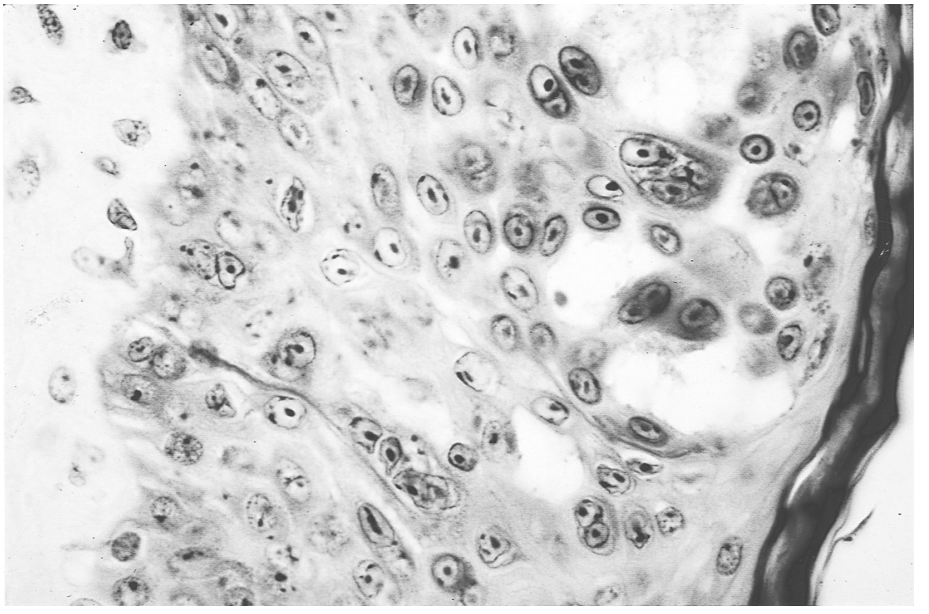

Figure 1.1 Photomicrograph of a day 1 cutaneous varicella lesion prepared by Dr. Tyzzer on June 8, 1904 in the course of his study in the Philippines. Stain: Eosin–methylene blue.

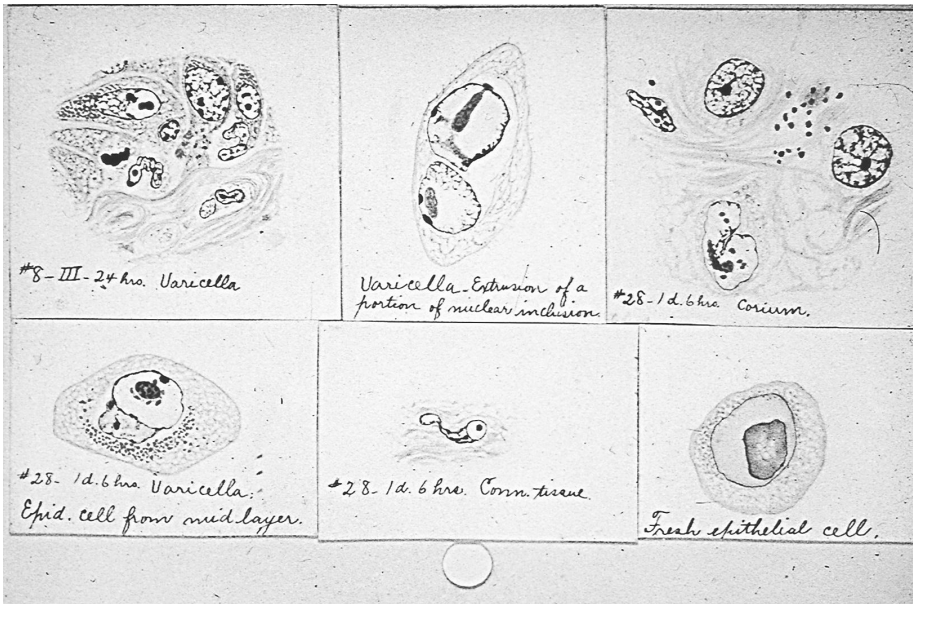

Figure 1.2 Camera lucida drawings made by Dr. Tyzzer of the nuclear changes observed in cells in the cutaneous lesions of varicella.

recorded the presence of intranuclear inclusions in the testicles of monkeys injected with the tissue of human varicella lesions (Rivers, 1926). For many years this was the only report of transmission of the etiologic agent to an experimental animal. However, various workers described what were called elementary bodies seen by light microscopy in samples of vesicle fluid. In a convincing study Amies demonstrated that such bodies were agglutinated by sera from patients convalescing from chickenpox (Amies, 1933). Examination of vesicle fluid by electron microscopy supported the concept that the bodies were viral in nature (Ruska, 1943). Use of a metal shadowing technique improved morphological details and demonstrated that the bodies were different from those of variola virus (Nagler & Rake, 1948).

The relationship of chickenpox and zoster

The question of whether chickenpox and shingles were etiologically distinct or were caused by the same virus remained unanswered for many years. That they were related was first suggested by clinical and epidemiological observations. In 1892 James Bokay, a professor of pediatrics in Budapest, published two reports describing five instances in which chickenpox had developed in individuals who had been in contact with a patient with zoster. With prescience he wrote "All the cases mentioned are very peculiar and I am reluctant to propose an explanation. However, I would like to bring up the question of whether or not the unknown infectious material of chickenpox could under certain circumstances manifest itself, instead of a generalized skin eruption, as a zoster eruption" (Jako & Jako, 1986). (While Bokay's article published in 1909 in German is usually cited, the Jakos published a translation of Bokay's 1892 Hungarian papers and kindly provided a reprint thereof.)

Many years elapsed before experimental and observational data began to accrue that supported Bokay's monistic theory of the etiology of varicella and zoster. In 1921, Lipschutz showed that the histopathology of the skin lesions of zoster was similar to that described by Tyzzer for varicella (Lipschutz, 1921). Kundratitz in 1925 experimentally transmitted the agent of zoster to volunteers with the production of varicelliform lesions, a finding confirmed by Bruusgaard (Kundratitz, 1925; Bruusgaard, 1932). By 1938, the School Epidemics Committee of Great Britain had linked 18 outbreaks of varicella in children to an exposure to zoster (School Epidemics Committee, 1938).

Using varicella and zoster vesicle fluids and crusts as antigen and convalescent phase sera from both entities in a complement fixation test, Netter and Urbain found almost identical reactions in the homologous and heterologous systems (Netter & Urbain, 1926). This finding was confirmed by Brain (1933). Amies found that some convalescent sera cross-agglutinated elementary bodies in vesicle fluid

samples from both entities (Amies, 1934). An electron microscopic study of vesicle fluids from the two entities revealed that the viral particles were morphologically identical (Rake et al., 1948).

By 1940 enough evidence supporting the monistic etiological theory had accrued to cause Zinsser (1940) and Sabin (1941) to comment on a close relationship, perhaps reflecting strains that were either dermatotropic or neurotropic. In 1943, Garland suggested that zoster reflected activation of a latent varicella virus, a situation similar to that observed with herpes simplex virus (Garland, 1943). Garland is credited with first expressing this now accepted view. Hope-Simpson elaborated on this concept, suggesting that after an attack of varicella, the virus persisted as a latent infection in the sensory ganglia (Hope-Simpson, 1965).

Cultivation of the varicella-zoster virus

As first shown by Tyzzer, the varicella-zoster virus could not be propagated in common laboratory animals. In 1944, Goodpasture and Anderson grafted fragments of human skin on the chorio-allantois of 9-day-old chick embryos and inoculated the fragments with zoster vesicle fluid. In a single experiment, histologic examination of fragments removed 4 to 8 days later showed intranuclear inclusions and multinucleated giant cells (Goodpasture & Anderson, 1944). This observation was confirmed by Blank et al. (1948).

In 1941, Dr. L. C. Kingsland and I, while interning at the Children's Hospital in Boston, attempted to grow varicella virus in cultures of human embryonic tissues. The effort was unsuccessful, reflecting the problems of contamination in the pre-antibiotic period, and enforced termination due to calls to active military duty.

In 1947, when I joined Dr. John F. Enders in organizing the Research Division of Infectious Diseases at the Children's Hospital, I returned to the problem of isolating the agent of varicella. Several unproductive months were spent using embryonated chicken eggs. Then, lacking the equipment for roller-tube cultures, but influenced by the obvious advantage of the prolonged maintenance of cultured cells, I altered the customary Maitland flask culture system. The nutrient medium was changed frequently and as a result the tissue fragments were left unchanged and remained viable. The technique proved of value and mumps virus was cultured for the first time (Weller & Enders, 1948). Then, influenced by the concept that varicella virus might be dermatotropic, on March 30, 1948 I prepared a series of cultures containing fragments of human skin–muscle tissue from a 4-month-old fetus. The majority of the cultures were inoculated with throat washings from a case of varicella and the few remaining were inoculated with a suspension of mouse brain containing Lansing poliomyelitis virus. The varicella cultures were negative, a finding that now would be expected, for virus can rarely be isolated from the oral secretions

of children with varicella. The poliomyelitis cultures were positive, thus initiating our collaborative study on the cultivation of the poliomyelitis viruses (Enders et al., 1949).

When I returned to the varicella problem and inoculated flask cultures of human embryonic tissues with varicella vesicle fluid samples from four different patients, there were positive results in six consecutive experiments. On histological examination eosinophilic intranuclear inclusions were demonstrable (see Figure 1.3). However, when we attempted to subculture the inclusion-producing agent by transferring affected tissue fragments to fresh tissue cultures, all attempts at subculture were unsuccessful (Weller & Stoddard, 1952). This frustrating situation reflected the now established fact that varicella-zoster virus remains strongly cell associated in tissue cultures.

In 1952, we began to use roller-tube cultures of human embryonic tissue and of foreskin tissue. In such cultures, the inoculation of vesicle fluid samples from six cases of varicella and from two cases of zoster resulted in slowly enlarging foci of swollen refractile cells that could be seen under low magnification in the living cultures. When stained, the swollen cells characteristically had intranuclear inclusions, and multinucleated giant cells were a common feature (see Figure 1.4). Subculture could be easily accomplished if the inoculum contained living infected cells. The foci appeared to develop as the result of transfer of infectious material from cell to contiguous cell. Similar cytopathic changes were induced by agents from the two clinical entities (Weller, 1953).

These findings initiated a 5-year study of the viruses. Strains of virus from 14 cases of varicella and from eight cases of zoster were propagated serially. Various types of cells of human origin and several of monkey origin were susceptible to infection in cultures. Again, strains of virus from the two clinical entities had similar cultural characteristics (Weller et al., 1958).

The unusual cell-associated behavior of the agents in vitro precluded the immediate application of the usual serological approaches to investigate the relationship. The fluorescent antibody technique then under development by Coons was therefore applied. Using preparations of the infected cells as antigen, fixation of antibody from human sera was detected by use of a fluorescent antihuman gamma globulin conjugate. Antibody reacting to the varicella and to the zoster antigens to an almost identical degree appeared during convalescence in serum specimens from the two diseases. This evidence supported the view that the etiological agents of the two diseases had been isolated and that they were closely related immunologically (Weller & Coons, 1954).

Concentration of the fluid phase of infected cultures yielded a workable complement-fixing antigen and by introducing convalescent-phase sera as a component of the medium, a neutralization test was developed. Convalescent-phase

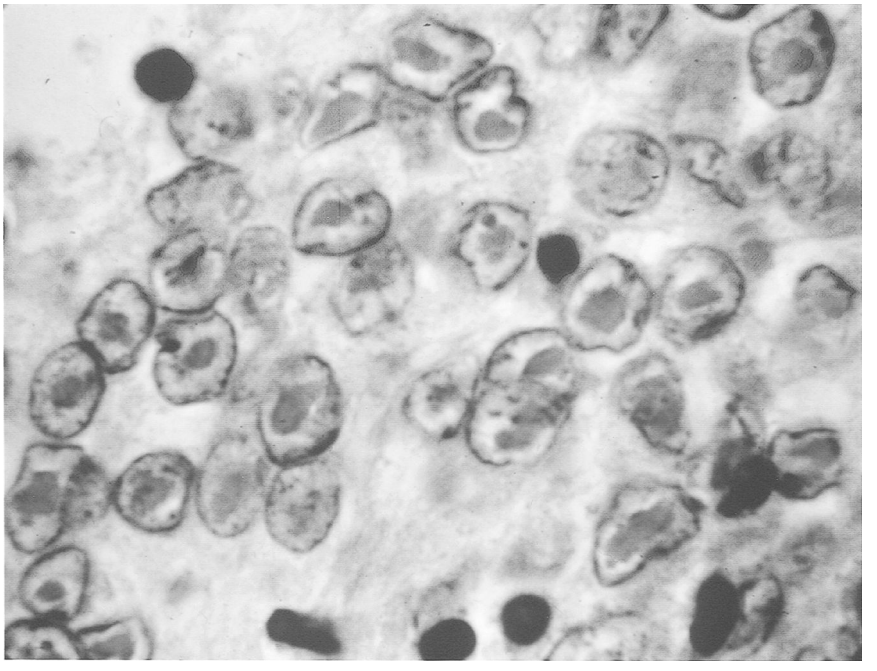

Figure 1.3 Fragment of human embryonic tissue from a Maitland type culture inoculated 14 days earlier with varicella vesicle fluid, showing nuclear inclusion bodies. From the first successful suspended cell culture; prepared March 19, 1949. Stain: hematoxylin–eosin.

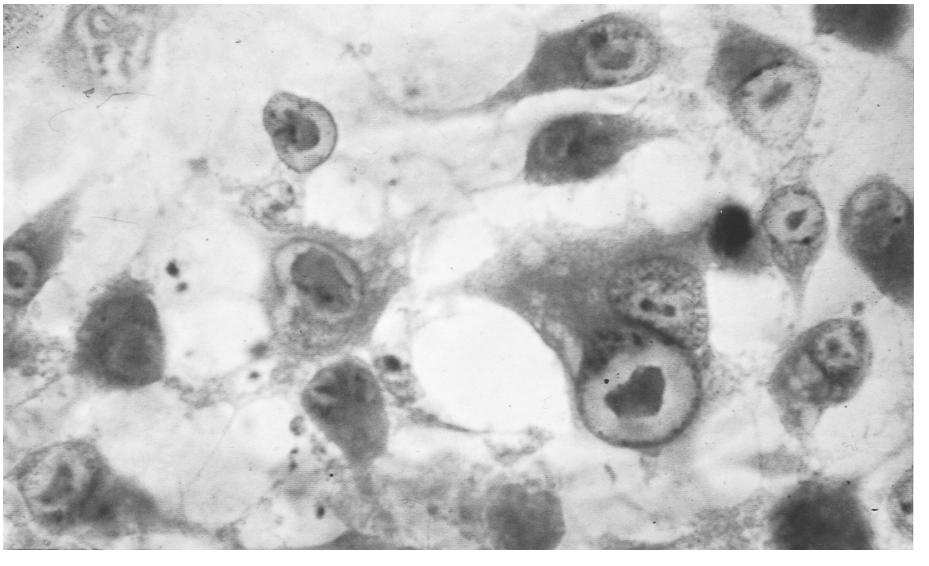

Figure 1.4 Edge of a focal lesion in the first successful roller-tube experiment. Tissue harvested 13 days after inoculation with varicella vesicle fluid (PWel.strain) showing numerous intranuclear inclusion bodies. Prepared November 19, 1952. Stain: hematoxylin–eosin.

sera from cases of varicella and from cases of zoster reacted similarly in both tests with the homologous and the heterologous antigens. We coined the phrase "varicella-zoster virus" and concluded "the accumulation of epidemiological and laboratory evidence in support of the hypothesis that a single etiologic agent is responsible for varicella and herpes zoster appears so impressive that the burden of proof must now shift to those who desire to refute the monistic concept" (Weller & Witton, 1958).

The availability of cultured virus permitted the application of the techniques of molecular biology as they evolved. While there proved to be only one type of varicella-zoster virus, application of restriction-endonuclease techniques revealed genomic differences between epidemiologically unrelated isolates (Straus et al., 1983). Using this approach, Straus and his coworkers provided proof of the coidentity of the etiological agents. Isolates were obtained from a patient with varicella and from the same patient who later developed zoster; on molecular characterization the isolates were identical (Straus et al., 1984).

In 1986, Davison and Scott reported the complete DNA sequence of varicella-zoster virus (Davison & Scott, 1986).

The increasing social significance of varicella-zoster virus

Heberden in his lecture in 1767 stated that illnesses caused by varicella "occasion so little danger or trouble to the patients, that physicians are seldom sent for to them, and have therefore very few opportunities of seeing this distemper. Hence it happens that the name of it is met with in very few books, and hardly any pretend to say a word of its history". This view of a benign illness persisted for almost 200 years. In medical school my pediatric textbook gave brief mention to varicella, referring to its mild constitutional symptoms and the fact that serious complications and sequelae were very rare (Holt & McIntosh, 1936)

Shortly thereafter increasing knowledge and changes in the human host altered the prevalent concept. Additionally, and paradoxically, medical progress *per se* enhanced the potential lethality of varicella-zoster virus. In 1942, as reviewed by Feldman (1994), it was recognized that varicella in adults was a more serious disease than in children, with viral pneumonia a common presentation. In 1947, Laforet and Lynch described the congenital varicella syndrome (Laforet & Lynch, 1947).

The changing age and nature of the population acquired importance as it was recognized that zoster increased in frequency with advancing age. In Hope-Simpson's classical study it was observed that in a cohort of 1000 people who lived to be 85 years old, 500 would have had one attack of zoster and ten would have had two attacks (Hope-Simpson, 1965). It is now known that this finding reflects the

gradual decay of cellular immunity in old age. Currently, the indigenous population is aging; one estimate is that in the next 40 years in the United States the number of persons over 85 years of age will increase from 3.5 to to 8.8 million (Gilford, 1988).

The nature of the population is also changing due to the immigration of adults from tropical areas, many of whom have not had varicella. The Census Bureau reported that in the United States between 1983 and 1992, the Hispanic population increased by 42% to 22.8 million people (US Census Bureau. 1994). Thus was introduced a large group of adults in whom varicella would be more severe. Of more import is the fact that these individuals may acquire caretaking jobs in hospitals and, if incubating varicella, may expose high risk patients.

In 1956, we described two cases of varicella from which we isolated virus at autopsy; one was a child on steroid therapy and the other a child undergoing treatment for malignancy (Cheatham et al., 1956). As similar cases were observed, a category of high risk patients subject to a severe or fatal varicella-zoster virus infection was recognized. All were immunosuppressed. One form of immunosuppression was biological as in those with reticuloendothelial or hematopoietic cancer, or with a concurrent infection such as the human immunodeficiency viruses (HIV). It was recognized that depression of cellular rather than of humoral immunity was important (Arvin et al., 1978). The other type of immunosuppression was iatrogenic as in the chemotherapeutic immunosuppression procedures essential in the burgeoning organ transplant field. In such individuals, either a primary infection or the reactivation of a latent infection could lead to a severe disseminated lethal process. With recognition of the high risk group, the virus lost its benign characteristics and the search for improved therapeutic and preventive measures was stimulated.

Modification, prevention, and treatment

Passive immunization

In 1962, Ross summarized the then limited literature on cases of severe varicella and conducted a classical study on the use of gamma globulin to modify the illness (Ross, 1962). Counts of pox proved to be a useful index of modification of varicella, and it was concluded that gamma globulin was an effective modifier if given within three days of exposure. A significant advance in providing an increased supply of high potency gamma globulin resulted from the selective use of outdated blood bank lots shown by complement fixation to have significant levels of varicella antibodies (Zaia et al., 1978).

Chemotherapy

Specific therapy for varicella developed in the 1970s. Interferon and transfer factor proved to be of some value in treating infections in high risk patients. Concurrently, compounds that interfered with the synthesis of viral DNA were introduced. Of the early compounds studied, adenine arabinoside, ARA-A, a purine nucleoside, was the most promising; a multi-institutional study demonstrated its value in the treatment of herpes zoster in high risk patients (Whitley et al., 1976).

As reviewed by Whitley & Gnann (1992), the synthesis of acyclovir by Gertrude Elion and her group in 1977 was a striking advance. The virus-encoded thymidine kinases present in infected cells convert acyclovir to its monophosphate derivative, which is subsequently converted to acyclovir triphosphate, a substance that inhibits viral DNA synthesis. This was the first of the currently available highly effective drugs.

Active immunization

In 1974, Takahashi and his co-workers reported that a live virus vaccine developed by them had prevented the spread of varicella in a hospital (Takahashi et al., 1974). The virus, the Oka strain, had been obtained from the vesicles of a typical case of varicella in a 3-year-old boy. Attenuation of the strain followed 11 passages in cultures of human embryonic lung cells at 34°C and 12 passages in guinea pig embryo cells at 37°C (Takahashi et al., 1975).

In retrospect, it is of interest that in spite of innumerable attempts no similar attenuated strain has been developed. Thus, the Oka strain remains the essential element of current vaccines. Takahashi's vaccine produced by the Biken Institute was used extensively in Japan and other far eastern countries. In 1984, Varilrix, a Smith–Kline Beecham product, was first licensed in Europe and is now licensed in about 40 countries (Francis Andre, personal communication). In the 1980s Pasteur Merieux Serums and Vaccins S.A. initiated studies of a vaccine in France. Varivax, produced by Merck and Company, was licensed in the United States in 1995 following 14 years of extensive collaborative studies organized by Dr. Anne Gershon (Gershon et al., 1984, 1989; Hardy et al., 1991). Thus, vaccines are now universally available. While the need to prevent varicella in the expanding population at high risk is obvious, studies by Preblud (1986) indicated that normal children would benefit from vaccination "not by virtue of the severity of the disease but rather because of the inevitability of the disease and its associated expense". Historical reviews of the varicella vaccines have been published (Gershon, 1995; White, 1997).

Acknowledgments

The author is indebted to Drs. Henry Balfour, Jr. and Janet A. Englund, who provided photocopies of some early historical papers.

When Dr. Ernest Tyzzer retired at Harvard in 1942 he gave me the microscopic slides and drawings that he had made in 1904–5 during his studies of the outbreak of varicella in Bilibid prison in the Philippines. Representative items have been deposited in the Registry of the Armed Forces Institute of Pathology and in the historical archives of the Countway Library at Harvard.

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Part II

Molecular Biology and Pathogenesis

2

Molecular evolution of alphaherpesviruses

Andrew J. Davison

Overview

Most modern virology revolves around determination of gene function. As a result, narrow perspectives abound – focused, for example, on a single host, a single virus, a single gene, a single motif. Work on viral evolution provides a constrasting perspective, where single entities take their place as equals among myriad others. It tells us what viruses share, and therefore what their history has been over broad sweeps of time. It tells us what is old and what is new, what is general and what is specific, what has happened once and what many times, which hurdles have been successfully overcome by different routes and which thus far only by one. Understanding viral evolution thus provides a context in which to view the origins, pervasiveness and flexibility of viral functions and the forces that have operated to shape viruses.

One feature that is pivotal to understanding the evolution of herpesviruses is their high degree of host specificity. Most herpesviruses infect a single species in nature, and a single animal species may be host to several distinct herpesviruses. This has long implied that herpesviruses have evolved in association with their hosts, and that interspecies spread may be relatively unimportant. Phylogenetic studies have generally confirmed this supposition, and have allowed a timescale to be inferred for herpesvirus evolution from knowledge about the evolution of their hosts.

In the context of herpesvirus evolution, the theme of this chapter is limited. Extant mammalian α-herpesviruses share the great majority of their gene functions and, according to current understanding, have histories as distinct lineages of up to 75 million years. Avian α-herpesviruses take the timescale back another 40 million. The α-herpesviruses separated from the other two subfamilies of mammalian herpesviruses perhaps 210 million years ago, and yet share over half of their gene functions. Looking back further, the barely detectable relationship between fish and mammalian herpesviruses probably represents over 400 million years of divergence. Finally, recent characterization of herpesviruses that infect

invertebrates is likely to take the documented history of the family back two or three times as far as this. Consequently, although this chapter deals largely with the α-herpesviruses, cognizance is taken of the family as a whole so that it may be seen that the modest evolutionary features of this subfamily are evident to much greater extent on the broader canvas.

The herpesviruses

Fundamentals

Well over a hundred herpesviruses have been described, infecting a range of vertebrates from humans to fish (Roizman et al., 1992). Also, at least one invertebrate, the oyster, is host to a herpesvirus (Le Deuff et al., 1994). On the scale of viruses, herpesviruses are complex entities. Their genomes are large, linear, double-stranded DNA molecules ranging in size from 125 to 245 kbp and containing from around 70 to 200 genes. The genome is packaged inside an icosahedral capsid of about 125 nm, which is contained within an amorphous layer of proteins called the tegument. The external surface of the virus particle consists of a lipid envelope carrying glycoproteins.

The most intensely studied herpesviruses are those with mammalian and avian hosts. Biologically, these viruses exhibit a wide range of properties consistent with extensive evolutionary divergence. One shared property is the ability to establish inapparent or latent infections and thus to infect an individual for life, punctuated by occasional reactivation episodes, which give opportunity for further transmission. The molecular mechanisms that enable latency to occur, however, vary widely. This striking property indicates that latency is a fundamental property, but that systems responsible for latency have arisen several times during herpesviruses evolution, presumably replacing previous ones. However, the fact that mammalian and avian herpesviruses have common mechanisms to achieve certain ends in their life cycles is indicated by the shared inheritance of a proportion of their genes.

Focusing further on one of the three lineages of the mammalian and avian herpesviruses, it is notable that even though the α-herpesviruses display a wide range of biological behaviours, they share most of their gene functions. Given the availability of extensive genome information, discovery of the genetic causes of biological differences might be judged a realistic target; whether, for example, the finger may be pointed at those few genes that are specific to a particular virus or whether the explanation lies in genes that are conserved in different viruses but exhibit subtle functional differences. It is sobering to note, however, that we have practically no understanding of how genetic differences between α-herpesviruses relate to pathogenesis.

Taxonomy

Taxonomy is (or should be) a reflection of the genetic, and hence evolutionary, relationships between organisms. The taxonomic structure of the herpesviruses was originally extablished from biological criteria before extensive sequence data were available, and has since been bolstered by genomic and genetic data (Roizman et al., 1981, 1992, 1995). In the ICTV (International Committee on Taxonomy of Viruses) system, three subfamilies are recognized: the Alpha-, Beta- and Gammaherpesvirinae. Each is divided into two or more genera; for example, the Alphaherpesvirinae into the genera Simplexvirus and Varicellovirus. However, the relentless push towards divesting the classification scheme of all reliance on biological properties, and assessing phylogeny solely on the basis of genetic relationships, has created an alternative system that is in widespread use. The genetic lineages equivalent to subfamilies are termed α-, β- and γ-herpesviruses (or the anglicized form, alphaherpesviruses, etc.), with further divisions indicated by subscripts. As the ICTV system has taken on board genetic data and misclassifications have been corrected, it has become essentially identical to the alternative system. Thus, viruses in the genera Simplexvirus and Varicellovirus are commonly termed α_1- and α_2-herpesviruses, respectively. Similarly, many individual herpesviruses are denoted in the literature, as in this chapter, by their common names (e.g. varicella-zoster virus) rather than by the ICTV names (e.g. human herpesvirus 3).

Most identified α_1-herpesviruses have primates (including humans) as their natural hosts. One exception, bovine herpesvirus 2 (BHV-2), has been noted thus far. The key viruses in this group (in the sense of pertinence to this chapter) are herpes simplex virus types 1 and 2 (HSV-1, HSV-2), both human pathogens. The α_2-herpesviruses are more widespread, occurring in primates (including humans) and also in other mammals including cattle, deer, horses, goats, pigs, cats, dogs and seals. The key viruses are varicella-zoster virus (VZV), equine herpesviruses 1 and 4 (EHV-1, EHV-4), bovine herpesvirus 1 (BHV-1) and pseudorabies virus (PRV), which infects pigs. Members of two other lineages in the α-herpesviruses infect birds: Marek's disease virus (MDV) and infectious laryngotracheitis virus (ILTV). It appears probable that the α-herpesviruses also count among their number members that infect turtles (Quackenbush et al., 1998). In their occurrence in mammals, birds and reptiles, α-herpesviruses are more widespread than β- or γ-herpesviruses, which have been found thus far only in mammals. In the wider scheme, it is important to emphasize that the current classification works for herpesviruses with mammalian, avian and reptilian hosts, but does not accommodate those that infect fish, amphibians or invertebrates. This point is returned to near the end of this chapter.

α-Herpesvirus genomes

Features

Characterised α-herpesvirus genomes range in size between 125 kbp (VZV) and 175 kbp (MDV), and in average nucleotide composition between 32% (canine herpesvirus) and 75% G + C (PRV) (Honess, 1984). The HSV-1, VZV, BHV-1 and EHV-1 genomes are known to have a single nucleotide extension at the 3′ ends (Mocarski & Roizman, 1982; Davison, 1984; Hammerschmidt et al., 1988), and are presumed to circularize after infection by direct ligation of the termini. Free genome ends are generated from head-to-tail concatemers produced during DNA replication by a cleavage event prior to packaging of unit-length genomes into capsids (Jacob et al., 1979). Cleavage involves sequence motifs present at each genome end (Stow et al., 1983; Nasseri & Mocarski, 1988).

Substructure

The genomes of α_1-herpesviruses may be considered as consisting of two major components, each comprising a unique sequence flanked by substantial inverted repeats (Figure 2.1; Sheldrick & Berthelot, 1974). Thus, U_S is flanked by TR_S and IR_S and U_L by TR_L and IR_L. The genomes of α_2-herpesviruses are similar, but TR_L/IR_L is much smaller (at its largest, 88 bp, in VZV, compared with 9212 bp in HSV-1) or absent (PRV). Genomes of α_1-herpesviruses are terminally redundant, having a sequence of a few hundred base pairs which is repeated directly at the genome termini and is also present in inverse orientation at the L–S junction (Wadsworth et al., 1975). Concatemeric DNA thus apparently contains two cleavage signals per genome length. In contrast, concatemeric α_2-herpesvirus DNA contains a single cleavage signal per genome length.

The presence of a large inverted repeat flanking U_S in all α-herpesvirus genomes facilitates recombination in concatemeric molecules that can lead to inversion of U_S relative to U_L. This, in combination with the presence of two cleavage sites per genome length in concatemeric DNA, gives rise to a total of four genome isomers in equimolar amounts in the virion DNA of α_1-herpesviruses, differing in the relative orientations of U_L and U_S (Hayward et al., 1975). In contrast, the presence of a single cleavage site per genome length yields a total of two genome isomers in most α_2-herpesviruses, each with U_L in the same orientation but differing in the orientation of U_S. Exceptionally, VZV U_L is found in a minor proportion of virion DNA molecules in the inverse orientation, presumably as a result of inefficient cleavage of concatemers at the alternative L–S junction (Davison, 1984). Although they belong to separate lineages within the α-herpesviruses, the genome of MDV (175 kbp) has an α_1 structure (Cebrian et al., 1982), and that of ILTV (155 kbp) has an α_2 structure, including a short TR_L/IR_L sequence (Leib et al., 1987; Ziemann et al., 1988b).

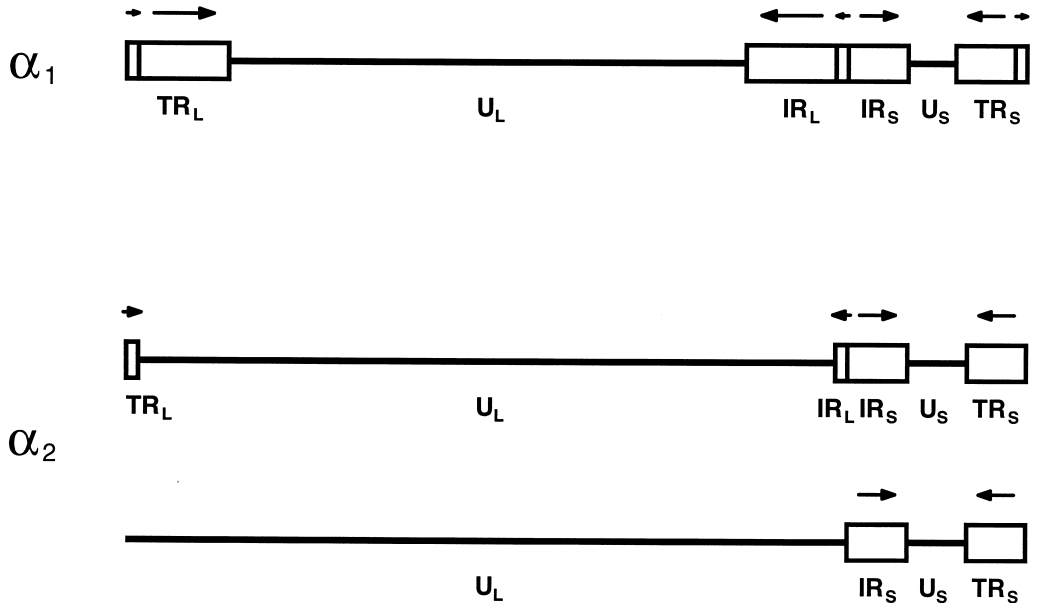


Figure 2.1 Structures of α-herpesvirus genomes. The structure of α_1-herpesvirus genomes and the two types of α_2-herpesvirus structure are shown, with unique sequences depicted as horizontal lines and repeats as rectangles oriented by arrows. The locations and orientations of the terminal redundancy are indicated in the α_1-herpesvirus genome by smaller rectangles and arrows. The nomenclature of parts of the genomes is explained in the text. The drawing is not to scale.

These features certainly add to the complexity of α-herpesvirus genomes. There is, however, no convincing evidence that either the genome structures of the α-herpesviruses or their ability to generate genome isomers is of biological consequence. It is intriguing, nonetheless, that the α_1-herpesvirus genome structure appears to have evolved independently in human cytomegalovirus, a β_1-herpesvirus (Weststrate et al., 1980) and the α_2-herpesvirus genome structure in an unrelated fish herpesvirus, salmonid herpesvirus 1 (Davison, 1998).

The wide variation in nucleotide composition of α-herpesvirus genomes has long been of interest. Genomes with high G + C content exhibit the greatest proportion of G and C residues in the third codon position, as would be expected in order to maintain coding potential (Murchie & McGeoch, 1982). In addition, the inverted repeats have higher G + C contents than unique regions, regardless of overall nucleotide composition, in some cases to such an extent that the amino acid compositions of encoded proteins are significantly biased (McGeoch et al., 1986). The forces that have driven nucleotide composition over genomes as a whole, and in particular in the inverted repeats, remain unidentified. Speculations have

involved sizes of nucleotide pools, DNA polymerase bias and the effects of recombination (Honess, 1984).

Sequences

The complete DNA sequences of two α_1-herpesviruses (McGeoch et al., 1988; Dolan et al., 1998) and four α_2-herpesviruses (Davison & Scott, 1986; Telford et al., 1992, 1998; accession AJ004801) have become available since 1986 (Table 2.1). Substantial amounts of sequence information are available for PRV, MDV and ILTV, and limited amounts for other members of the subfamily. This dataset is the essential to most experimental research on the α-herpesviruses, and also forms the basis for our current understanding of the evolution of the subfamily.

α-Herpesvirus genes

Genes

The gene sets for sequenced α-herpesviruses are listed in Figure 2.2, and the gene layout is shown for VZV and HSV-1 in Figures 2.3 and 2.4.

Various criteria are employed to identify genes from DNA sequence data. The end result inevitably leaves some uncertainty as to the total number of genes in a genome, especially with respect to small genes that lack homologs in related viruses. The current best estimate for the number of different protein-coding genes in the genome of the most studied α-herpesvirus, HSV-1, is 74 (Figures 2.2, 2.4). The great majority of the genome encodes proteins, and genes are arranged about equally between the two strands, generally with little or no overlap of protein-coding regions. There is, however, one gene (UL26.5) located completely within another (UL26) on the same strand, so that it shares its 3′ terminus with UL26 but has its 5′ end within the UL26 coding region (Liu & Roizman, 1991). The UL26.5 protein is thus an N-terminally truncated form of the UL26 protein. Because of this nested arrangement, the UL26.5 gene was identified not from sequence analyses but from experimental detection of its expression and function. The UL26.5 protein forms the major scaffold protein involved in capsid formation (Figure 2.2).

The discovery of UL26.5 implied that the HSV-1 genome may contain other genes that are not obvious because of substantial overlap. At least four candidates (UL8.5, UL12.5, US1.5 and UL15.5) in the UL26.5 class have since been proposed from experimental data (Draper et al., 1986; Baradaran et al., 1994; Carter & Roizman, 1996; Baines et al., 1997). The lack of a described phenotype for any (although it must be said that constructing mutants without affecting the larger, overlapping gene is problematic), and in some cases the absence of counterparts in other herpesviruses, currently excludes them from the HSV-1 gene set.

Experimental data have also demonstrated expression of sequences that are

Table 2.1 Completely sequenced α-herpesvirus genomes

Lineage	Common name and abbreviation	ICTV name	Genome size (bp)	% G + C	Gene number[a]	Accession number	Reference
α_1	Herpes simplex virus type 1 (HSV-1)	Human herpesvirus 1	152261	68.3	74	X14112	McGeoch et al. (1988)
	Herpes simplex virus type 2 (HSV-2)	Human herpesvirus 2	154746	70.4	74	Z86099	Dolan et al. (1998)
α_2	Varicella-zoster virus (VZV)	Human herpesvirus 3	124884	46.0	70	X04370	Davison & Scott (1986)
	Equine herpesvirus 1 (EHV-1)	Equid herpesvirus 1	150224	56.7	76	M86664	Telford et al. (1992)
	Equine herpesvirus 4 (EHV-4)	Equid herpesvirus 4	145597	50.5	76	AF030027	Telford et al. (1998)
	Bovine herpesvirus 1 (BHV-1)	Bovine herpesvirus 1	135301	72.4	71[b]	AJ004801	None

Notes:

[a] Genes present twice in the genome because they are present in repeated regions are counted only once.

[b] It is questionable whether three of these genes encode functional proteins.

VZV[a]	EHV-1 EHV-4	BHV-1	HSV-1 HSV-2	Function or other characteristic	Essential[b]
—	1	—	—	Possible membrane protein	− (EHV-1)
1	2	—	—	Membrane protein	− (VZV)
2	3	circ	—	Myristylated tegument protein	− (BHV-1)
—	—	—	UL56		−
3	4	—	UL55		−
4	5	UL54	UL54	Post-transcriptional regulator of gene expression	+
5	6	UL53	UL53	Virion glycoprotein gK	−
6	7	UL52	UL52	Component of DNA helicase-primase complex; primase	+
7	8	UL51	UL51	Tegument protein	−
8	9	UL50	UL50	Deoxyuridine triphosphatase	−
9A	10	UL49.5	UL49A	Non-glycosylated envelope protein disulphide-linked to gM	−
9	11	UL49	UL49	Tegument protein	− (BHV-1)
10	12	UL48	UL48	Tegument protein; transactivator of immediate-early genes	+
11	13	UL47	UL47	Tegument protein	−
12	14	UL46	UL46	Tegument protein	−
13	—	—	—	Thymidylate synthase	− (VZV)
—	15	—	UL45	Tegument/envelope protein	−
14	16	UL44	UL44	Envelope glycoprotein gC; role in cell entry	−
15	17	UL43	UL43	Probable integral membrane protein	−
16	18	UL42	UL42	Processivity subunit of DNA polymerase; complexes with UL30 protein	+
17	19	UL41	UL41	Tegument protein; host shut-off factor	−
18	20	UL40	UL40	Small subunit of ribonucleotide reductase	−
19	21	UL39	UL39	Large subunit of ribonucleotide reductase	−
20	22	UL38	UL38	Capsid protein; component of intercapsomeric triplex	+
21	23	UL37	UL37	Tegument protein	+?
22	24	UL36	UL36	Tegument protein	+
23	25	UL35	UL35	Capsid protein; located on tips of hexons	−
24	26	UL34	UL34	Membrane-associated phosphoprotein	+?
25	27	UL33	UL33	DNA packaging	+
26	28	UL32	UL32	DNA packaging	+
27	29	UL31	UL31	Nuclear matrix protein	−
28	30	UL30	UL30	Catalytic subunit of DNA polymerase; complexes with UL42 protein	+
29	31	UL29	UL29	Single-stranded DNA-binding protein	+
30	32	UL28	UL28	DNA packaging	+
31	33	UL27	UL27	Envelope glycoprotein gB	+
32	34	—	—		− (VZV)
33	35	UL26	UL26	N-terminal domain acts in capsid maturation and is a capsid protein; C-terminal domain is the minor capsid scaffold protein	+
33.5	35.5	UL26.5	UL26.5	Major capsid scaffold protein	−
34	36	UL25	UL25	DNA packaging; minor capsid protein	+
35	37	UL24	UL24		−
36	38	UL23	UL23	Deoxypyrimidine kinase	−
37	39	UL22	UL22	Envelope glycoprotein gH; complexes with gL; role in cell entry	+
38	40	UL21	UL21	Tegument protein	−

Figure 2.2 Gene functions of α-herpesviruses.

VZV[a]	EHV-1 EHV-4	BHV-1	HSV-1 HSV-2	Function or other characteristic	Essential[b]
39	41	UL20	UL20	Integral membrane protein; role in virion egress	−
40	42	UL19	UL19	Major capsid protein; component of hexons and pentons	+
41	43	UL18	UL18	Capsid protein; component of intercapsomeric triplex	+
42/45	44/47	UL15	UL15	DNA packaging; putative terminase	+
43	45	UL17	UL17	DNA packaging; tegument protein	+
44	46	UL16	UL16	Tegument protein	−
46	48	UL14	UL14	Tegument protein	−
47	49	UL13	UL13	Serine-threonine protein kinase; tegument protein	−
48	50	UL12	UL12	Deoxyribonuclease; role in maturation/packaging of DNA	−
49	51	UL11	UL11	Myristylated tegument protein; role in virion envelopment	−
50	52	UL10	UL10	Envelope glycoprotein gM	−
51	53	UL9	UL9	Binds to origins of DNA synthesis; helicase	+
52	54	UL8	UL8	Component of DNA helicase-primase complex	+
53	55	UL7	UL7		−
54	56	UL6	UL6	DNA packaging; minor capsid protein	+
55	57	UL5	UL5	Component of DNA helicase-primase complex; helicase	+
56	58	UL4	UL4		−
57	59	UL3.5	—	Tegument protein; role in virion egress	− (VZV)
58	60	UL3	UL3		−
59	61	UL2	UL2	Uracil-DNA glycosylase	−
60	62	UL1	UL1	Virion glycoprotein gL; complexes with gH	+
—	—	UL0.7	—		
—	—	UL0.5	—		
—	—	LRORF2	—	Proposed product of latency-related gene	
61	63	BICP0	RL2	Modulator of cell state and gene expression	−
—	—	—	RL1	Neurovirulence factor	−
62	64	BICP4	RS1	Transcriptional regulator	+
63	65	BICP22	US1	Host range factor	−
64	66	—	US10	Tegument protein	−
—	67	US1.67	—	Virulence factor	− (EHV-1)
	68	US2	US2	Virion protein	−
66	69	US3	US3	Serine-threonine protein kinase	−
—	70	US4	US4	Envelope glycoprotein gG	−
—	71	—	US5	Envelope glycoprotein gJ	−
—	72	US6	US6	Envelope glycoprotein gD	+
67	73	US7	US7	Envelope glycoprotein gI; complexes with gE in Fc receptor	−
68	74	US8	US8	Envelope glycoprotein gE; complexes with gI in Fc receptor	−
—	75	—	US8A		−
65	76	US9	US9	Ubiquitinated tegument protein	−
—	—	—	US11	RNA-binding protein	−
—	—	—	US12	Inhibitor of peptide transport by TAP, and of antigen presentation	−

Notes:

[a] Genes that have counterparts in α-, β- and γ-herpesviruses are shaded.

[b] The + symbol indicates that the gene function is absolutely essential for growth of in cell culture, the − symbol indicates that it is not. Queries indicate doubt. Data are for HSV-1 unless indicated otherwise.

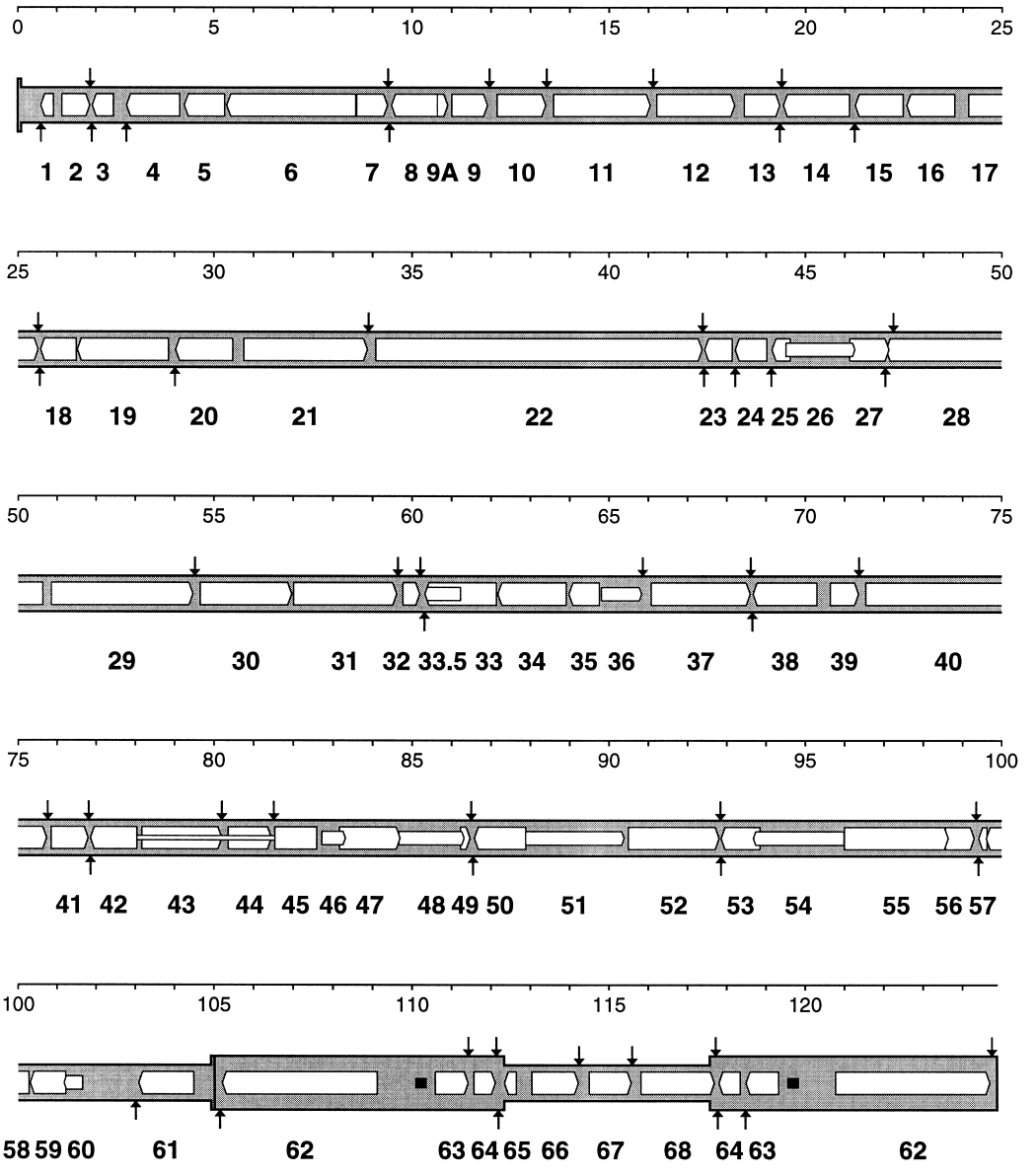


Figure 2.3 Predicted arrangement of protein-coding genes in the VZV genome. The genome is shaded, the thinner and thicker portions denoting the unique regions (U_L, U_S) and inverted repeats (TR_L/IR_L, TR_S/IR_S), respectively. The scale is in kbp. Protein-coding regions are shown as open arrows above the gene nomenclature. ORFs 42 and 45 are predicted to be expressed as a spliced mRNA; the intron is indicated by a narrow bar. Candidate polyadenylation sites are indicated by vertical arrows in the appropriate strand. The origins of DNA replication are denoted by small black rectangles.

wholly or largely antisense to accepted HSV-1 genes (Lagunoff & Roizman, 1994; Ward et al., 1996; Bruni & Roizman, 1996; Randall et al., 1997; Chang et al., 1998). However, HSV-2 counterparts of these entities (ORF P, UL43.5, ORF O and UL27.5) would require unusual transcriptional properties to be expressed, and none is conserved in other α-herpesviruses. Moreover, all apparent amino acid sequence conservation between these HSV-1 "genes" and their HSV-2 counterparts is due to conservation of the recognized gene on the other strand. Also, at least three lack a detectable phenotype. These features currently prompt exclusion of this class of antisense gene from the HSV-1 gene set. With the exception of UL26.5 counterparts, the expression of other α-herpesviruses has been too little studied to provoke description of overlapping "genes" similar to those reported for HSV-1.

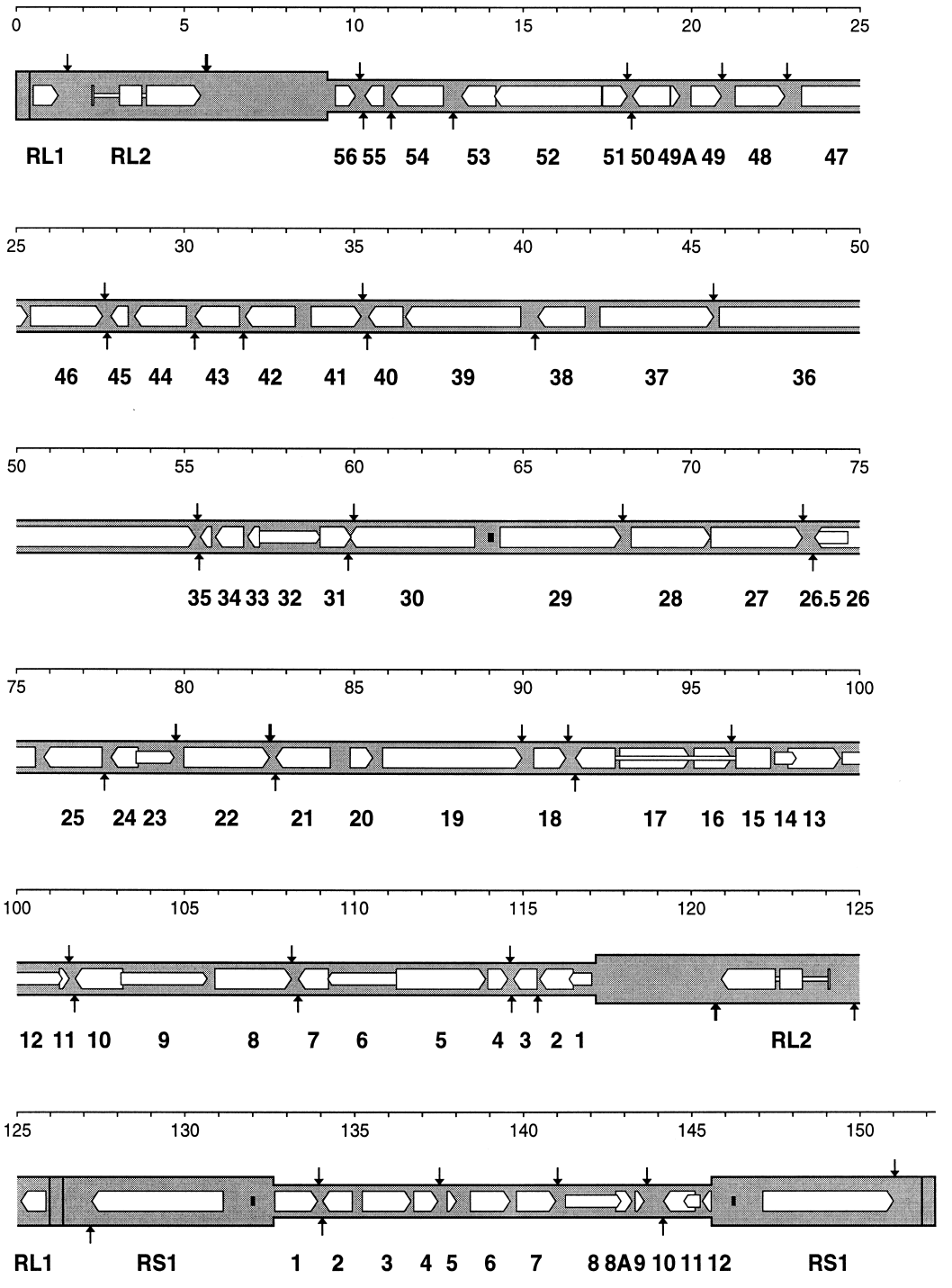
Expression

Most, if not all, α-herpesvirus genes are expressed from their own promoters. It is common for a family of genes arranged adjacently on the same strand to share a polyadenylation site downstream from the most 3′ member. Examples are VZV genes 46 to 49, where the shared polyadenylation site is downstream from gene 49 (Figure 2.3), and their HSV-1 counterparts, genes UL14 to UL11, where the site is downstream from UL11 (Figure 2.4).

Splicing is employed relatively rarely by α-herpesviruses; only four HSV-1 genes have been convincingly shown to be expressed by this means. Two (US1 and US12) share an untranslated exon in TR_S/IR_S, one (UL15) consists of two protein-coding exons, and one (RL2) consists of three protein-coding exons. In other α-herpesviruses, the UL15 counterparts are spliced, and there is evidence for splicing in certain EHV-1 and BHV-1 immediate-early genes (Harty et al., 1989; Schwyzer et al., 1993, 1994; Fraefel et al., 1993). Splicing is also involved in transcription of RNAs that are expressed from TR_L/IR_L during HSV-1 latency (Wagner et al., 1988), and from the equivalent region during PRV (Cheung, 1991) and BHV-1 latency (Hossain et al., 1995; Devireddy & Jones, 1998).

Relationships

Figure 2.2 lists the correspondence between α-herpesvirus genes. Given the relatively large divergence between the DNA sequences of these viruses, relationships between genes are generally assessed at the level of encoded amino acid sequence. However, HSV-1 is sufficiently closely related to HSV-2, and EHV-1 to EHV-4, for DNA sequence similarities to be obvious. The degree of amino acid sequence relatedness varies widely from gene to gene, presumably depending on how flexible a sequence can be while still retaining function. Indeed, the counterparts of a few genes, such as HSV-1 US5 and US8A, are so weakly related that their listing is tentative and depends on features such as position or orientation rather than on


0 5 10 15 20 25
RL1 RL2 56 55 54 53 52 51 50 49A 49 48 47
25 30 35 40 45 50
46 45 44 43 42 41 40 39 38 37 36
50 55 60 65 70 75
35 34 33 32 31 30 29 28 27 26.5 26
75 80 85 90 95 100
25 24 23 22 21 20 19 18 17 16 15 14 13
100 105 110 115 120 125
12 11 10 9 8 7 6 5 4 3 2 1 RL2
125 130 135 140 145 150
RL1 RS1 1 2 3 4 5 6 7 8 8A 9 10 11 12 RS1

convincing sequence similarity. Others, such as DNA polymerase (UL30), uracil–DNA glycosylase (UL2) and the UL15 protein are highly related. Nevertheless, it is clear that the great majority of genes have counterparts in all the α-herpesviruses examined. This indicates that the α-herpesviruses have evolved from a common ancestor.

Functions

The predictive value of the general correspondence in the genetic content of α-herpesviruses was highlighted by Davison & Wilkie (1983) and, as sequence data have accumulated, has been useful in inferring the functions of genes in less well characterized viruses from those in HSV-1. In evolutionary terms, however, the assumption of common function may be an oversimplification that fails to do justice to herpesvirus biology, since it is likely that certain genes have adapted to different roles in different viruses. This line of thought may be extended by considering the broad criterion of whether a gene is "essential"; that is, required for viral growth in cell culture (as indicated in the last column of Figure 2.2).

Most essential genes are directly involved in transcriptional control, capsid structure, DNA replication and packaging, or entry into and egress from the cell. The available information generally indicates that genes that are essential in one α-herpesvirus are also essential in others. Nevertheless, there are instances where this appears not to hold true. Glycoprotein D is required for cell-to-cell spread of HSV-1 (Ligas & Johnson, 1988) and BHV-1 (Schröder et al., 1997), but not of PRV (Rauh & Mettenleiter, 1991), MDV (Parcells et al., 1994) or VZV (Davison, 1983). Also, PRV UL3.5 (Fuchs et al., 1996) is essential whereas its VZV counterpart, gene 57, is not (Cox et al., 1998), and HSV-1 UL48 is essential (Ace et al., 1988) whereas its VZV homologue, gene 10, is not (Cohen & Seidel, 1994). It seems probable that more examples will emerge.

Essentiality in cell culture is rather a coarse measure, and it is likely that many

Figure 2.4 (*left*) Predicted arrangement of protein-coding genes in the HSV-1 genome. The genome is shaded, the thinner and thicker portions denoting the unique regions (U_L, U_S) and inverted repeats (TR_L/IR_L, TR_S/IR_S), respectively. In order to show the genes in the same general order as they are in the VZV genome, the HSV-1 U_L region is depicted in the orientation inverse to that of the prototypic isomer. The scale is in kbp. Protein-coding regions are shown as open arrows above the gene nomenclature. The "UL" prefix has been omitted from genes UL1–UL56, and the "US" prefix from genes US1–US12. Genes RL2 and UL15 are expressed as spliced mRNAs; the introns are indicated by narrow bars. Two other genes, US1 and US12, are also spliced, but as only the second exon is translated the intron is not shown. Candidate polyadenylation sites are indicated by vertical arrows in the appropriate strand. The origins of DNA replication are denoted by small black boxes.

genes sharing evolutionary ancestry exhibit subtler functional differences. Most mutants lacking a nonessential gene are compromised in cell culture, the extent depending on the gene. Thus, "essentiality" actually forms a spectrum rather than two extreme categories. Some HSV-1 or HSV-2 mutants, such as those lacking UL12 (Weller et al., 1990) or UL31 (Chang et al., 1997), are extremely debilitated, whereas others appear not to be affected at all, such as that lacking US3 (Nishiyama et al., 1992). It is also clear that the ability of certain mutants to grow is dependent on cell type or state. Examples of HSV-1 genes in this category include some that function at the interface between virus and cell, such as those involved in nucleotide metabolism (UL23 encoding thymidine kinase, Jamieson et al., 1974; UL39 encoding the large subunit of ribonucleotide reductase, Goldstein & Weller, 1988) or viral egress and maturation (UL20, Baines et al., 1991; UL53 encoding gK, Jayachandra et al., 1997; RL1, Brown et al., 1994).

In this connection, it is necessary to exercise a degree of caution in defining genes as essential, since this depends on characterization of mutants. The basic approach is to make a deletion of the majority of the target gene. In some cases, it is difficult to do this without potentially affecting neighbouring genes. However, modest deletions potentially result in truncated forms of the protein, which may retain function (and thus make an essential gene seem nonessential) or act as dominant inhibitors (and thus make a non-essential function seem essential). An example of the latter has been reported for HSV-1 UL53 (Jayachandra et al., 1997). There has also been an unsatisfactory tendency to denote genes as essential because of failure to generate viable mutants (e.g. HSV-1 UL34; Purves et al., 1991).

Regardless of whether a gene is essential in cell culture, or whether related genes encode proteins exhibiting different properties, it is axiomatic that all have biological function. Indeed, many genes that are nonessential in cell culture are severely compromised in animal models of infection (for example, several genes in U_S; Meignier et al., 1988). This phenomenon is likely to be even more accentuated in the context of the life cycle of the virus in the natural host.

Knowledge of the genetic basis for establishment, maintenance and reactivation of α-herpesvirus latency is rudimentary, despite intensive research. The role of the spliced latency-associated transcripts (LATs) expressed by HSV-1 in a proportion of infected neurons is not known. Doerig et al. (1991) detected a protein produced from these RNAs in infected neuronal cell cultures, but it has not been detected in latently infected tissues. Arguments from sequence data indicate that these transcripts are probably not translated into functional proteins (McGeoch et al., 1993). There is evidence, however, that RNAs expressed from the corresponding region in the latent PRV and BHV-1 genomes may function via translation products (Cheung, 1991; Hossain et al., 1995; Schang et al., 1996). Latency in VZV appears to be different, involving expression of certain lytic cycle genes (Croen et al., 1988).

Given the uncertainties, there is little understanding of the evolution of α-herpesvirus latency systems or how they relate to the corresponding processes in β- and γ-herpesviruses.

Collinearity

In the genomes dealt with in Figure 2.2, the order of genes is generally conserved. Local irregularities are present around the junctions between U_S and TR_S or IR_S, owing to recombination events that have resulted in movement of genes to and from the repeats (Davison & McGeoch, 1986). In an extreme case, this has resulted in transfer of VZV gene 65 from one end of U_S to the other. In PRV, a substantial region of U_L is inverted (Davison & Wilkie, 1983; Ben-Porat et al., 1983). Similarly, an independent inversion of part of U_L has occurred during evolution of ILTV (Ziemann et al., 1998a). In addition, one gene (UL47) has been transferred to U_S. Such large-scale recombination events resulting in permanent gene rearrangement are rare, but have occurred throughout herpesvirus evolution, as is evident from the more dramatic comparisons between different subfamilies (see below). Their rarity may indicate that most rearrangements were disadvantageous and did not survive. Rearrangements that have survived may have done so through inherent advantage or by coincident linkage to other beneficial mutations.

α-Herpesvirus evolution

Pathways

During their evolution, α-herpesviruses have employed a range of mechanisms to generate genetic diversity. The gradual processes of nucleotide substitution, insertion and deletion, coupled with the mysterious pressures that make their presence known at the level of nucleotide composition, have in general produced genomes that share little DNA sequence similarity while retaining closely similar gene complements. In one case where direct DNA sequence comparisons can be made (HSV-1 and HSV-2), no evidence has emerged for positive selection of any gene (Dolan et al., 1998). Positive selection is indicated when mutations promoting amino acid substitution are in excess of silent mutations, and is a feature of certain genes in other organisms, often associated with immune system functions. It can result in rapid divergence of DNA and encoded amino acid sequences.

In addition to these incremental modes, larger scale evolutionary processes have been at work. Gene duplications specific to the α-herpesviruses appear to have occurred in generating the glycoprotein genes present in HSV-1 as US4, US6 and US7 (McGeoch, 1990b). Gene duplication and inversion was possibly involved in generation of EHV-1 genes 1 and 2 (Telford et al., 1992). Gene loss also seems to have occurred; the U_S region in VZV lacks genes present in other α-herpesviruses,

including that encoding gD (Davison, 1983; McGeoch & Cook, 1994). Some genes have evidently been captured from the host cell genome. The most obvious examples within the α-herpesviruses are thymidylate synthase, encoded by VZV gene 13 (Thompson et al., 1987), and the HSV-1 RL1 gene (McGeoch & Barnett, 1991). Recombination has also played its part in α-herpesvirus genome evolution, as described above, both in local events at the ends of U_S and in large-scale rearrangements in U_L. However, the ultimate origins of most α-herpesvirus genes remain obscure. There is no evidence that the genes specifying proteins that make up the structure of the virus were derived from the host cell, although it seems more likely that they are highly diverged forms of such genes rather than genes generated de novo. Evidence is thin on the ground for de novo development of genes. There is at present a persuasive case only for the HSV-1 US12 gene, based on its extensive overlap with the US11 gene, the location of its promoter in TR_S and IR_S (where it controls expression of the US1 gene) and the small size and unstructured nature of the encoded protein (McGeoch & Davison, 1999). It is possible that some of the other smaller genes that are specific to one or two viruses also arose de novo.

Phylogeny

The availability of extensive DNA sequence data for the α-herpesviruses has enabled the construction of detailed, reliable phylogenetic trees. The analysis of McGeoch & Cook (1994) was based upon several genes from a wide range of α-herpesviruses, but particularly on the gene encoding the highly conserved glycoprotein, gB (UL27 in HSV-1). This analysis showed that the mammalian α-herpesviruses fall into two major groups, with MDV and ILTV forming two separate offshoots. The positions of most viruses in the phylogenetic tree accord with a model of coevolution with the hosts, with the exception of BHV-2. This general correlation was used to derive a timescale for α-herpesvirus evolution, and to estimate approximate nucleotide substitution rates, which are of the order of 20 times greater than that of the host genome. The analysis was subsequently refined and extended to the β- and γ-herpesviruses (McGeoch et al., 1995), as summarized in Figure 2.5.

Within this framework, development of lineages within the same host has also occurred more than once: HSV-1 and VZV at the time when α_1- and α_2-herpesviruses began to diverge in an ancestor of modern mammals about 75 million years ago, HSV-1 and HSV-2 in a higher primate around 8.4 million years ago, and EHV-1 and EHV-4 in an equid at a slightly more recent date.

Comment may also be passed on those hosts in which α-herpesviruses have not been found, with the caveat that absences may eventually be remedied by further sampling. Thus, α_2-herpesviruses occupy niches in many mammalian hosts, but α_1-herpesviruses only in primates. BHV-2 is thought to represent transmission

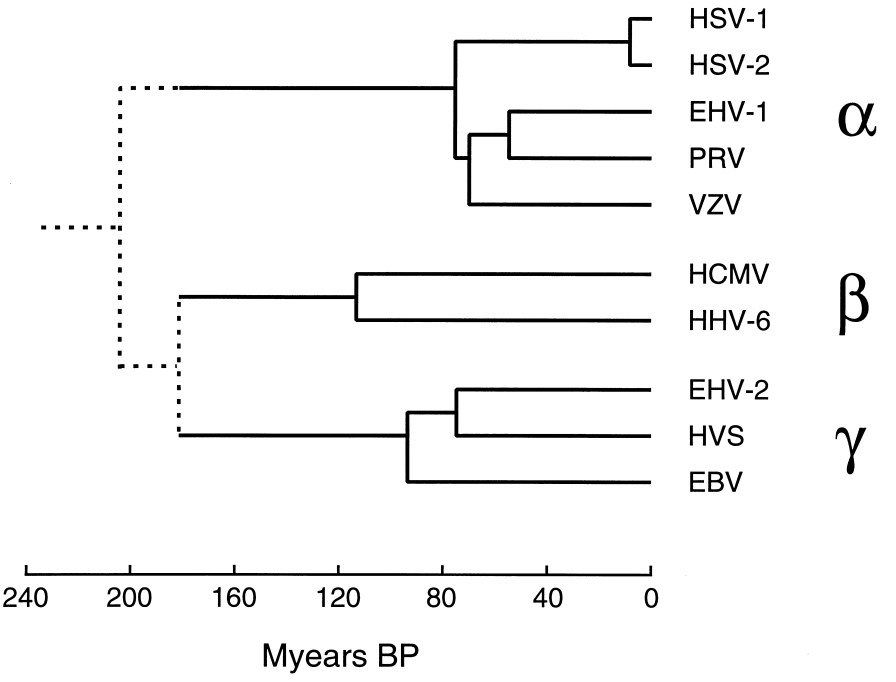


Figure 2.5 Phylogenetic tree for selected mammalian herpesviruses deduced by McGeoch et al. (1995) from the combined amino acid sequences encoded by several well-conserved genes. HSV-1 and HSV-2 are shown as representatives of the α_1-herpesviruses, and EHV-1, PRV and VZV as representatives of the α_2-herpesviruses. HCMV and human herpesvirus 6 (HHV-6) are β-herpesviruses, and equine herpesvirus 2 (EHV-2), herpesvirus saimiri (HVS) and Epstein-Barr virus (EBV) are γ-herpesviruses. A proposed timescale inferred from host palaeontology is shown in millions of years before present (Myears BP). The oldest part of the tree is shown as a broken line to indicate lower confidence. Reproduced from McGeoch et al. (1995) with permission of Academic Press.

from a primate to a bovine host about 33 million years ago (McGeoch & Cook, 1994). A similar explanation could apply to two marsupial herpesviruses whose gB sequences were recently reported by Mahony et al. (1999) to have unexpectedly close relationships to those of α_1-herpesviruses. The observation that α-herpesviruses are not represented in some groups of mammals, such as rodents, indicates that they may have suffered extinction in certain animal lineages.

α-, β- and γ-herpesvirus evolution

Phylogeny

In extending their findings to the herpesviruses as a whole, McGeoch et al. (1995) indicated that the α-herpesviruses separated from the lineage that gave rise to the

β- and γ-herpesviruses about 210 million years ago, and that the β- and γ-herpesviruses diverged 180 million years ago (Figure 2.5). These authors rightly pointed out the tentative nature of this timescale, given that extrapolations of evolutionary rates derived from the α-herpesviruses to increasingly remote times are prone to greater error. Nonetheless, genetic comparisons of the three lineages make it clear that relationships are much more distant than they are within the α-herpesviruses.

Whereas the great majority (62) of HSV-1 genes are conserved in α-herpesviruses, only 43 have counterparts in β- and γ-herpesviruses. These are shown by shading in Figure 2.2. It is notable that few of the genes that are specific to α-herpesviruses are essential for growth of HSV-1 in cell culture. Of the known exceptions UL48, US6, RS1 and UL9, the first two are not essential in some α-herpesviruses, as mentioned above. RS1 is the major transactivator of α-herpesvirus transcription, and its function is supplied in β- and γ-herpesviruses by unrelated genes. UL9 binds to α-herpesvirus origins of DNA replications, and, along with the origin of DNA replication itself, is the only element of the DNA replication machinery that is not common to α-, β- and γ-herpesviruses. These elements are, however, found in β_2-herpesviruses (Gompels et al., 1995; Nicholas, 1996), indicating that they may predate alternatives developed by β_1- and γ-herpesviruses (McGeoch & Davison, 1999).

As in the α-herpesviruses, β-herpesviruses share the same general order of genes (Gompels et al., 1995; Rawlinson et al., 1996). The same is true of γ-herpesviruses (Albrecht et al., 1992). From comparisons between members of different lineages, however, it is evident from the differing gene orders that multiple large-scale rearrangements have taken place at times predating divergences that took place within each lineage to give rise to extent herpesviruses (i.e. between 210 and 75 million years ago) (Davison & Taylor, 1987; Chee et al., 1990; Hannenhalli et al., 1995). In addition, each of the three major lineages has accumulated a sizeable number of genes specific to that lineage.

The mechanisms that have operated to produce the α-, β- and γ-herpesvirus lineages are the same as those apparent within the α-herpesviruses, but on a grander scale. They include gradual mutation, gene capture and gene duplication. The β_1-herpesviruses contain substantial arrays of duplicated genes (Chee et al., 1990; Rawlinson et al., 1996). There is good evidence in the deoxyuridine triphosphatase gene (UL50 in HSV-1) of an ancient gene capture event, followed by duplication and fusion, that preceded separation of the three lineages (McGeoch, 1990a). There are several other examples of genes whose capture predated separation: DNA polymerase, uracil-DNA glycosylase, thymidine kinase, ribonucleotide reductase, protein kinase, primase and DNA helicase. Phylogenetic considerations indicate that gene capture has operated in herpesviruses throughout evolution, from very

ancient periods (e.g. DNA polymerase) to more recent times (e.g. HSV-1 RL1 and some of the captured genes with which the γ_2-herpesviruses are particularly well endowed). There are also examples of independent capture of the same host gene (e.g. thymidylate synthase) in different lineages. Moreover, there is evidence that certain captured genes have lost their original functions and, by implication, gained new ones (McGeoch & Davison, 1999). In addition, there are striking examples in a γ_2-herpesvirus, human herpesvirus 8, of positive selection of the K1 gene (Nicholas et al., 1998; P.M. Cook, D. Whitby, D. Nalwanga-Kakoola, H. Hjalgrim, M. Melbye, P. Monini, A. Hatzakis, A.J. Davison & T.F. Schulz, unpublished data) and gene swapping of the K15 or LAMP gene (Nicholas et al., 1998; M.A. Glenn, L. Rainbow, A.J. Davison & T.F. Schulz, unpublished data).

The bigger picture

As stated at the outset, this chapter focuses on one corner of the herpesvirus world, the α-herpesviruses. The evolutionary changes that have occurred in giving rise to today's α-herpesviruses may seem large, particularly from the point of view of viral biology, and there is no denying that differences between members of this group are extensive at the sequence level. The mechanisms by which even closely related viruses exert differing pathogenic effects remain poorly understood. Moreover, the genetic differences among the α-herpesviruses are minor in comparison with those between the α-, β- and γ-herpesviruses, and seem minimal when set in the context of herpesviruses as a whole.

Despite the differences resulting from long periods of separate evolution, α-, β- and γ-herpesviruses share a substantial genetic heritage and thus may be presumed to have evolved from an ancestor that would have been clearly recognizable as a herpesvirus. In contrast, herpesviruses of amphibians and bony fish are related to each other but share very little genetic similarity with the α-, β- and γ-herpesviruses (Davison, 1992, 1998; Davison et al., 1999). The two groups of viruses recognizably share only a single gene (UL15 in HSV-1) that lacks a cellular counterpart and therefore may have been derived from a common ancestor. Nevertheless, the one fish herpesvirus that has been examined in detail (channel catfish virus) has a characteristic herpesvirus capsid (Booy et al., 1996) and probably utilizes processes similar to those of the α-, β- and γ-herpesviruses to form the capsid and package DNA (Cebrian et al., 1983; Davison, 1992; Davison & Davison, 1995).

Given this state of apparent genetic isolation, these two major groups of herpesviruses (mammalian, avian and reptilian herpesviruses on the one hand, and fish and amphibian herpesviruses on the other) sit together rather uncomfortably. On balance, it appears most likely that they diverged from a common ancestor, recognizably a herpesvirus, at the time when bony fish separated from other

vertebrates about 400 million years ago, but that evolutionary processes have proceeded to the point where detectable sequence similarity has been lost. Two processes that are inapparent in α-herpesvirus evolution but which have occurred in recent evolution of the γ-herpesviruses – positive selection and gene replacement – may have played significant roles over this much greater length of time. There is also the possibility that the balance of evolutionary mechanisms may have varied over such lengthy periods and over such a wide range of organisms.

To go one step further, evidence is now accumulating for the existence of herpesviruses of shellfish, and it appears that these may fall into a third major group. Oyster herpesvirus appears to lack significant genetic relationship to the other two groups (A.J. Davison, unpublished data), as would be expected if this virus has evolved separately from vertebrate herpesviruses for perhaps a billion years (Wray et al., 1996). Lastly, it is intriguing that the one gene apparently shared by all herpesviruses, UL15 in HSV-1, has a distant counterpart in bacteriophage T4 (Davison, 1992). It is stimulating to speculate that this may be a relic of a very ancient evolutionary divergence, but sobering to realize that establishing a link of such antiquity is likely to remain beyond our powers.

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3

DNA replication

William T. Ruyechan and John Hay

Structure and physical properties of VZV DNA

The varicella-zoster virus (VZV) genome is a linear double-stranded DNA molecule consisting of approximately 125 000 base pairs with an average G + C content of 46%. Computer analysis of the sequence predicted the presence of approximately 70 open reading frames (ORFs). The genome is similar in overall structure to other alphaherpesvirus DNAs and its significant colinearity with the herpes simplex virus type 1 (HSV-1) sequence facilitated assignment of the ORFs (Davison & Scott, 1986). The VZV genome consists of two covalently linked segments, L and S, which are in turn composed of unique sequences U_L and U_S. These unique regions are bounded by inverted repeat sequences IR_L/TR_L and IR_S/TR_S, respectively (Figure 3.1). In the genome of the Dumas strain, which was completely sequenced by Davison & Scott (1986), the U_L region consists of 104 836 bp flanked by 88.5 bp inverted repeats and the U_S region consists of 5232 bp flanked by 7319.5 bp inverted repeats. These data are consistent with earlier estimates of the size of the genome derived from electron microscopic measurements, restriction enzyme analysis, and the overall G + C content estimated by bouyant density (Ludwig et al., 1972; Dumas et al., 1980, 1981; Straus et al., 1981; Davison & Scott, 1983).

Analysis of restriction enzyme digests of DNA derived from purified virions of numerous strains indicated that unlike the herpes simplex genome, which has a distinctive strain-dependent restriction fragment fingerprint, only a few "geographic" restriction fragment polymorphisms present or absent in VZV DNA genomes isolated in Asia or western Europe and the US were observed. These polymorphisms have subsequently been extremely useful in delineating the Oka vaccine strain from other VZV strains (Martin et al., 1982; Hayakawa et al., 1986; LaRussa et al. 1992). In addition to these restriction site polymorphisms, it was noticed that certain DNA fragments showed strain dependent differences in their migration rates (Straus et al., 1983). DNA heteroduplex experiments and sequencing showed that these differences were the result of the presence of varying numbers of copies of repetitive elements. Davison & Scott (1986) identified four such repeat regions: R1,

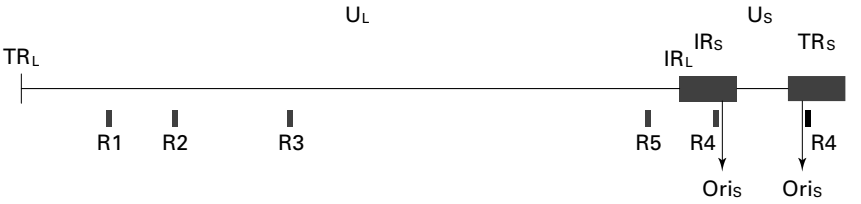


Figure 3.1 General structure of VZV DNA. The structural organization of the prototype (P) isomeric form of the VZV genome is depicted schematically. The positions of the five repeat elements and the locations of the origins of DNA replication are represented by solid rectangles and arrows, respectively. The G + C content of the major structural elements is as follows: U_L, 44.3%; U_S, 42.8%; IR_L/TR_L, 68.4%; and IR_S/TR_S, 59.1%.

R2, R3, and R4, as a result of their sequencing of the genome of the Dumas strain. A fifth repeat element R5 was subsequently identified by Hondo & Yogo (1988). R1 to R3 occur in the ORFs encoding VZV genes 11, 14, and 22 respectively. Two copies of R4 occur within the repeat regions bounding the unique short region, thus they are identical within the context of the sequence of the genome of a specific strain, one being the inverse of the other. The R5 repeats fall within an intragenic region within U_L.

R1 is an extensive, complex repeat made up of varying numbers and arrangements of two 18 bp elements, two 15 bp elements and a 3 bp GGA element. Within all strains currently analyzed the nucleotide sequence differences are in multiples of three, thus resulting in no frame shifts within ORF11. The R2 repeat consists of tandem arrays of two 42 bp sequences, which differ only by an A/T substitution at position 21. A third sequence, comprising the first 32 bases of the longer sequences, is present as the final element in these repeats. As with the R1 repeat, the entire set of R2 sequences remain in-frame, resulting in the generation of proteins of different size encoded by ORF14 in various strains (Kinchington et al., 1986).

The R3 repeat consists of multiple copies (up to 100) of a 9 bp element. The number of these repeats, at least in part, contributes to the instability of cloned fragments of this part of the genome. Again, as was the case with R1 and R2, the repeats are in-frame, resulting in variation in size of the ORF22 protein in various VZV strains. It is not clear if the presence of more or less of these repeat elements results in a biological advantage for a given strain. Similarly the significance, if any, of the fact that these repeats occur within proteins present in the virus particle (ORFs 11 and 22 encode putative tegument proteins, ORF14 encodes glycoprotein C) has not yet been investigated.

The two copies of the R4 are made up of variable numbers of a 27 bp element (Casey et al., 1985; Davison & Scott, 1986), which are located in an intragenic region adjacent to the two copies of the viral origin of DNA replication (see below)

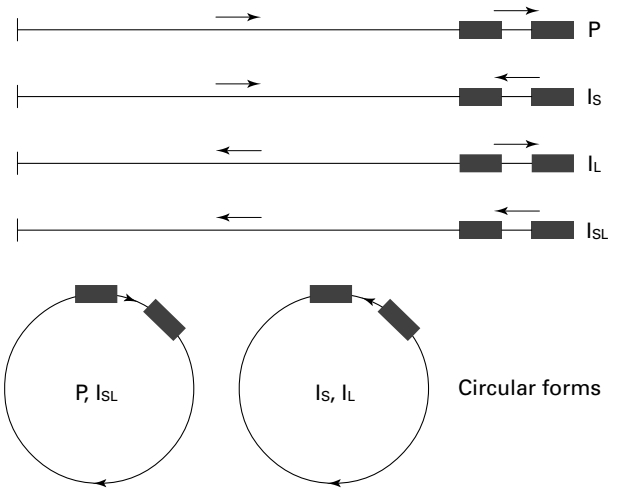


Figure 3.2 Forms of VZV DNA. The major and minor isomeric forms of VZV DNA and the circular forms of the genome are depicted schematically. The P and I_S isomers make up over 95% of the packaged viral DNA. The two circular forms shown would result from the circularization of the P or I_{SL} and I_L or I_S isomers, respectively.

within the IR_S/TR_S repeats. The R5 repeats are located between coding sequences for the ORF60 and 61 genes and consist of tandem arrays of an 88 bp element followed by a 24 bp element (Hondo & Yogo, 1988). Davison & Scott (1986) indicated that these reiterated elements are unique to VZV with respect to their sequence and location, with the possible exception of the R4 repeats, which show some homology to a similarly located repeat element within the HSV genome.

VZV DNA derived from purified virions is present in two predominant isomeric forms designated arbitrarily as P (prototype) and I_S (the S region inverted with respect to the prototype form). Restriction enzyme analysis of virion-purified DNA shows bands at concentrations of 0.5, 1.0 and 2.0 M., in keeping with the two-isomer model of VZV DNA (Dumas et al., 1981; Straus et al., 1981, 1982; Ecker & Hyman, 1982; Davison & Scott, 1983). However, additional restriction enzyme analyses targeted to the termini of the genome indicate the presence of novel DNA fragments at very low abundance, which would result from the presence of two other isomeric forms of the DNA in which the L region is inverted with respect to the S region in the P and I_S configurations (Davison, 1984). These isomeric forms are designated I_L and I_{SL} and are estimated to be present at levels representing 2–5% of the total virion DNA (Figure 3.2).

Evidence has also been presented for the existence of small numbers of closed circular molecules present in virion DNA. This evidence includes the presence of novel DNA fragments present in restriction enzyme digests which would be

generated by the presence of structures with the characteristics of closed circular DNA molecules and the presence of VZV DNA migrating on agarose gels at a position corresponding to the position of circular DNA 125 000 bp in size (Straus et al., 1982; Ecker et al., 1984; Ruyechan et al., 1984; Kinchington et al., 1985). The circular DNA molecules are estimated to comprise between 0.1 and 5% of nucleocapsid DNA. Since these DNA preparations were subjected to extensive protease treatment and phenol chloroform extraction, it is believed that the molecules observed represent covalently closed circular DNA. The role of these circular DNAs in the viral infectious cycle is currently unknown. They could potentially play a role in DNA replication (see below) or may represent a subpopulation of molecules that is energetically more favorable to transcription and thus would act as a template for the earliest transcriptional events in VZV infection.

VZV DNA purified from nucleocapsids is infectious. The ability to transfect susceptible cells with purified VZV DNA resulting in the generation of mature infectious virions was first demonstrated by Dumas et al. (1980). The specific infectivity of the DNA was determined to be 80–140 plaque forming units (pfu)/μg. This level of infectivity is one to two orders of magnitude lower than that observed with HSV DNA. It is possible that the nature or mechanism of replication of purified transfected VZV DNA is intrinsically different (and less efficient) than that for HSV DNA (Sheldrick et al., 1973). This is a formal possibility that cannot be discounted based on the data reported to date. It is known that cotransfection with plasmids expressing the VZV ORF62 protein enhances the infectivity of the DNA by 10–100 fold (Moriuchi et al., 1994) and that the ORF62 protein is present in significant amounts in the virus tegument and thus probably enters the nucleus along with the viral DNA (Kinchington et al., 1992). These findings suggest that the ORF62 transactivator plays an important role in the infectious process, most likely by increasing transcription of important viral gene products (see below) although an as yet unknown activity of this protein may also be important.

An additional factor in the low specific infectivity of VZV DNA observed may lie in the nature of virion purified DNA. The data from electron microscopic studies of the size of VZV DNA are based on measurements of small numbers of intact molecules (Dumas et al., 1980; Straus et al., 1981, 1982; Ecker & Hyman, 1982) and sometimes show the presence of molecules that are less than unit length and appear to contain single-stranded regions (Ruyechan, unpublished observations), suggesting that packaged VZV DNA is relatively fragile and the low levels of infectivity observed may be related to the number of genomes remaining intact after purification and transfection.

The fact that VZV DNA is infectious in the context of transfection of susceptible cells raised the possibility that mutations could be introduced directly into the viral genome by recombination with mutagenized fragments of VZV DNA, as was

the case with HSV. Unfortunately the difficulty in generation of large amounts of purified DNA from infected tissue culture and the low and variable levels of infectivity obtained precluded the use of this method as a viable approach. Cohen & Seidel (1993) have pioneered the development of cosmid systems for generation of viral mutants. In these systems, large overlapping portions of the VZV genome, typically 20–30 kbp in length, were cloned into cosmid vectors. Transfection of recombinant cosmid DNA containing all four VZV DNA segments resulted in the production of infectious virus via general recombination events that regenerated intact VZV genomes. Thus the combination of the intrinsic infectivity of VZV DNA and recombinant DNA technology led to the generation of a system that can be used to generate specific viral mutants, since the same strategy of transfecting the recombinant cosmid DNA containing specifically mutagenized VZV DNA sequences could be used. This approach has been employed extensively by the laboratories of Cohen and Arvin to generate numerous deletion mutants in specific genes, the majority of which have been shown to be dispensable for growth of the virus in tissue culture but important to viral pathogenesis (Cohen & Seidel, 1995; Mallory et al., 1997; Moffet et al., 1998). In the future, these techniques should prove equally useful in the introduction of small deletions and point mutations in both ORFs and in the cis-acting elements that control expression from those ORFs. Finally, and most directly related to the subject matter of this chapter, the infectious nature of purified viral DNA and the success of the cosmid systems in the generation of viral particles indicates that viral functions transcribed from the input DNA as well as some host cell activities are sufficient for the replication of viral DNA.

The viral origin of DNA replication

The locations and structure of the viral origins of DNA replication and the function of various elements contained therein have been determined by Stow and coworkers (Stow & Davison, 1986; Stow et al., 1990). The VZV genome contains two copies of a sequence that acts as a replication origin in the context of VZV infection. These sequences occur within the inverted repeats flanking the U_S region of the genome. They consist of a 46 bp palindromic sequence, the center of which is composed of 16 TA dinucleotide repeats, and three sites recognized by the VZV ORF51 viral origin binding protein (OBP). These three sites, designated A, B, and C, consist of 10 bp elements and all three are located upstream of the AT-rich palindrome with A being the most proximal site and B the distal site (Figure 3.3). All three sites conform to the concensus binding site 5′-YGYTCGCACT-3′ identified for the HSV-1 OBP (Koff & Tegtmeyer, 1988). The site A and B sequences are identical (5′-CGTTCGCACT-3′) with the C site having a single G/A substitution at the second position (5′-CATTCGCACT-3′). Stow et al.(1990) showed that the A site is

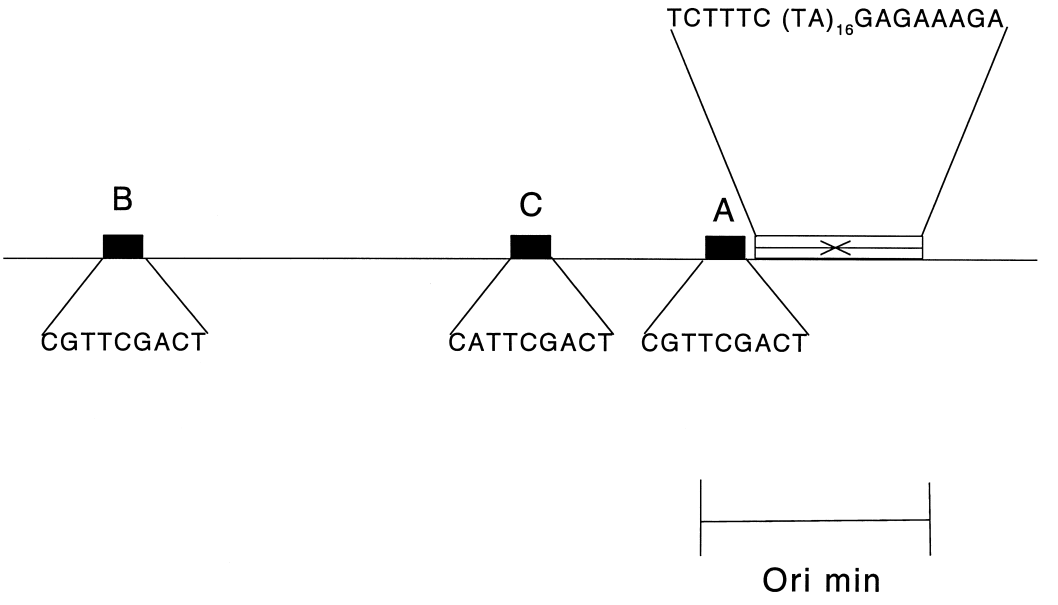


Figure 3.3 Structure of the VZV DNA replication origin. The organization of the elements making up one of the two identical VZV replication origins is depicted. The binding sites for the VZV ORF51 origin binding protein are represented as solid rectangles and the A/T-rich palindrome by the open rectangle. Ori min refers to the minimal set of elements required for replication of plasmids containing VZV origin sequences.

absolutely required for replication and that deletion of the C site results in a decreased level of replication. In contrast, deletion of the B site showed no effect on the extent of replication of an origin containing plasmid transfected into VZV infected cells. Thus the minimal VZV origin consists of the A site and the AT-rich palindrome.

Several points differentiating the location and structure of the VZV replication origins versus these elements in HSV DNA should be made. Firstly, the VZV genome contains two origins of replication whereas the HSV genome contains three (reviewed by Boehmer & Lehman, 1997). Two of the HSV origins are present in analogous sites in the HSV genome in the IR_S/TR_S repeat regions (Ori_S). The third is located in the U_L region between the divergent coding regions for the HSV DNA polymerase and major DNA-binding protein (Ori_L). Experiments assessing the presence of a functional origin element in this region in VZV DNA have proven negative. Rather this region in VZV DNA is comprised of a bidirectional promoter sequence that regulates the transcription of the VZV DNA polymerase and DNA-binding protein genes (Meier & Straus, 1993). Secondly, the structure of the analogous VZV and HSV Ori_S regions is different. While both are palindromic

Table 3.1 Varicella-zoster virus DNA replication genes and their functions

VZV gene	Predicted mol. wt.	Function	HSV homolog
ORF28	134041	Polymerase catalytic subunit	UL30
ORF16	46087	Polymerase processivity factor	UL42
ORF55	98844	Helicase	UL5
ORF6	122541	Primase	UL52
ORF52	86343	Helicase/primase accessory	UL8
ORF29	132133	Single-strand DNA binding	UL29
ORF51	94370	Origin binding/helicase	UL9

Note: The functions of the proteins encoded by VZV ORFs 6, 16, 52, and 55 are inferred based on the properties of their HSV homologs (Boehmer & Lehman, 1997).

sequences 45–46 bp in length with a $[TA]_{16}$ element at their cores, all of the binding sites for the VZV OBP are upstream of the AT-rich palindrome and all are in the same orientation. In contrast, the HSV Ori_S has OBP binding sites both upstream and downstream of the AT-rich core and these sites are oriented in opposite directions. These differences suggest that the binding of the respective OBPs may be different, as would be the unwinding of the origin and development of the replication fork. Another possibility, raised by Stow et al. (1990) could be that an as yet unknown viral or cellular protein binds downstream of the TA-stretch in the VZV promoter. As precedence for this it has been shown that both HCMV and Epstein Barr Virus require additional viral proteins that specifically activate origin-dependent replication (Iskendarian et al., 1996; Sariskey & Hayward, 1996; Sariskey et al., 1996). A third possibility would involve an alternative binding sequence for the VZV OBP in this region, resulting in an overall mechanism similar to that observed for HSV.

Viral proteins involved in DNA replication

Comparison of the sequences of the VZV and HSV genomes revealed that VZV encodes homologs of the seven viral proteins that are required for origin-dependent DNA synthesis in HSV (Challberg, 1986; Davison & Scott, 1986; Boehmer & Lehman, 1997). As summarized in Table 3.1, these include the viral DNA polymerase catalytic subunit encoded by ORF 28 and its accessory factor the ORF 16 protein, the major DNA binding protein encoded by ORF 29, a putative heterotrimeric helicase/primase complex encoded by ORFs 6, 52, and 55, and the ORF 51 protein [OBP], which binds specifically to the viral DNA replication origin sequences. Homologs of the first six of these proteins are encoded by all human

herpesviruses. The current data describing the properties of these VZV DNA replication proteins are summarized below.

Biochemical characterization of these activities has been undertaken only in the case of the polymerase, the origin binding protein and the ORF29 protein (SSB). This is in part due to the fact that prior to the availability of DNA sequence information the amounts of infected cell material required for even partial purification of these activities were prohibitively large considering the inefficiency of growth of the virus in tissue culture. Subsequent to this, the lack of biochemical information available appears to be a combination of the small number of laboratories working on VZV worldwide and the erroneous assumption that HSV DNA replication is sufficiently similar to VZV DNA replication to make such investigations redundant.

VZV DNA Polymerase

The VZV DNA polymerase is predicted to be a heterodimer consisting of the ORF28 and ORF16 gene products. The catalytic subunit of the VZV DNA polymerase is a polypeptide with a predicted molecular weight of 134041 kDa and exhibits 67.7% similarity and 58.9% identity with its HSV homologue. The polymerase contains structural motifs similar to those found in the eukaryotic α and δ DNA polymerase families, a characteristic it shares with all known herpesvirus polymerases (Coen, 1996). The putative processivity factor encoded by the ORF16 gene has a predicted molecular weight of 46087 kDa. and shows less similarity (45.2%) and identity (31.2%) to its HSV homologue. This protein has not been characterized biochemically. However, based on its homology with the HSV UL42 protein it is predicted to interact with the ORF28 gene product and with duplex DNA thus tethering the catalytic subunit of the polymerase to the template strand of the DNA being replicated.

May et al. (1977) showed that replication of VZV in tissue culture was inhibited by phosphonoacetic acid, indicating that VZV encoded its own DNA polymerase. This compound had already been shown to be an inhibitor of other herpesviruses (Shipkowitz et al., 1973) with its target being the viral DNA polymerase (Mao & Robishaw, 1975). Partial purification and characterization of the VZV DNA polymerase was reported by Miller & Rapp (1977), Mar et al. (1978) and Mar & Huang (1979). These purifications were accomplished by ion exchange chromatography of infected cell extracts. Although no characterization of the polypeptides present in the active fractions was presented in either study, it is reasonable to assume that the activity observed represents that associated with the holoenzyme as opposed to the catalytic subunit alone. The data from both reports showed that the VZV DNA polymerase could be differentiated from host cell polymerase activities based on template specificity and salt optima. The VZV polymerase prefers homopolymer

templates [Poly(dC)/oligo(dG) and Poly(dA)/oligo(dT)] although it can also utilize activated calf thymus DNA. The polymerase exhibits optimum activity at 30–60 mM ammonium sulfate and 60–100 mM KCl. This latter property is in contrast to the HSV-1 polymerase, which exhibits optimal activity between 100 and 200 mM monovalent cation (Powell & Purifoy, 1977; J. W. Olson & W. T. Ruyechan, unpublished observations and Figure 3.4A). This difference may reflect the difference in the G + C contents of the genomes that these polymerases replicate (46% for VZV and 67% for HSV) or a difference in the local environment of the polymerase in the nucleus during infection. Data presented by Ertl et al. (1991) indicate that the observed differences in monovalent salt optima are probably due to a number of factors including the specific DNA template being used in the assay and the level of purity of the enzyme.

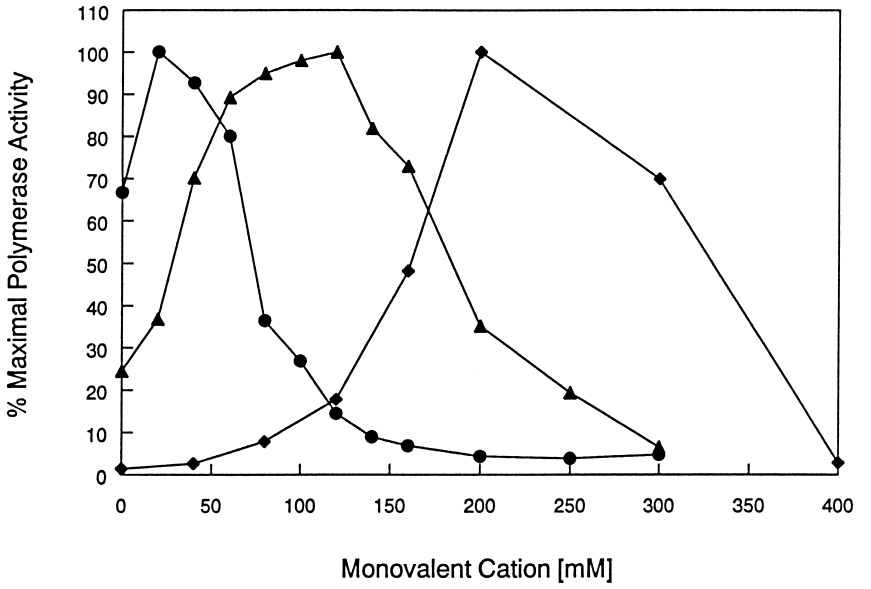
The primary reason that the VZV DNA polymerase has not been further characterized lies in the lability of the enzyme activity. Mar et al. (1978) noted that VZV DNA polymerase was more labile than DNA polymerases from HSV-1, HSV-2, and HCMV. All of these enzymes had been purified to a similar extent. More recent work has shown that even under circumstances where significant amounts of the VZV ORF29 protein were purified to homogeneity (see below) the VZV polymerase was present at relatively low levels and its activity was lost after storage for a short time at 4 °C (W. T. Ruyechan, J. W. Olson, & H. L. Cawley, unpublished observations). This lability appears to be an intrinsic property of the catalytic subunit since Ertl et al. (1991) reported that expression of the ORF28 gene product in baculovirus also resulted in a polymerase that rapidly lost activity. There have been no reported attempts to date to express the ORF28 and the ORF 16 proteins simultaneously.

The VZV ORF 29 protein

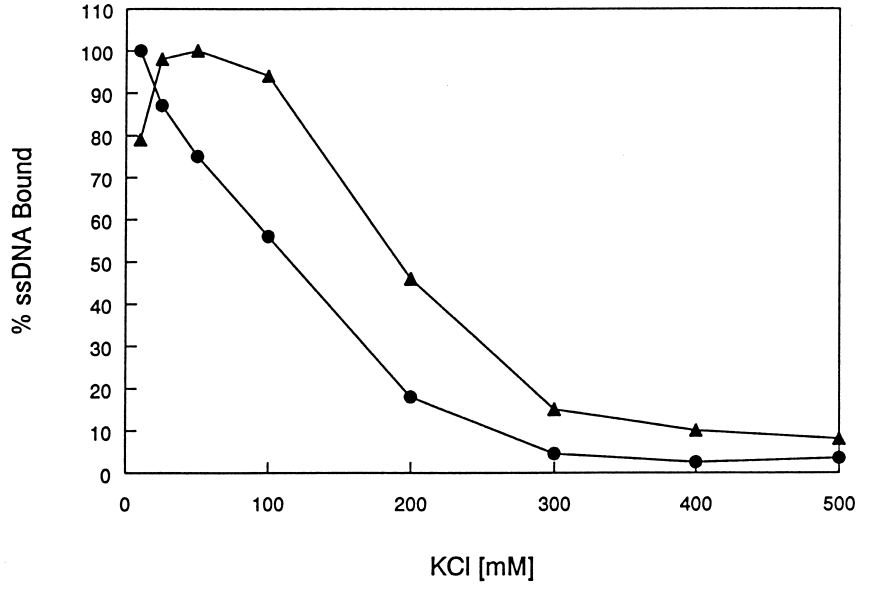
The VZV ORF29 protein is the homologue of the HSV major single-strand binding protein ICP8, which is coincidentally encoded by the UL29 gene of that virus. The ORF29 protein has a predicted molecular weight of 132 133 Da and shows 60% similarity and 50% identity with its HSV homologue. Examination of the predicted primary structure of the ORF29 protein shows that it contains a region showing homology to a putative single-strand DNA binding motif found in prokaryotic and eukaryotic SSBs (amino acids 801–847) (Wang & Hall, 1990) and a sequence (amino acids 497–509) that fits the consensus motif for a zinc binding site, although the presence of a metal ion in this protein has not been investigated (Kinchington et al., 1988).

Early studies involving the fractionation of VZV infected cell protein extracts on single and double-stranded DNA cellulose columns indicated the presence of a major single-strand binding species with an apparent molecular weight of

A


% Maximal Polymerase Activity
110
100
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
350
400
Monovalent Cation [mM]

B


% ssDNA Bound
110
100
90
80
70
60
50
40
30
20
10
0
0
100
200
300
400
500
KCl [mM]

125–130 kDa (Roberts et al., 1985). This protein eluted from ssDNA celluose at KCl concentrations of 0.6–1.0 M and was not present in protein extracts derived from purified virions. Antibodies raised against a synthetic peptide corresponding to the carboxy-terminal 12 amino acids of the predicted ORF29 amino acid sequence recognized a 130 kDa species present in infected cells. This protein bound to single-stranded DNA matrices and was eluted at salt concentrations similar to those observed in the previous study, thus confirming that the ORF29 gene product is the VZV major single-strand DNA binding protein (Kinchington et al., 1988). Immunofluorescence localization of the ORF 29 protein in infected cells displayed patterns similar to those observed for ICP8 in HSV infected cells. Specifically, these patterns included punctate nuclear staining (interpreted as prereplicative sites) as well as broader nuclear staining believed to represent active replication compartments. Thus the ORF29 protein exhibits numerous characteristics associated with a herpesvirus protein known to be required for origin-dependent replication of the viral DNA. Whether the ORF29 protein exhibits other properties of ICP8 such as the ability to stimulate the helicase activity of the putative VZV helicase/primase heterotrimer and the helicase activity of the origin-binding protein remains to be determined.

The ORF29 protein has been purified to homogeneity from VZV infected cells (W. T. Ruyechan, J. W. Olson & H. L. Cawley, unpublished data) and has been expressed and purified from recombinant baculovirus systems (Webster et al., 1995; Boucaud et al., 1998). One preliminary observation from using these sources of ORF29 shows that, in filter-binding assays, the single-strand DNA binding activity of the ORF29 protein is more salt sensitive than that of ICP8 (Figure 3.4B). This property appears to mirror the difference in salt sensitivities of the two respective DNA polymerases, further suggesting that the environment of the site of VZV DNA replication may be different than that of HSV.

Several studies have been done attempting to determine if the VZV DNA

Figure 3.4 (*left*) (A) Monovalent cation dependence of the VZV and HSV DNA polymerases. Typical data obtained with VZV and HSV DNA polymerases using activated calf thymus DNA as template. Closed circles and triangles illustrate the salt dependence of the VZV DNA polymerase at increasing concentrations of NH_4OAc and KCl, respectively. Closed diamonds illustrate the KCl dependence of HSV polymerase activity. The experimental data presented were obtained in the authors' laboratories using preparations of the viral enzymes purified to similar extents. (B) KCl dependence of DNA binding for the VZV ORF29 and HSV ICP8 single-strand binding proteins. Data from nitrocellulose filter binding assays illustrating the greater salt sensitivity of the VZV SSB activity (closed circles) as compared to the HSV SSB activity (closed triangles). Both proteins were purified to homogeneity in the authors' laboratories from infected cell lysates. The DNA used as a binding substrate was radioactively labeled, heat-denatured calf thymus DNA.

polymerase and the ORF29 protein can substitute for the their HSV homologs based on the substantial levels of identity and similarity observed in amino acid sequence. These have included unsuccessful complementation of thermolabile mutants of HSV (Felser et al., 1987; Ruyechan et al., 1991) with lesions in the genes encoding the catalytic subunit of the polymerase and ICP8. Other studies have involved the substitution of these VZV genes in in situ DNA replication assays utilizing either plasmids or recombinant baculovirus expressing all of the genes required for origin-dependent HSV DNA replication. Neither VZV protein was capable of subsitution or complementation of its homologous HSV function in these experimental systems (Webster et al., 1995; M. Sadeghi-Zadeh, W. T. Ruyechan, & J. Hay, unpublished observations). The differences in salt optima described above do not account for these results since there is substantial overlap in activity between the VZV and HSV proteins. Rather, these results strongly suggest that the assemblage of these proteins into a multi-component DNA replication apparatus requires specific protein–protein contacts. Thus regions of significant nonidentity and similarity are candidates for protein–protein interfaces.

The VZV origin binding protein

VZV ORF51 encodes a protein with a predicted molecular mass of 94370 Da and shows 54.8% similarity and 46.5% identity with its HSV-1 homologue, the UL 9 protein. The VZV ORF51 protein has been shown to interact with the VZV DNA replication origin at the three similar 11 bp sequences designated A, B, and C, all of which are upstream of the AT-rich palindromic sequence contained in the VZV DNA replication origin (see above). The site-specific DNA-binding activity is present in the carboxy-terminal 322 amino acids of the protein, which contain a helix-turn-helix DNA-binding element and a pseudo-leucine zipper, both of which may be important for site-specific binding. Site-specific binding was demonstrated by electrophoretic mobility shift assays using DNA fragments containing the binding sites and by DNAse footprinting assays (Stow et al., 1990; Chen & Olivo, 1994). The observed specificity of binding was the same for both a recombinant fusion protein containing only the carboxy-terminal third of the protein and for the full length protein expressed in an in vitro transcription/translation system. The ORF51 amino acid sequence contains a concensus ATP-binding site and six helicase superfamily motifs indicating that, like the UL9 protein, the ORF51 gene product acts as an ATP-dependent DNA helicase that unwinds the AT-rich origin element following site specific binding in the presence of the VZV single-strand binding protein. It is not known if the ORF51 protein forms homodimers as does its HSV counterpart. However, the leucine zipper motif believed to be responsible for dimerization of the HSV protein is conserved at the same location near the amino terminus of the ORF51 protein. Similarly it has not formally been shown

that the ORF51 and ORF29 proteins interact, although based on the results cited below it appears likely that they do.

The most interesting finding concerning the ORF51 protein thus far lies in the fact that it is the only one of the VZV replication proteins that has been able to be substituted in an HSV origin dependent DNA replication assay. Webster et al. (1995) showed that co-infection of a recombinant baculovirus expressing the ORF51 protein with baculoviruses expressing all of the HSV replication proteins except for UL9 resulted in replication of plasmids containing either the HSV or VZV origins of replication. No replication was observed with either of these plasmids when a recombinant baculovirus expressing the VZV ORF29 protein was substituted for the equivalent virus expressing the ICP8 gene. These results indicate that, as anticipated, the ORF 51 protein can bind to the concensus binding sites present at both origins and furthermore that there is sufficient sequence and structural homology within the ORF51 protein to allow interaction with the HSV ICP8 and UL8 proteins for replication to occur.

The VZV helicase/primase complex

Neither the putative VZV helicase/primase complex nor its individual components (encoded by ORFs 6, 52, and 55) have been purified or characterized. Rather, they have been identified based on their amino acid homologies and respective genome location to the homologous HSV-1 genes (Boehmer & Lehman, 1997). By analogy with HSV-1, the ORF6, 52 and 55 proteins are predicted to assemble into a heterotrimeric complex exhibiting both helicase and primase activity. The complex would translate along the lagging strand where the helicase activity would unwind the DNA at the replication fork and the primase would synthesize RNA primers, which would prime the synthesis of Okazaki fragments.

The putative helicase is encoded by VZV ORF55 and its predicted amino acid sequence shows over 68% similarity and 59% identity with its HSV-1 homologue including conserved ATP-binding and helicase motifs (Gorbalenya et al., 1989). The ORF55 protein has a predicted molecular weight of 98 844 Da. The VZV primase is predicted to be encoded by ORF 6, which has a predicted molecular mass of 122 541 Da and exhibits 48% similarity and 40.6% identity with its HSV-1 homologue. Amino acids 651–676 contain a proposed divalent metal binding site which is present in the HSV-1 primase and conserved in primases and DNA polymerases (Klinedinst & Challberg, 1994). The third component of the VZV helicase /primase complex is predicted to be encoded by ORF52 and to have a molecular mass of 86 343 Da. The ORF52 protein shows 36.3% similarity and 27.6% identity with its HSV homologue the UL 8 protein. Thus the ORF52 protein shows the least conservation of all seven of the predicted VZV DNA replication proteins with its HSV homologue. No enzymatic activity has been associated with the HSV-1 UL8

protein. However, this protein stimulates primer synthesis, is required for optimal DNA helicase, DNA-dependent nucleotide triphosphatase, and primase activity in the presence of ICP8, and interacts with the HSV OBP. Currently, the VZV ORF52 protein is predicted to exhibit all of these properties.

The numerous functions and interactions predicted to be associated with the ORF52 protein may explain in part why, with the exception of the ORF51 OBP, no other VZV functions have been able to substitute in in situ origin dependent HSV replication assays. The low level of similarity and identity seen at the level of the predicted amino acid sequence of the VZV and HSV proteins may result in significant differences in the tertiary structure of the protein or in the amino acid sequences that form interfaces with the other replication proteins. As a consequence, these interactions may be incapable of occurring or would be suboptimal, ultimately resulting in a nonfunctional or extremely inefficient replication apparatus.

A model for VZV DNA replication

A model for replication of VZV DNA was proposed by Davison (1984) and was based on information specific for VZV DNA as well as elements of a general mechanism proposed for HSV DNA replication. A modified version of this model, which includes VZV specific data published subsequently, is illustrated in Figure 3.5 and described below.

In step I, linear DNA from the infecting virion in one of the two major (P and I_S) or two minor (I_L and I_{SL}) isomeric arrangements circularizes in the infected cell's nucleus as an initial step to subsequent replication. Alternatively, the small fraction of circular molecules present in some virions and derived from all four isomeric forms of DNA represents the replicative form of the DNA. In either case, the circularization of the DNA molecules and the postulated stability of these molecules during additional replication steps would suggest that the termini are ligated and that this ligation is somehow facilitated by the presence of the unpaired nucleotide at the L and S termini of the DNA molecule. Presumably viral factors that recognize specific sequences would be involved in this ligation process, which could also involve a cellular ligase, since such an activity has not been shown to be coded by herpesviruses. In the case of virion-packaged circular DNA, the ligation event would occur just prior to, or possibly post-packaging. The argument for covalently closed circular molecules present in the infected cell is bolstered by the observations of Kinchington et al. (1986) who showed the presence of DNA with the characteristics of closed circles after extensive protease and denaturation treatment.

In step II, DNA replication is initiated at one of the two Ori_S loci (illustrated by the open repeat region) by binding of the ORF51 protein and possibly a cellular factor(s).

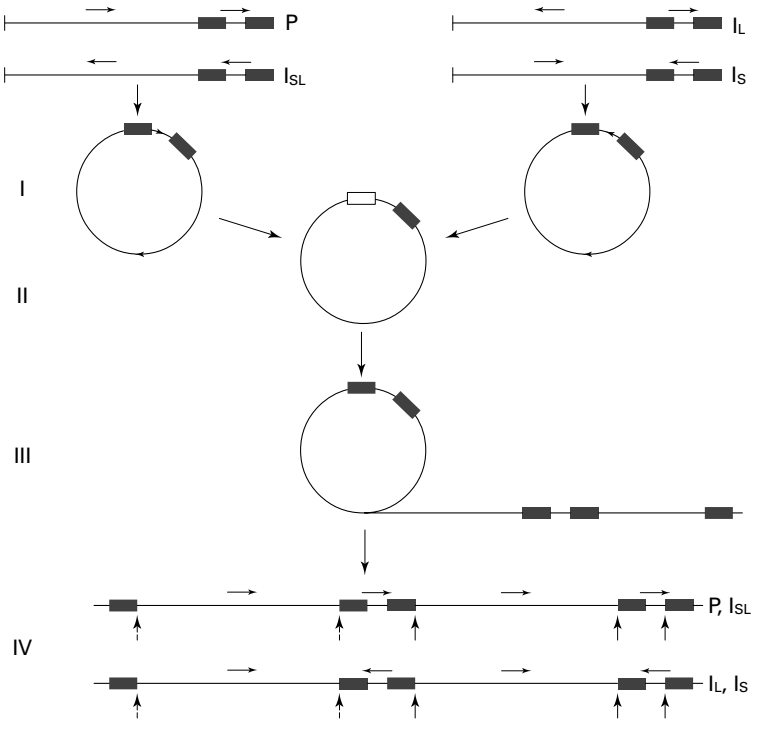


Figure 3.5 Model for VZV DNA replication. Schematic diagram of the replication model described in the text. Note that the structures illustrated in steps II and III are representative of replication intermediates which would result from all isomeric forms of the DNA. In step IV, the cleavage sites generating the major and minor isomeric forms of linear VZV genomes are indicated by solid and broken arrows, respectively.

Unwinding of the origin is probably facilitated by the ORF29 protein. The remainder of the replication proteins are then recruited to the origin and limited replication of the circular molecules occurs, presumably by means of theta intermediates. During this phase, segment inversion takes place by intramolecular recombination between inverted repeats. It is not currently known if the recombination is general or site specific in VZV. However, in HSV, such recombination events do not appear to be site specific and are mediated by the viral DNA replication apparatus (Weber et al., 1988).

In step III, a nick is introduced in the replicating DNA and replication shifts to a rolling circle mechanism, by analogy with HSV-1 and Pseudorabies virus, resulting in the generation of head to tail concatemers (Ben Porat & Rixon, 1979; Jacob et al., 1979).

In step IV, the concatemers are cleaved at specific sites by viral factors in order to generate unit length genomes, which can then be packaged. Cleavage usually occurs at the novel L–S joint corresponding to the fusion of the L and S termini of linear

molecules upon circularization. This cleavage would generate the major isomeric forms of the DNA. Occasionally, however, cleavage occurs at the normal L–S junction giving rise to the minor isomeric forms. As Davidson (1984) indicated, the ratio of the major and minor isomeric forms of the DNA would be determined by the relative efficiency of recognition of sequences at the novel L–S joint as compared to the normal L–S joint by the cleavage apparatus. This implies that the recognition sequence at the novel L–S joint must in part be present in the region of U_L adjacent to that joint. Since the L region contains identical 88 bp sequences at its ends, the site specific cleavage signal must begin at least 90 bp from the joint taking into account the additional unpaired base at the normal terminus. The other portion of the recognition sequence would reside in either the IR_L/TR_L or IR_S/TR_S sequences and recognition of this element alone may occasionally result in cleavage at the normal L–S joint.

Davison also considered two formal, but unlikely hypotheses concerning the distribution of the isomeric forms of VZV DNA. In the first, only the novel L–S joint is cleaved and then the mature, linearized DNA molecules undergo recombination in order to produce the minor isomers. This is unlikely since cleavage and packaging of herpesvirus DNA appear to be closely linked (Ladin et al., 1980), thus making a temporal scheme for such recombination difficult to imagine. Moreover, as was mentioned above, in HSV, recombination appears to be mediated by the replication apparatus, which would probably not have access to mature DNA. The second hypothesis involved the possibility that the DNA of certain strains of virus contains predominantly I_L and I_{SL} isomeric forms of DNA and that sufficient numbers of VZV strains had not as yet been analyzed in 1984 to preclude this possibility. Some 15 years later, with the DNA from numerous clinical isolates from varied geographical areas having been analyzed, there is at at present no evidence that this situation exists.

Antiviral drugs targeted to DNA replication

This section will be relatively brief since the subject of anti-VZV drugs is treated in a subsequent chapter. Thus far, all effective anti-VZV drugs have proven to be targeted to the viral DNA polymerase and elongation of newly synthesized viral DNA. These drugs include phosphonoacetic acid (PAA) and its less cytotoxic pyrophosphate analog foscarnet, vidarabine or adenine arabinoside, acyclovir (ACV) and its valine derivative valacyclovir, and bromvinyl arabinosyl uracil (BV-araU).

PAA and foscarnet competitively inhibit the VZV and other herpesvirus polymerases by blocking the pyrophosphate binding site, and as a result, inhibiting the formation of the 3′ to 5′ phosphodiester bond between the growing DNA strand and the next nucleotide to be added, thus resulting in chain termination. These

compounds act directly on the polymerase and require no additional viral or cellular enzymes for activation (Leinbach et al., 1976; Chrisp & Clissold, 1991).

Adenine arabinoside is a nucleoside analogue that is phosphorylated intracellularly to yield its active triphosphate derivative. This compound is a competitive inhibitor of the activities of both the viral and cellular replicative DNA polymerases, although it has a significantly higher affinity for the viral polymerase. Adenine arabinoside is also incorporated into the growing DNA chains and acts as a terminator of elongation (Miller et al., 1968).

Acyclovir, or acycloguanosine, is perhaps the most recognized of the anti-VZV drugs based on its widespread use and efficacy in the treatment of herpes simplex virus infections. ACV is phosphorylated to its monophosphate derivative by the HSV and VZV thymidine kinase and pyrimidine kinases respectively. It is then di- and triphosphorylated by cellular enzymes. This initial phosphorylation event does not take place to a significant extent in uninfected cells, making ACV a highly specific antiviral. The triphosphate derivative acts as a competitive inhibitor of the viral DNA polymerase by competing for the nucleotide binding site resulting in chain termination (Elion, 1983). While ACV is used as an anti-VZV drug, it is considerably less effective against the VZV polymerase as compared to the HSV polymerase. This is due to the weaker binding of the drug to the VZV DNA polymerase as compared to the HSV polymerase. The mechanism of action of valacyclovir is essentially the same as that of ACV, the difference between the two drugs being that valacyclovir has a much higher bioavailability via the oral route. Once absorbed, the valine moiety is cleaved away and inhibition by the resultant ACV triphosphate proceeds as described above (Beutner et al., 1995).

Like acyclovir and valacyclovir, BV-AraU is phosphorylated by the viral thymidine or pyrimidine kinases and its triphosphate form inhibits the viral DNA polymerase and acts as a chain terminator. This drug is reported to be over 1000 times more effective than ACV against VZV in vitro. BV-AraU overcomes the bioavailability problems associated with ACV but its toxicity in combination with 5-fluorouracil has prevented licensure in the United States (Machida, 1986; Machida & Sakata, 1984).

The greatest problem observed with these drugs lies in the fact that they are all directed at a single component of the DNA replication apparatus. Thus resistant viral polymerases or pyrimidine kinases that no longer metabolize them can and do arise (Boivin et al., 1994). Therefore attempts should be made to identify drugs that interfere with other replication functions such as the viral helicase /primase, or for compounds that form adducts specifically with VZV replication proteins and interfere with the assembly of the viral replication machinery.

Future directions

The unique biology of varicella-zoster infection has long been appreciated. The work of the past ten to 15 years in the area of VZV molecular biology has underscored this fact, demonstrating that there are significant differences in the mechanisms of viral propagation and reactivation as compared to other alphaherpesviruses. These differences allow for both expansion of our knowledge base concerning herpesviruses and for the development of newer, more efficient antiviral strategies. In this regard there are numerous worthwhile areas for continued investigation into the mechanism of VZV DNA replication. First, an in situ DNA replication assay must be established for VZV. Based on the current published data as well as extensive but unsuccessful attempts in our own laboratories (M. Sadeghi-Zadeh, W. T. Ruyechan, & J. Hay, unpublished observations) the six replication functions common to herpesviruses (the polymerase holoenzyme, the helicase/primase complex, and the major single-strand binding protein) may not be sufficient for origin dependent replication even in the presence of the VZV origin-binding protein. Data supporting this hypothesis include the differences in origin structure between HSV and VZV described above, and the relatively inefficient activation of the VZV origin by HSV gene products. Stow et al. (1990) have postulated that another viral, or perhaps cellular, protein binds downstream of the VZV origin and aids in unwinding of the AT-rich region. Thus the situation in VZV could be analogous to that found in HCMV and EBV in that other viral functions are required for full activation of the origin. Such a mapping could readily be done with the current cosmid technology.

The second area in VZV DNA replication that needs to be reassessed and completed is the expression of the individual replication proteins and the characterization of their specific biochemical properties. The first part of this task may not be trivial. The large average size of these proteins and the fact that some undergo post-translational modification by analogy with their HSV counterparts makes their expression in bacteria problematic. The ORF29 protein has been expressed in baculovirus in a soluble form. However, neither the VZV ORF51 protein nor the enzymatic subunit of the polymerase encoded by ORF28 have been produced in significant amounts in soluble form from recombinant baculovirus cultures. Thus far, only the ORF29 protein has proven amenable to this expression system. It is possible that these difficulties are due to some intrinsic property of the VZV proteins. More likely, the problems lie in the expression systems, strategies, and expression levels achieved. For example, the lack of solubility and stability observed with the ORF28 polymerase subunit might be alleviated by coexpression of this protein with the ORF16 gene product, hopefully leading to the generation of a stable and active holoenzyme. Similarly it may be reasonable to attempt to coexpress the helicase/primase components. The lack of success with the ORF51 OBP may be due

simply to expression at levels which induce the protein to aggregate, thus significantly enhancing problems with precipitation. Therefore, choice of expression vectors that allow for an attenuation of the level of expression may be appropriate. Alternatively, other expression systems such as the Semliki Forest virus expression system could be used. This system has recently been used to express several components of the HCMV replication apparatus (including the viral DNA polymerase) in soluble form, which proved refractory in both bacterial and baculovirus expression systems (McCue & Anders, 1998).

The availability of large quantities of purified proteins is essential for the complete characterization of the activity of the viral DNA polymerase and for the determination of the enzymatic properties of the putative helicase/primase complex, of which nothing is currently known. Based on the differences observed between the VZV and HSV DNA polymerases, it is likely that differences, both in enzymatic properties and susceptibility to antiviral drugs, will also be observed with the VZV helicase /primase.

Third, an effort should be made to elucidate the cleavage and packaging machinery of VZV. As a first step, one could use DNA fragments that encompass the novel L–S joint and its flanking regions in an attempt to identify viral proteins that bind to the proposed cleavage recognition sequence.

One additional area of investigation that would add to our understanding of the molecular biology of VZV DNA replication is the regulation of expression of the replication genes. Little has been done in this area in any of the alphaherpesvirus systems. The most extensive work has been done with HCMV, where Iskendarian et al. (1996) have shown that four of 11 loci required for transient complementation of HCMV DNA replication are involved in the activation of expression of replication genes. The situation in VZV is particularly interesting due to the identification of a novel bidirectional promoter that controls the expression of both the ORF28 and ORF29 genes (Meier & Straus, 1993; Meier et al., 1994). This promoter is activated by the action of the VZV major transcriptional transactivator, the IE62 protein, and the cellular factor, USF. It is unique to VZV, this region of the HSV genome being occupied by a third origin of replication. Thus other aspects of the expression of VZV DNA replication genes may also be novel and an elucidation of the mechanisms and temporal sequence of these regulatory events could yield information leading to the development of new antivirals and insight into the mechanism of the establishment and reactivation of VZV latency.

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4

Viral proteins

Paul R. Kinchington and Jeffrey I. Cohen

Introduction

While the clinical aspects of diseases caused by varicella zoster virus (VZV) have been well documented and recognized for decades, the functional biology and biochemistry of VZV proteins have proven more difficult to elucidate. This is largely due to the strong association of progeny virus with the infected cell, the low titres of cell-free virus that can be obtained and, until recently, the scarcity of viral mutants with defects in specific genes. However, the landmark paper describing the DNA sequence (Davison & Scott, 1986) revealed the coding potential of the VZV genome and demonstrated that most VZV putative proteins possessed homology to herpes simplex virus type 1 (HSV-1) proteins. The VZV sequences enabled prediction of most VZV protein functions based upon their better characterized HSV-1 counterparts. The DNA sequence also accelerated studies of the expression of VZV proteins outside of the limitations of the virus, and enhanced the development of specific immunological reagents to VZV proteins. A more recent important development in the understanding of the roles of VZV proteins and their functions during VZV growth was the development of a cosmid-based system for the generation of recombinant VZV, which enabled gene specific genetic manipulation of the VZV genome (Cohen & Seidel, 1993). The use of cosmids to generate recombinant VZV effectively surmounted problems encountered in plaque purification from wild type virus, since the recombinant VZV only contains sequences present in the cosmids. A second, independently derived set of infectious VZV cosmids has been developed (Mallory et al., 1997).

A growing number of VZV proteins have been characterized and these will be the focus of this chapter. In particular, the unique features of VZV proteins which are not shared with other alphaherpesvirus homologs are highlighted. While comparisons are usually made to HSV-1 because of more extensive studies of HSV-1 protein function, it is important to note that VZV is more closely related to three animal herpesviruses, equine herpesvirus type 1 (EHV-1), pseudorabiesvirus

(PRV), and the less well-characterized simian varicella virus (McGeoch & Cook, 1994).

Coding potential

The VZV genome contains at least 69 unique open reading frames (ORFs), which are predicted to encode proteins during lytic infection (Davison & Scott, 1986). Three genes are diploid, lying within the large repeats bounding the short unique region. Two VZV ORFs (ORFs 42 and 45) are probably spliced together to form one protein, based upon similar gene arrangement in HSV-1. Comparisons reveal that five of the VZV ORFs are not found in HSV-1 (ORFs 1, 2, 13, 32, 57), although some are found in EHV-1. At least one protein (ORF33.5) is derived from an overlapping protein (ORF33) by proteolytic processing. Table 4.1 shows the 69 unique VZV proteins and their predicted functions.

Proteins involved in the regulation of transcription

VZV transcription kinetics

Experimental demonstration of the kinetics of viral transcription has been difficult, because the low titres of cell-free VZV do not allow high multiplicity synchronized infections. However, VZV genes are almost certainly expressed in a temporal, transcription controlled cascade like other herpesviruses (Honess & Roizman, 1974). Immediate early (IE) gene transcription occurs upon infection and without the need for prior new viral protein synthesis. Early gene transcription, on the other hand, requires functional IE proteins, and late gene transcription requires both IE proteins and ongoing viral DNA replication to obtain maximal levels of transcription. Experiments showing time-dependent differential protein expression in VZV infected cells have been reported (Ruyechan et al., 1991).

While it has been assumed that each VZV gene is temporally regulated in the same transcriptional group as its HSV-1 homolog, this may not be the case, particularly for IE gene expression. VZV contains homologs to four of the five HSV-1 IE genes (VZV ORFs 4, 61, 62 and 63). However, the VZV genome is more homologous to genomes of PRV and EHV-1 than to that of HSV-1, and these animal herpesviruses only express a single IE gene (the respective homologs of VZV ORF62). To date, three VZV genes have been shown to be expressed under IE conditions, namely ORF62 (Forghani et al., 1990), ORF63 (Debrus et al., 1995) and ORF4 (Defechereux et al., 1997).

Activation of VZV IE gene expression also appears to differ from the mechanisms used by HSV-1, PRV and EHV-1. In the latter viruses, all IE genes contain upstream promoter elements known as TAATGARAT motifs, through which a

Table 4.1 VZV genes with their HSV-1 homologs

VZV gene	Function	HSV-1 homolog
1	membrane protein, NE[a]	none
2		none
3		UL55
4	transactivator, tegument protein	UL54 (ICP27)
5	gK, E[b]	UL53 (gK)
6		UL52 (helicase-primase)
7		UL51
8	dUTPase, NE	UL50 (dUTPase)
9		UL49 (VP22)
9A	syncytia formation, NE	UL49A
10	transactivator, tegument protein, NE	UL48 (VP16)
11		UL47(VP13–14)
12		UL46
13	thymidylate synthetase, NE	none
14	gC, NE	UL44 (gC)
15		UL43
16		UL42 (polymerase processivity)
17		UL41 (viral host shutoff)
18	ribonucleotide reductase, small subunit	UL40 (RR2)
19	ribonucleotide reductase, large subunit, NE	UL39 (RR1)
20		UL38 (VP19C, ICP32)
21		UL37 (VP7)
22		UL36 (VP1–2)
23		UL35 (VP26)
24		UL34
25		UL33
26		UL32
27		UL31
28	DNA polymerase	UL30 (DNA polymerase)
29	ssDNA binding protein	UL29 (ICP8)
30		UL28
31	gB	UL27 (gB)
32	probable substrate for ORF47 kinase, NE	none
33	protease	UL26 (protease, VP24)
33.5	assembly protein	UL26.5 (VP22)
34		UL25
35		UL24
36	thymidine kinase, NE	UL23
37	gH	UL22 (gH)

Table 4.1 (*cont.*)

VZV gene	Function	HSV-1 homolog
38		UL21
39		UL20
40	major nucleocapsid protein	UL19 (VP5)
41		UL18 (VP23)
42/45		UL15
43		UL17
44		UL16
46		UL14
47	protein kinase, NE	UL13 (VP18.8)
48		UL12 (deoxyribonuclease)
49		UL11
50		UL10 (gM)
51	origin-binding protein	UL9
52		UL8 (helicase/primase)
53		UL7
54		UL6 (VP11–12?)
55		UL5 (helicase/primase)
56		UL4
57	NE	none
58		UL3
59	uracil-DNA glycosylase, NE	UL2
60	gL	UL1 (gL)
61	transactivator, transrepressor, NE	ICP0 (Vmw110)
62, 71	transactivator, tegument protein	ICP4 (Vmw 175)
63, 70	tegument	US 1 (ICP22, Vmw 68)
64, 69		US10
65		US9
66	protein kinase, NE	US3 (protein kinase)
67	gI, NE	US7 (gI)
68	gE	US8 (gE)

Notes: [a] NE, not essential for growth in cell culture; [b] E, essential for growth in culture

virion transactivator protein (homologous to VZV ORF10) activates transcription. While the ORF62 promoter contains such elements (Disney et al., 1990; Moriuchi et al., 1995a), the VZV IE genes ORF4 and ORF63 do not (Moriuchi et al., 1994c; Kinchington et al., 1994, 1995). The latter genes may be activated directly by other transactivators in the virion, such as the ORF62 protein (Kinchington et al., 1992; Moriuchi et al., 1994c).

ORF62–IE62, the major transcriptional regulatory protein

ORF62 is a diploid gene and is predicted to encode a 140 kDa protein of 1310 amino acids. ORF62-specific polyclonal and monoclonal antibodies recognize a series of phosphoproteins in VZV infected cells between 175 and 180 kDa termed collectively as IE62 (Arvin et al., 1986; Forghani et al., 1990; Kinchington et al., 1992; Ng et al., 1994). IE62 is found predominantly in the nucleus of infected cells, although some cytoplasmic forms accumulate late in VZV infection (Forghani et al., 1990; Kinchington & Turse, 1998, 2000).

Functionally, IE62 is similar to HSV-1 ICP4, PRV IE175 and EHV-1 EP0 in that it is a potent transcriptional activator. IE62 can stimulate the transcription of all VZV genes studied to date in transient transfection assays (Inchauspe et al., 1989; Perera et al., 1992a; Moriuchi et al., 1994c). IE62 can also repress its own transcription (Disney et al., 1990), although in neural cells IE62 can enhance transcription from its own promoter (Perera et al., 1992b). IE62 binds to specific DNA sequences similar to those that bind HSV-1 ICP4 and PRV IE175, although there is a wide divergence from a consensus sequence motif (Wu & Wilcox, 1991; Tyler & Everett, 1993, 1994). The DNA binding and dimerization domains of VZV IE62 and HSV ICP4 can interact with each other to form heterodimers (Tyler & Everett, 1994). The functional conservation between these proteins is sufficient to enable IE62 to complement the growth of HSV-1 ICP4 mutants (Felser et al., 1987, 1988) and to substitute for ICP4 in the HSV-1 genome (Disney & Everett, 1990).

IE62 can be delineated into five regions based upon homology to other alphaherpesvirus homologs, corresponding approximately to amino acids 1–460 (region I), 460–630 (II), 630–750 (III), 750–1170 (IV), and 1170–1310 (V) (Cheung, 1989). Regions II and IV show a high degree of amino acid similarity with other alphaherpesvirus homologs, whereas regions I, III and V are more divergent. Region II includes the domains involved in DNA binding and dimerization (Wu & Wilcox, 1991; Tyler & Everett, 1994). A nuclear localization signal, similar to that of SV40 T antigen, is present within region III of IE62 and includes the arginine/lysine rich region within amino acids 677–685 (Kinchington & Turse, 1998). A nucleolar localization signal for IE62 has also been mapped just upstream of these amino acids (Baudoux et al., 1995; Piette et al., 1995).

IE62 interacts with additional VZV and cellular proteins during VZV infection.

IE62 can mediate the nuclear localization of the ORF4 protein (Defechereux et al., 1996), and recent experiments suggest a physical interaction between ORF4 protein and IE62 (J. Hay, W. T. Ruyechan, personal communication). These interactions may be relevant to the observation that the activation of promoters by IE62 is synergistically enhanced in cotransfection assays by the ORF4 protein (Perera et al., 1992b; Defechereux et al., 1993). Coprecipitation experiments suggest that IE62 may also interact with and be phosphorylated by the ORF47 protein kinase *in vitro* (Ng et al., 1994). IE62 also cooperates with the cellular transcription factor USF to activate the promoters of ORF28, ORF29 (Meier et al., 1994; Meier & Straus, 1995) and ORF4 (Michael et al., 1998).

Four unusual features of IE62 differentiate it from other herpesvirus homologs. First, amino acids 1–90 of IE62 contain the transcriptional activation domain (Cohen et al., 1993; Perera et al., 1993), while the corresponding domain in ICP4 is near the amino terminus but lies internally (Xiao et al., 1997). Second, the nuclear localization of IE62 is influenced by the phosphorylation mediated by the ORF66 protein kinase (Kinchington & Turse, 1998, 2000). A similar interaction has not been identified in the homologous proteins of other alphaherpesviruses to date, but nuclear localization for many cellular proteins is regulated by phosphorylation (Jans & Hubner, 1996). Third, abundant levels of IE62 protein are found associated with purified VZV virions, most likely within the tegument (Kinchington et al., 1992, 1995). In contrast, only a small fraction of ICP4 is associated with HSV-1 virions (Yao & Courtney, 1991). Fourth, RNA transcripts derived from ORF62 have been detected in latently infected human ganglia (Croen et al., 1988; Meier et al., 1993).

IE62 is a major target for the cellular immune response to VZV. Both CD4+ and CD8+ VZV specific cytotoxic lymphocytes are induced by VZV infection which target the IE62 protein to a similar extent as the major VZV glycoprotein, gE (Arvin et al., 1986, 1987; Bergen et al., 1991; Arvin et al., 1991). In addition, the immune response to IE62 protects against live virus challenge in the guinea pig model of VZV infection (Lowry et al., 1992; Sabella et al., 1993; Lowry et al., 1997), indicating that IE62 might be a useful component for a candidate subunit vaccine.

ORF4 – Transcriptional activator

VZV ORF4 encodes a 51 kDa phosphoprotein (Ng et al., 1994; Kinchington et al., 1995), which is expressed under immediate-early conditions (Defechereux et al., 1997). The protein is localized predominantly in the cytoplasm of VZV-infected cells, although some protein is detected in the nucleus early in infection. ORF4 protein is associated with the tegument of purified virions (Kinchington et al., 1995).

Functionally, VZV ORF4 protein is a transcriptional activator in transient transfection assays, upregulating several (but not all) promoters from each of the three

putative classes of VZV genes (Inchauspe et al., 1989; Perera et al., 1992a; Defechereux et al., 1993; Perera et al., 1994; Moriuchi et al., 1994b). The ORF4 protein also augments IE62-mediated transactivation in a cooperative fashion. ORF4 protein acts at a transcriptional level by increasing levels of mRNA, possibly acting through sequence-specific elements in promoters (Perera et al., 1994). Recent evidence indicates that the ORF4 protein also acts at a post-transcriptional level (Defechereux et al., 1997).

VZV ORF4 and its homolog, HSV ICP27, have considerable sequence homology at their carboxy termini, but have much lower homology over the remainder of the proteins. In transfection assays, the VZV ORF4 protein transactivates VZV promoters and has no transrepressing activity. This contrasts with HSV-1 ICP27, which has little activity on its own in transfection assays, but in combination with other HSV IE proteins can act as a transactivator or transrepressor, depending on the type of polyadenylation signal in the target gene. The amino terminus of VZV ORF4 can functionally substitute for the corresponding portion of HSV ICP27, which is required for its transactivation and transrepression activities (Moriuchi et al., 1995b). Conversely, the amino terminus of ICP27 can partially replace the corresponding region of ORF4 which is required for transactivation. Cell lines expressing VZV ORF4 protein do not efficiently complement HSV ICP27 mutants (Moriuchi et al., 1994b), indicating some divergence in function between the corresponding proteins.

ORF61 – Transcriptional activator

VZV ORF61 encodes 62 to 66 kDa phosphoproteins (Ng et al., 1994; Kinchington et al., 1995), which localizes to the nucleus of VZV-infected and transfected cells (Stevenson et al., 1992). ORF61 protein is a transactivator, enhancing expression of VZV immediate-early and putative early promoters in transient expression assays and enhancing the infectivity of VZV DNA (Moriuchi et al., 1993b). VZV ORF61 protein can also transactivate or transrepress the function of other VZV transactivators (ORF4, ORF62) on viral promoters, depending on the cell line and transfection conditions used (Nagpal & Ostrove, 1991; Perera et al., 1992a; Moriuchi et al., 1993b). The ORF61 protein shows relatively low homology to its HSV-1 counterpart ICP0, but can complement an HSV-1 ICP0 mutant (Moriuchi et al., 1992). Unlike other VZV transactivators, ORF61 protein is not associated with purified virions (Kinchington et al., 1995).

The amino terminus of ORF61 contains a RING finger domain, composed of histidine and cysteine residues, that binds zinc (Everett et al., 1993). This domain is found in several other viral proteins, such as PRV EP0 and CMV IE2, that transactivate gene expression (Freemont, 1993) and is thought to be important for

protein–protein interactions. The amino terminal RING finger domain of ORF61 protein is required for its transactivating ability; however, a carboxy-terminal truncation mutant of ORF61 that retains the RING finger domain acts as transrepressor and dominant negative mutant in the presence of full length ORF61 in transient expression assays (Moriuchi et al., 1994a). The amino portions of VZV ORF61 and HSV ICP0 can substitute for each other to transactivate gene expression in vitro (Moriuchi et al., 1994a).

VZV deleted for ORF61 is impaired for replication in vitro, but can be complemented by certain cell lines (Cohen & Nguyen, 1998). Cells infected with the VZV ORF61 deletion mutant express normal levels of ORF62, but have reduced levels of gE and show abnormal syncytia formation.

ORF63

ORF63 is a diploid gene which is located completely within the short repeat regions of the VZV genome. ORF63 encodes an IE protein of 47 kDa (Debrus et al., 1995; Kinchington et al., 1995; Stevenson et al., 1996), which is larger than that predicted (30.5 kDa) from its amino acid sequence. A 28 kDa species has also been found in some studies (Kinchington et al., 1995). The protein is extensively phosphorylated, with most phosphorylation sites located between residues 142 and 210. Casein kinase II phosphorylates the ORF63 protein in vitro (Stevenson et al., 1996). While the HSV homolog of ORF63, ICP22, is phosphorylated by an HSV protein kinase (Leopardi et al., 1997), there is no evidence to suggest that ORF63 protein is phosphorylated by the homologous VZV protein kinase (Heineman & Cohen, 1995). In VZV infected and transfected cells, ORF63 protein is predominantly located in the nucleus (Debrus et al., 1995; Stevenson et al., 1996), and a nuclear localization signal lies within amino acids 210–278. The protein is also associated with purified virions (Kinchington et al., 1995). The mRNAs encoding ORF63 have been mapped in detail, and while they are not spliced like their HSV counterparts, they are complex with multiple initiation and polyadenylation sites (Kinchington et al., 1994, 1995; Kost et al., 1995).

The function of ORF63 protein remains unclear. Initial studies showed that ORF63 protein upregulates the thymidine kinase promoter and downregulates the IE62 promoter in transient transfection assays (Jackers et al., 1992). However, a more recent study showed minimal activity of the ORF63 protein on the activity of IE and early promoters (Kost et al., 1995). ORF63 protein has been detected in animal and human ganglia, suggesting a role in latency (Debrus et al., 1995; Mahalingam et al., 1996), but unlike the predominantly nuclear location of ORF63 in infected cells, ORF63 protein in latently infected ganglia is largely distributed in the cytoplasm (Lungu et al., 1998).

Proteins involved in DNA replication, nucleotide metabolism and phosphorylation

Most alphaherpesvirus early proteins are involved in DNA replication or modification of the levels of nucleotides in infected cells. VZV has homologs for each of the seven core proteins involved in origin-dependent DNA replication of HSV (Wu et al., 1988) and these are encoded by VZV ORF28 (DNA polymerase), ORF29 (single-stranded DNA binding protein), ORF16 (polymerase accessory protein), ORF51 (origin-binding protein) and ORFs 6, 52 and 55 (helicase/primase complex). However, these seven VZV proteins have not yet been shown to replicate DNA in a VZV origin-dependent fashion. The VZV origins of replication are located within the short repeat regions of the genome, between ORF62 and ORF63 (and ORFs 70 and 71). Unlike HSV, VZV does not possess an origin of replication in the long unique region of the genome. VZV has homologs for all of the HSV-1 enzymes involved in nucleotide metabolism which have been identified to date, and also encodes a thymidylate synthetase which is not present in HSV-1.

ORF28 – DNA polymerase

The 1194 amino acid DNA polymerase, a 130 kDa protein, is the target for all antiviral agents used in the clinical treatment of VZV infections. It shows several chemical differences from the cellular polymerase, particularly an increased sensitivity to phosphonoacetic acid (Miller & Rapp, 1977; Mar et al., 1978) and to acyclovir triphosphate. Partially purified VZV polymerase (Abele et al., 1988) and highly purified polymerase expressed from baculovirus recombinants (Ertl et al., 1991) have been used to determine the inhibitory effects of novel antiviral compounds. Resistance to the antiviral agent acyclovir occasionally arises through mutation of the DNA polymerase (Hasegawa et al., 1995), although most acyclovir-resistant VZV isolates arise due to mutation(s) in the viral thymidine kinase.

The promoter of the DNA polymerase gene is coordinately regulated with the adjacent ORF29 promoter. Both promoters are activated by the IE62 protein and the cellular transcription factor USF (Meier & Straus, 1993; Meier et al., 1994; Meier & Straus, 1995).

ORF29 – Single-stranded DNA binding protein

ORF29 encodes a 1204 amino acid protein with approximately 58% amino acid identity to the HSV-1 single-stranded DNA binding protein ICP8. The 130 kDa protein shows considerable biochemical similarities to HSV-1 ICP8, in that both proteins bind tightly to single-stranded DNA and are predominantly located in subnuclear compartments in infected cells (Kinchington et al., 1988). In addition to its presumed role in DNA replication, recent evidence has suggested that the

ORF29 protein influences transcription from certain VZV promoters in transfection assays (Boucaud et al., 1998). The VZV ORF29 protein cannot substitute for ICP8 in an HSV origin-dependent DNA replication assay using the six remaining HSV core replication proteins (Webster et al., 1995a). This suggests that specific interactions probably exist between VZV ORF29 and other viral proteins involved in VZV replication. Transcription from the ORF29 gene is tightly coordinated with that of ORF28 during lytic infection, as described above (Meier & Straus, 1993; Meier et al., 1994; Meier & Straus, 1995). However, mRNA transcripts corresponding to ORF29 (but not from ORF28) have been detected in human ganglia, suggesting neural-specific expression of this gene during latency (Croen et al., 1988; Croen & Straus, 1991; Meier et al., 1993; Cohrs et al., 1996).

ORF51 – The origin-binding protein

The origin binding protein, predicted to be 95 kDa, has 44% amino acid identity and 53% similarity to the HSV origin-binding protein UL9, and has similar functional properties. VZV ORF51 protein expressed by in vitro translation or in *E.coli* as a fusion protein binds to three specific sequences within the VZV DNA origin of replication (Stow et al., 1990; Chen & Olivo, 1994). These sequences are similar to those that bind the HSV-1 origin-binding protein and include a requirement for a CGC triplet within the 10 bp consensus binding sequence. VZV ORF51 protein can functionally complement the growth of an HSV-1 UL9 null mutant (Chen et al., 1995) and can substitute for the UL9 protein in combination with other HSV replication proteins in an origin-dependent DNA replication assay. A plasmid containing the VZV origin of replication can also substitute for a plasmid-born HSV origin using HSV proteins in replication assays (Stow et al., 1990; Webster et al., 1995b).

ORF8 – dUTPase

VZV ORF8 encodes the viral dUTPase which catalyzes the hydrolysis of dUTP to dUMP and pyrophosphate. A fusion protein expressed in *E. coli* containing ORF8 has dUTPase activity in vitro (Ross et al., 1997). Deletion of the gene from VZV results in loss of dUTPase activity in VZV infected cells, but does not impair growth of virus in vitro.

ORF 36 – Thymidine kinase

ORF36 encodes the viral thymidine kinase, which catalyzes the phosphorylation of thymidine to thymidylate (Sawyer et al., 1986). The 341 amino acid protein exists as a 70 kDa non-covalent homodimer composed of two 36 kDa subunits (Mahalingam et al., 1990). The enzyme has been characterized from VZV infected cells (Shiraki et al., 1986; Talarico et al., 1993), in vitro translation products

(Mahalingam et al., 1990) and from proteins expressed in *E. coli*. The VZV enzyme has greater deoxycytidine kinase activity than thymidine kinase activity, and is therefore frequently referred to as a deoxypyrimidine kinase (Fyfe et al., 1982). The VZV enzyme also has greater thymidylate kinase activity than its HSV homolog. Substitution of the HSV thymidine kinase gene with the VZV enzyme results in an HSV mutant with an antiviral sensitivity pattern that resembles VZV more than HSV (Bevilacqua et al., 1995). The ORF36 mRNA has been mapped and has a surprisingly long 5′ untranslated region which places the ORF36 promoter within the coding sequences of the upstream ORF35 gene (Davison & Scott, 1986).

The viral thymidine kinase is dispensable for VZV replication in cell culture. Resistance to acyclovir and other antivirals such as BvaraU (soruvidine) is usually due to point mutations or premature truncations of the ORF36 coding sequence (Sawyer et al., 1986; Mori et al., 1988; Lacey et al., 1991; Talarico et al., 1993; Koyano et al., 1996). Missense mutations are often located within ATP or nucleotide-binding sites and cause a decreased affinity for antiviral substrates (Shiraki et al., 1986; Suzutani et al., 1992). However, point mutations at other sites have also resulted in antiviral resistance. While acyclovir resistance arises readily in cell culture, clinically significant resistant VZV is not usually detected in healthy patient populations, but has been found following long-term acyclovir therapy in patients with AIDS (Linnemann et al., 1990; Boivin et al., 1994; Fillet et al., 1998).

ORF13 – Thymidylate synthetase

ORF13, the viral thymidylate synthetase, has homologs in some of the gammaherpesviruses, but not in HSV. Thymidylate synthetase catalyzes the methylation of deoxyuridylate to thymidylate. VZV ORF13 is not required for growth of the virus in cell culture (Cohen & Seidel, 1993).

ORF18 and 19 – Ribonucleotide reductase

The VZV ribonucleotide reductase consists of two subunits encoded by ORF18 (small subunit) and ORF19 (large subunit), which together catalyze the reduction of ribonucleotides to deoxyribonucleotides (Spector et al., 1989). VZV deleted for ORF19 is impaired for growth in cell culture, especially under conditions of low serum (Heineman & Cohen, 1994). The ORF19 deletion virus is more sensitive to acyclovir, with an IC_{50} about three-fold lower than parental virus, probably due to reduction of nucleotide pools that compete with acyclovir triphosphate for binding to the viral DNA polymerase. Similarly, a ribonucleotide reductase inhibitor has been developed that inhibits growth of VZV in vitro and potentiates the antiviral activity of acyclovir (Spector et al., 1989).

ORF59 – Uracil DNA glycosylase

VZV ORF59 encodes the viral uracil DNA glycosylase that removes uracil residues from DNA (Reddy et al., 1998). VZV deleted for the viral uracil DNA glycosylase is unimpaired for growth in cell culture. Expression of a uracil DNA glycosylase inhibitor protein in VZV abolishes detectable enzymatic activity in vitro and indicates that neither the cellular or viral enzyme are required for growth of virus in vitro.

ORF47 – Protein kinase

ORF47 encodes a serine/threonine protein kinase and has a predicted amino acid sequence which matches the consensus sequence derived from catalytic domains of other serine/threonine protein kinases. The 54 kDa protein is located in both the cytoplasm and nucleus of infected cells and is associated with the capsid/tegument fraction of virions (Ng & Grose, 1992; Stevenson et al., 1994). The ORF47 protein phosphorylates itself and ORF62 protein in protein kinase assays in vitro (Ng et al., 1994); however, ORF47 protein is not required for phosphorylation of ORF62 protein in VZV-infected cells (Heineman & Cohen, 1995). Several phosphoproteins of viral or cellular origin are present in cells infected with parental virus which are absent in cells infected with an ORF47 mutant. Recently, the ORF32 protein has been shown to be a possible substrate for the ORF47 protein kinase, since ORF32 protein is less phosphorylated in cells infected by a VZV ORF47 mutant than in cells infected with wild-type VZV (Reddy et al., 1998). While VZV unable to express ORF47 is not impaired for replication in vitro, the same mutant cannot replicate in human fetal T cells or skin implanted into SCID-hu mice (Moffat et al., 1998b) or cord blood lymphocytes (Soong et al., 2000). Thus, while ORF47 is not required for growth in cell culture, it is essential for growth in certain tissues in vivo.

ORF66 – Protein kinase

ORF66 is also predicted to be a serine/threonine protein kinase and shares the greatest homology with a cellular cyclin-regulated protein kinase known as CDC28. The VZV ORF66 protein has protein kinase activity, like its homologous proteins in HSV-1, PRV and HSV-2 (Frame et al., 1987; Leader et al., 1991; Daikoku et al., 1994; Kinchington & Turse, 2000). The ORF66 protein is a 46 kDa polypeptide which is not required for growth of VZV in cell culture (Heineman et al., 1996), although a mutant unable to express ORF66 is partially impaired for growth in human fetal T cells in SCID-hu mice (Moffat et al., 1998b) and cord blood lymphocytes (Soong et al., 2000).

One target for the ORF66 protein kinase is the IE62 protein. Unlike its other alphaherpesvirus homologs, ORF66 protein phosphorylates IE62 and inhibits the nuclear localization of the major regulatory protein IE62, causing accumulation of IE62 in

Table 4.2 Varicella-zoster virus glycoproteins

Glycoprotein (ORF)	Prior nomenclature[a]	Predicted size (kDa)	Approx. size (kDa) in VZV-infected cells	Target for neutralizing antibodies
gB (ORF31)	gpII	98.1	57–65[b], 62–68[b], 125–140	Yes (+/−C′)[d]
gC (ORF14)	gpV	61.4	95–105, up to >200[c]	Yes
gE (ORF68)	gpI	67.0	85–110	Yes (+/−C′)
gH (ORF37)	gpIII	93.6	105–118	Yes
gI (ORF67)	gpIV	39.4	45–50, 50–55	Yes (+/−C′)
gK (ORF5)		38.5	40	ND
gL (ORF60)		17.6	20	ND

Notes:

[a] As defined by Davison et al. (1986). See this reference for earlier nomenclatures.

[b] Upon reducing conditions

[c] Size variable; see text

[d] Monoclonal antibodies have been isolated which do or do not require complement for neutralization

ND, not determined

the cytoplasm of infected cells (Kinchington & Turse, 1998, 2000). In addition, a 48 kDa protein is not phosphorylated in cells infected with a virus unable to express both the ORF47 and ORF66 protein kinases, but is phosphorylated in cells infected with VZV mutants unable to express either the ORF47 or the ORF66 protein (Heineman et al., 1996). These results indicate that the ORF66 protein has, or can induce, a novel protein kinase activity in infected cells (Heineman et al., 1996).

Glycoproteins

VZV contains homologs for each HSV-1 glycoprotein located within the unique long region of the genome, but VZV lacks several of the HSV-1 glycoproteins in the short region. In particular, VZV lacks a homolog for HSV-1 glycoprotein gD, which is essential for HSV-1 infection and is conserved in all other known alphaherpesviruses. The nomenclature for VZV glycoproteins was changed in 1993 to reflect that of the HSV glycoprotein homologs. The seven known VZV glycoproteins are gB, gC, gE, gH, gI, gK and gL. Table 4.2 summarizes the VZV glycoproteins and previous nomenclatures.

ORF31 – gB

VZV gB is a type I glycoprotein of 125–140 kDa with ten potential N-linked glycosylation sites and hydrophobic regions near the amino and carboxy termini. Unlike its HSV-1 homolog, VZV gB is a disulfide-linked glycoprotein which

migrates as 65 and 68 kDa species upon reducing conditions (Montalvo & Grose, 1987). On the surface of cells, VZV gB also forms dimeric and multimeric forms like its HSV-1 homolog. VZV gB contains both N- and O-linked carbohydrates which become sialated, sulfated (Montalvo & Grose, 1987; Edson, 1993), and acylated (Namazue et al., 1989). The gB proteins of alphaherpesviruses are the most highly conserved glycoproteins and it is likely that VZV gB, like its HSV-1 homolog, is probably important for adsorption and fusion of the viral membrane to the cell membrane. VZV gB is a target for neutralizing antibodies (Massaer et al., 1993; Jacquet et al., 1995; Zhu et al., 1996).

VZV gB has an unusually short leader sequence, which has made expression in heterologous systems problematic (Massaer et al., 1993). However, VZV gB has been expressed in both vaccinia virus and in CHO cells with a view to its potential use as a candidate subunit vaccine (Grose et al., 1984; Massaer et al., 1993; Jacquet et al., 1995; Zhu et al., 1996).

ORF14 – gC

ORF14 is a type I glycoprotein which contains five possible sites for N-linked carbohydrate addition (Kinchington et al., 1986). ORF14 is encoded by two RNAs with colinear 5′ ends and different polyadenylation sites (Ling et al., 1991). Monospecific antibodies to gC recognize a 95–105 kDa protein in some American VZV isolates although the size may vary considerably from strain to strain, due to an unusual in-frame repeating structure of 14 amino acids near the amino terminus of the protein. This is encoded by DNA repeating elements of unusually high (67%) G + C content. The number of repeats vary from isolate to isolate, from 3 and 2/3 repeats in a VZV Oka variant (Kinchington et al., 1986) to over 14 repeats in a Japanese VZV isolate, Kawaguchi (Kinchington et al., 1990a). The function of the repeated elements is unknown, but they bear similarity to a repeating structure found in mucin which may be responsible for extension of the protein from the membrane surface. VZV gC is a target for neutralizing antibodies (Kinchington et al., 1990a,b).

The functions of VZV gC have not been identified, but its HSV homolog is important for attachment of virus to specific cell types through heparan sulphate moieties. While HSV-1 gC can bind the C3b component of complement, experiments suggest that VZV gC cannot bind any of the components of C3 (P. R. Kinchington, G. H. Cohen, unpublished data). VZV gC is not essential for growth of the virus in cell culture and both spontaneous and recombinant gC negative mutants have been isolated (Kinchington et al., 1990a,b; Cohen & Seidel, 1994b). Recent evidence indicates that gC is important for virus growth in fetal skin implanted into SCID mice (Moffat et al., 1998a). Most VZV clinical isolates express similar levels of gC, but highly passaged virus stocks may accumulate variants with

reduced expression of gC (Kinchington et al., 1990b; Ling et al., 1991; Moffat et al., 1998a). Two variants with reduced expression of gC are derivatives of vaccine Oka; however, the commercial vaccine expresses apparently normal levels of gC (P. R. Kinchington, A. A. Gershon, unpublished data). The variants expressing low levels of gC possess a defect at the level of transcription of the glycoprotein (Ling et al., 1991; Moffat et al., 1998a). The underlying mechanism for the transcriptional defect is not known.

ORF68 – gE

While gE of HSV-1 is a minor glycoprotein, VZV gE is the major glycoprotein expressed on VZV-infected cells. VZV gE has four N-linked glycosylation sites and possesses amino and carboxy terminal hydrophobic sequences typical of type I glycoproteins. Specific antibodies to gE recognize two or more forms of gE of 85–110 kDa. VZV gE contains both N-linked and O-linked carbohydrates, is heavily sialated and sulfated, and is covalently attached to palmitate in VZV infected cells (Namazue et al., 1989; Harper & Kangro, 1990; Litwin et al., 1992; Edson, 1993). VZV gE is one of the most immunogenic proteins of VZV, and is a prominent target for the cellular and humoral arms of the immune response (Arvin et al., 1986, 1987, 1991). Neutralizing antibodies to gE recognize three distinct domains (Wu & Forghani, 1997). In animal models, the immune response to gE antigens can protect animals from virus challenge (Lowry et al., 1992) and can mediate partial, but not complete, viral clearance from the eye after inoculation of VZV in the guinea pig (Kimura et al., 1998). Like its HSV-1 homolog, VZV gE binds the Fc receptor of IgG, although binding appears to be weaker than that of the HSV-1 homolog (Litwin et al., 1992).

VZV gE recycles in cells from the surface of the cytoplasmic membrane through internalization, possibly through endocytic vesicles and the transGolgi network (Zhu et al., 1995; Alconada et al., 1996; Zhu et al., 1996). It is phosphorylated on multiple sites (Grose et al., 1989; Yao et al., 1992) and the 63 amino acids in the cytoplasmic tail of gE are a target for several protein kinases, including casein kinase II and casein kinase I. The serine and threonine residues at amino acids 596 and 598 are major targets of phosphorylation (Grose et al., 1989; Yao et al., 1993b; Alconada et al., 1996). Recent evidence strongly suggests that the cycling of gE is dependent on the state of phosphorylation, in a manner similar to the phosphorylation-dependent sorting signals used by the cellular receptor pathway system. The cytoplasmic tail can direct intracellular trafficking when transferred to other surface proteins (Zhu et al., 1995; Alconada et al., 1996; Zhu et al., 1996). VZV gE expressed in baculovirus may exist in monomeric and dimeric forms, with each form serving as substrate for different kinases. A tyrosine kinase activity is thought to target the dimeric form of gE expressed in baculovirus and mammalian cells,

which enables endocytosis of the glycoprotein through a YXXL motif (Alconada et al., 1996; Olson & Grose, 1997; Olson et al., 1997). Tyrosine 569 in the motif AYRV is critical for targeting gE to the transGolgi network (Zhu et al., 1996). Evidence has also suggested that gE is the target for a viral induced protein kinase (Petrovskis et al., 1986).

VZV gE is noncovalently linked to gI, as are gE and gI of other herpesviruses (Yao et al., 1993a) and a monoclonal antibody to gE coprecipitates gI (Vafai et al., 1989). The ability of gE and gI to form a complex may mediate and accelerate gI transport to the transGolgi network (Yao et al., 1993a; Alconada et al., 1996; Kimura et al., 1997) where it has been hypothesized that VZV acquires its envelope (Zhu et al., 1995, 1996). VZV gI may also be recycled from the cell surface due to its association with gE.

ORF37 – gH

VZV ORF37 is predicted to encode a typical type 1 glycoprotein with nine potential N-linked glycosylation sites. Antibodies to VZV gH recognize a 105–118 kDa glycoprotein which is sulfated in VZV-infected cells, but does not contain O-linked sugars (Edson, 1993). Antibodies to VZV gH effectively neutralize the virus, prevent virus-induced cell-to-cell fusion, and prevent virus egress from the infected cell (Keller et al., 1987; Rodriguez et al., 1993). Mouse and human monoclonal antibodies have been isolated which specifically recognize a conformational epitope on gH following its coexpression with gL (Sugano et al., 1991; Forghani et al., 1994; Nemeckova et al., 1996). Like other herpesviruses, VZV gH forms a complex with gL and requires gL for its correct maturation and cell surface expression (Duus et al., 1995; Nemeckova et al., 1996). Without gL expression, VZV gH localizes in the cell diffusely and fails to appear at the cell surface. Recent evidence suggests that gH can also interact with gE and gI in complexes which can enable maturation of the gH protein (Duus et al., 1995).

ORF67 – gI

ORF67 encodes a class I glycoprotein which has two forms in VZV-infected cells; the larger 50–55 kDa form is also present on the virion envelope (Montalvo et al., 1985). VZV gI contains N-linked and O-linked sugars and, like gE, is phosphorylated in its cytoplasmic tail in VZV infected and transfected cells (Yao et al., 1993b; Ye et al., 1999). Recently, it has been shown that a cyclin dependent kinase, possibly CDK1, is able to phosphorylate the protein and the kinase targets serine 343 in the internal cytoplasmic tail (Ye et al., 1999). As described above, gI forms a noncovalent complex with gE in the endoplasmic reticulum and co-localizes with gE on the surface of VZV-infected cells (Yao et al., 1993a). The N terminus of gI is required for complex formation with gE (Kimura et al., 1997). Recent studies based

on VZV gI deletion mutants indicate that gI is required for both the proper distribution of gE in the cell membrane and for processing of gE to its mature form (Mallory et al., 1997; Cohen & Nguyen, 1997). A VZV gI mutant is slightly impaired for adsorption to human cells, impaired for growth in these cells in vitro, and cannot grow in Vero cells. Syncytia formation is also disrupted in VZV gI deletion mutants.

VZV gI induces complement-dependent neutralizing antibodies (Forghani et al 1990) and is a target of cytotoxic T cells (Huang et al., 1992). Immunization of guinea pigs with gI protects them from challenge with VZV, suggesting a potential use as a candidate subunit vaccine (Lowry et al., 1992).

ORF5 – gK

ORF5 is predicted to encode a hydrophobic protein which contains only two potential sites for N-linked glycosylation near its amino terminus. It encodes a 40 kDa structural glycoprotein which is present on virions and in the cytoplasm of VZV-infected cells (Mo et al., 1999). Virus deleted for gK cannot grow on noncomplementing cell lines, indicating gK is an essential glycoprotein. HSV-1 gK cannot substitute for VZV gK gene in the VZV genome.

ORF60 – gL

ORF60 is predicted to encode an 18 kDa protein with one N-linked glycosylation site. Cotransfection of ORF60 and gH in mammalian cells results in expression of a 20 kDa protein identified as gL (Forghani et al., 1994). VZV gL does not possess a typical hydrophobic signal sequence, but contains a putative endoplasmic reticulum targeting sequence which is common to many chaperone proteins (Duus et al., 1995). While VZV gL is not highly conserved with gL proteins of other herpesviruses, it appears to have a similar function in chaperoning gH. Thus, VZV gL and EBV gL can substitute for each other and chaperone the respective heterologous gH proteins to enable correct processing (Li et al., 1997).

Structural proteins and virus maturation

The structural proteins of VZV have not been as well defined as those of HSV-1. Three VZV nucleocapsid proteins have been partially characterized, including the major capsid protein encoded by ORF40, the protease encoded by ORF33.5, and the assemblin protein encoded by ORF33. The product of ORF21 appears tightly associated with the capsid. In addition, proteins encoded by ORFs 20, 23 and 41 are likely capsid proteins based on the function of their HSV homologs.

Several studies have followed maturation of VZV in the infected cell to determine the cause of the unusual degree of cell association of progeny virus in culture.

While high titres of HSV-1 are released from cells, virtually no infectious VZV is released from infected cells in vitro, regardless of cell type. Some virions are released into the media at lower temperatures, but the virus appears to be damaged and is not infectious (Grose & Brunel, 1978). Currently, VZV maturation is thought to occur by one of three pathways. One model proposes that VZV maturation uses similar cellular pathways required for melanogenesis in melanocytes (Harson & Grose, 1995). Viral nucleocapsids form within the nucleus, acquire tegument through the nuclear envelope, and subsequently mature through the endoplasmic reticulum into vacuoles. These fuse with Golgi-derived vesicles containing mature glycoproteins and subsequently travel to the cell surface. However, fusion of the viral vesicles with lysosomal vesicles causes the action of proteolytic enzymes to disrupt and enzymatically degrade virions prior to their appearance on the cell surface (Jones & Grose, 1988). The reason for incomplete release of virions is not understood.

A second pathway, proposed by Gershon et al. (Gershon et al., 1994; Zhu et al., 1995) suggests that the VZV nucleocapsid buds through the nuclear envelope and is released into the cytoplasm as a naked nucleocapsid. Reenvelopment occurs within the transGolgi network, where vesicular structures containing viral tegument proteins and glycoproteins attach and wrap around the nucleocapsid. Subsequent maturation into intracellular vacuoles occurs, which delivers membrane-attached particles to the cell surface. A third pathway must occur in human skin in vivo, where the degradative processes which render virus generated in culture noninfectious are avoided, resulting in the release of infectious virus. Such a pathway must occur in vivo, since infectious cell-free virus isolated from vesicles is more stable and higher in titre than that obtained from cell culture. Cell-free VZV is also released from VZV-infected human T cells (Moffat et al., 1995).

ORF10 – Virion associated transactivator

VZV ORF10 encodes a 47 kDa protein located in the tegument of VZV virions (Kinchington et al., 1992). VZV ORF10 protein transactivates expression from the ORF62, but not the ORF4, ORF61 or ORF63 promoters (Moriuchi et al., 1993a) and enhances the infectivity of transfected VZV DNA by three-fold. While VZV ORF10 shows considerable homology to the HSV virion associated IE activator VP16, ORF10 lacks the carboxy terminal transcriptional activation domain present in VP16. Instead, the activation domain of VZV ORF10 is located at the amino terminus of the protein, and a motif centered at phenylalanine 28 of ORF10 resembles a similar transactivating domain surrounding phenylalanine 442 of HSV VP16 (Moriuchi et al., 1995c). ORF10 protein forms a complex with cellular proteins and sequence-specific elements on the ORF62 promoter (Moriuchi et al., 1995a). The ORF62 promoter contains a TAATGARAT-like element with an overlapping

octamer-like element (oct+/TAATGARAT), and two TAATGARAT-like elements without octamer-like elements (oct−/TAATGARAT). ORF10 forms a complex with Oct1 and HCF at either of the oct−/TAATGARAT elements, but not oct+/TAATGARAT. The Oct2 transcription factor also binds to the oct−/TAATGARAT element and inhibits transactivation of ORF62 by ORF10 protein.

Surprisingly, ORF10 is dispensable for VZV replication in cell culture (Cohen & Seidel, 1994a), while its HSV counterpart VP16 is essential for HSV replication and assembly of virus. However, cell lines expressing ORF10 protein complement an HSV-1 VP16 mutant that lacks transactivating activity.

ORF21

VZV ORF21 encodes a 115 kDa protein that is expressed in the cytoplasm and nucleus of infected cells (Mahalingam et al., 1998). The protein is also tightly associated with the VZV nucleocapsid. Messenger RNA corresponding to ORF21 has been detected in latently infected trigeminal ganglia (Cohrs et al., 1992, 1996)

ORF33 – Viral protease

VZV ORF33 is the assemblin gene which encodes the precursor of the viral protease. Expression of ORF33 in baculovirus results in a protein that is autocatalytic and cleaved into four different polypeptides (Garcia-Valcarcel et al., 1997; McMillan et al., 1997). The amino terminal cleavage product is the mature 31 kDa form of the protease and this cleaves the ORF33.5 protein. Because of the antiviral potential of protease inhibitors, the crystal structure of the mature VZV protease has been determined (Qiu et al., 1997). The structure has an overall fold different from most other serine proteases, but similar to that of the cytomegalovirus protease. The catalytic site of the VZV protease consists of a serine and two histidines. The active form of the VZV protease is predicted to be a dimer.

ORF33.5 – Assembly protein

VZV ORF33.5 encodes the 40 kDa precursor of the viral assembly protein and is located in frame within the 3′ half of the ORF33 gene. Coexpression of the ORF33 and ORF33.5 proteins results in processing of the latter protein into the mature 37 kDa assembly protein (McMillan et al., 1997; Preston et al., 1997). Both the precursor and mature assembly proteins have been detected in VZV-infected cells. By analogy with other herpesviruses, the assembly protein of VZV is presumed to act as a scaffolding protein for assembly of the nucleocapsid. Expression of ORF33.5 protein in cell culture results in the formation of long, hollow rods predominantly in the nucleus of cells (Preston et al., 1997). ORF33.5 can partially complement the homologous HSV protein and act as a scaffolding protein for HSV capsid shell proteins. Mature VZV nucleocapsids contain viral DNA, but lack 32 kDa and

36 kDa proteins, presumed to be the mature viral protease and assembly proteins, while immature capsids lack viral DNA and contain both proteins (Harper et al., 1995).

ORF40 – The major nucleocapsid protein

ORF40 encodes the 155 kDa major nucleocapsid protein of VZV, which is readily detectable in preparations of purified virions and nucleocapsids (Vafai et al., 1990; Kinchington et al., 1992, 1995). Sera from convalescent zoster patients contain antibodies which recognize ORF40 protein. A monoclonal antibody to ORF40 has been obtained which cross-reacts with the major capsid protein of HSV and recognizes an antigenic site common to both proteins (Vafai et al., 1990).

Other viral proteins

ORF1

VZV ORF1 encodes a protein that has no homolog in HSV, but does have homologs in equine herpesviruses. Immunoprecipitation of epitope-tagged ORF1 protein shows three polypeptides of 17 to 21 kDa located in the membrane of infected cells (Cohen & Seidel, 1995). ORF1 is not required for growth of VZV in vitro.

ORF9A

VZV ORF9A encodes a 7 kDa protein located in the membranes of infected cells (Ross et al., 1997). VZV unable to express both ORF9A and the adjacent ORF8 protein is impaired for syncytia formation and grows to lower titres than wild-type virus in vitro.

ORF32

ORF32 encodes 16 and 18 kDa phosphoproteins that are expressed in the cytoplasm of virus-infected cells (Reddy et al., 1998). The larger 18 kDa phosphoprotein is modified post-translationally by the VZV ORF47 protein kinase, since this species is not present in cells infected with a VZV ORF47 negative mutant. ORF32 is dispensable for replication in cell culture. However, since ORF47 protein is required for growth of the virus in human fetal T cells and skin (see above), the ORF32 protein may have a role for growth of the virus in these cells.

ORF57

ORF57 does not have a homolog in HSV, but has homologs with several animal herpesviruses. ORF57 encodes a 6 kDa protein in the cytoplasm of VZV-infected cells (Cox et al., 1998). While the homolog of VZV ORF57 in pseudorabiesvirus,

UL3.5, is critical for viral egress and growth in cell culture, VZV unable to express ORF57 is unimpaired for growth in cell culture.

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5

Pathogenesis of primary infection

Charles Grose, Ming Ye, and Jorge Padilla

Introduction

The pathogenesis of primary VZV infection can be considered in the broader context of new information about the evolution of the herpesviruses. On a geologic time scale, herpesviruses existed in the Jurassic and Cretaceous period 200 to 70 million years before the present time. Molecular phylogenetic analyses indicate that the origins of the ancestral VZV date from about 60–70 million years ago, during the Paleocene epoch, the era which followed the demise of the large dinosaurs and was associated with the expansion of mammals (McGeoch & Cook, 1994). The herpesviruses must have evolved in ancestral primates that derived from a small mammal between 70 to 50 million years ago. The dwarf lemur of Madagascar is an extant primate which may retain some characteristics of this ancestral Primate order (Martin, 1990) (Figure 5.1). As the primates assumed more simian features from 70 to 25 million years ago, the genus Aegyptopithecus appeared as the ancestral anthropoid and ancestral VZV presumably coevolved through the same genus (Fleagle, 1998; Stewart & Disotell, 1998). During the subsequent major branching of simians and great apes, ancestral VZV cospeciated with the apes and eventually cospeciated with hominoids, as they evolved in Africa over the prior 4 million years. Australopithecus was replaced by Homo erectus less than 2 million years ago. In turn, about 100 000 years ago, *Homo erectus* was succeeded by *Homo sapiens*. An increase in population of the highly adapted *Homo sapiens* in Africa is thought to have been followed by migrations, out of Africa, initially to the middle East, India and China, and subsequently to Europe. *Homo sapiens* arrived in North America via the Bering land bridge at least 10 000 years ago (Lahr & Foley, 1994). VZV and the other human herpesviruses are likely to have been dispersed globally during these migrations.

Concomitant with the evolution of the hominoids, the two phases of VZV disease may be considered to have emerged as a means to allow persistence of the virus in the population. The primary infection is chickenpox, or varicella, after which VZV remains in a dormant or latent state for decades. In adulthood, latent

Figure 5.1 Ancestral primate and ancestral VZV. Based on the analyses of several herpesvirus genes, the VZV genome evolved from a more ancient herpesviral genome about 70 million years ago. The ancestral primate in which VZV first appeared is not known; however, the primate may have closely resembled the Malagasy dwarf lemur.

VZV re-emerges as herpes zoster. Herpes zoster may perpetuate the virus since the individual with recurrent disease can transmit the virus to nonimmune members of the family or small community, who would contract chickenpox. Another 20–40 years could pass until one of the chickenpox cases grew older, developed herpes zoster, and transmitted the virus to individuals born after the last case of herpes zoster within the community. In this manner, VZV may have perpetuated itself through the last 4 million years. One observation supporting this survival strategy is that outbreaks of chickenpox in Tristan da Cunha, an extremely isolated community on a remote South Atlantic island, can be attributed to an occasional case of herpes zoster in an elderly resident (Taylor-Robinson & Tyrrell, 1963).

The Fenner–Grose model

The use of the term Fenner–Grose model, to describe the pathogenesis of primary VZV infection, is based on the adaptation of the Fenner model of mousepox or ectromelia to analyze the stages of VZV infection by Grose (Fenner, 1948; Grose,

1981; Fisher & Edwards, 1998). The description by Grose was entitled "Variation on a theme by Frank Fenner: the pathogenesis of chickenpox".

Fenner documented the pathogenesis of mouse pox or ectromelia by inoculating animals in the hind foot, sacrificing one animal each day for a month, and analyzing the individual organs for viral cytopathology and infectious virus. The viral titer rose for the first week in the inoculated foot, then plateaued for the second week before declining during the third and fourth weeks. Since the first sign of inflammation of the foot was observed at the end of the first week, this time point was designated as the end of the incubation period. In a supplementary experiment, Fenner detected virus in the regional lymph nodes as early as 8 hours after the initial inoculation whereas detection of virus in the spleen and liver on the fourth day was the first sign of systemic infection. The viral titer in liver and spleen increased markedly over the next 6 days and then fell rapidly. Virus was detected in the skin distant from the inoculation site on the sixth day and the titer increased until the ninth day. Based on these meticulous observations, Fenner proposed a model for poxvirus pathogenesis which includes an initial invasion, followed by two cycles of replication; the first replication site is local and leads to the primary viremia while the second replication site involves multiple organs and ends with the secondary viremia (Figure 5.2). Circulating virus from the secondary viremia enters the epidermis to cause the typical exanthem of mousepox. Antiviral antibodies were first detected on the seventh day and rose in titer for another week before declining slowly; the swelling of the foot detected on the sixth day may represent an initial delayed type hypersensitivity reaction. The highest titer of virus in the blood was observed on the ninth day and viremia resolved coincident with the peak titer of serum antibody around day 14. Virus disappeared from the spleen by day 18 and from the skin by day 24.

The adaptation of the Fennerian mousepox model to VZV, a completely unrelated virus, was suggested by extensive clinical observations about the incubation period of chickenpox, which is 14–15 days in the vast majority of cases (Grose, 1981). Nevertheless, some unusual circumstances suggested that primary VZV infection could have a shorter incubation period. In the early 1900s, physicians described the use of vesicular fluid obtained from children with chickenpox as a source of virus for intradermal vaccination of nonimmune children. Under these conditions, the period between inoculation of vesicular fluid and onset of chickenpox was frequently reduced to 8–10 days. A second situation concerned pregnant women who contracted chickenpox in the last week of gestation. If their newborn children developed chickenpox, the interval between onset of disease in mother and infant was often 9–10 days. In each of these clinical situations, the reduction of the usual incubation period by 4–6 days may be explained by the hypothesis that the incubation period includes two viremic phases. The direct inoculation of the

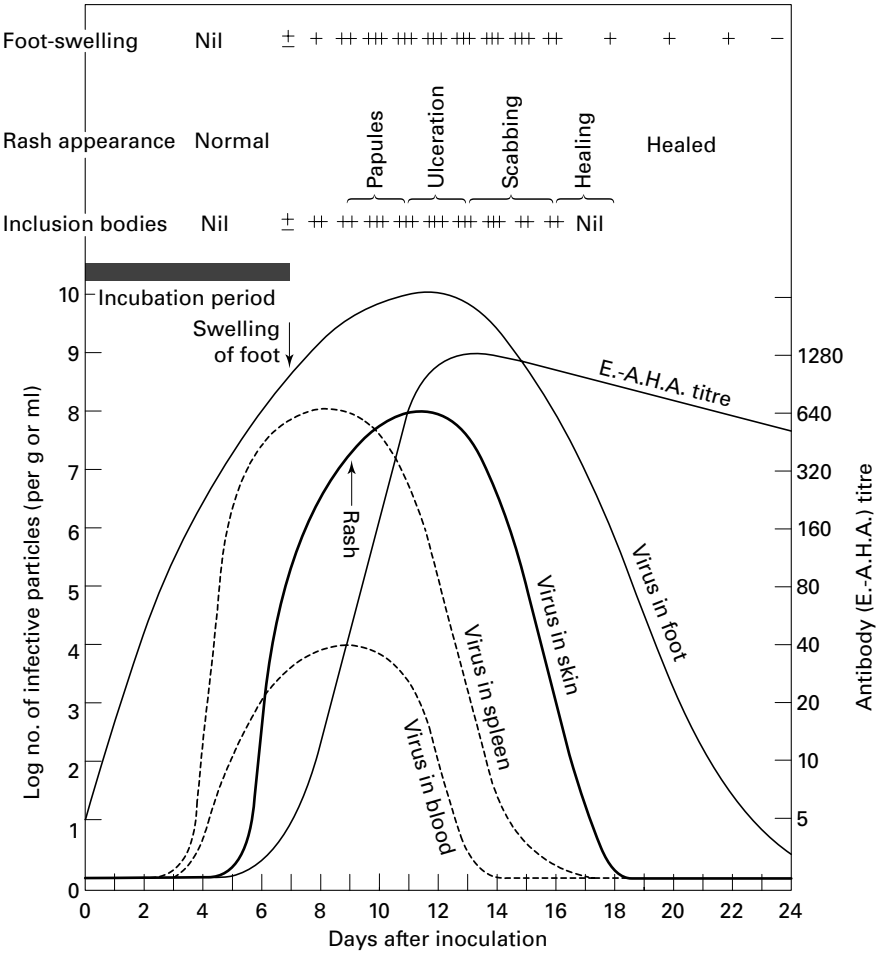

Figure 5.2 Original diagram for pathogenesis of ectromelia infection. The Fenner paper in 1948 proposed a schema for the pathogenesis of the disease known as mousepox or infectious ectromelia. The diagram includes a continuous curve representing the viremia; however, the raw data described in the text actually showed a biphasic viremia.

child by injection or the late gestation infant with virus of sufficiently high titer, may eliminate the first replication/viremia phase because the inoculum virus is distributed directly to the major organs.

The schema for pathogenesis of primary VZV infection that emerges from these observations and the evidence from the Fenner model, is illustrated in Figure 5.3. The model predicts that the method of contagion is primarily through virus in airborne water droplets (Sawyer et al., 1993) or by transmission through close contact. The typical incubation period for chickenpox is 14–15 days, twice as long as that for mousepox. Incorporating two viremic phases in the model suggests that the first

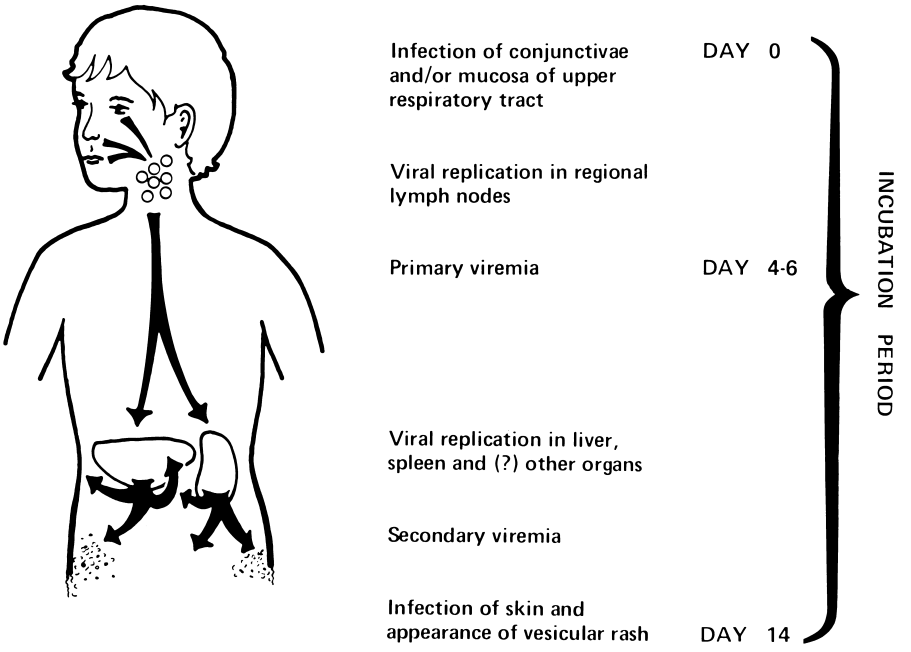

Figure 5.3 Pathogenesis of chickenpox. Schema for the pathogenesis of primary VZV infection was based on the observations of Fenner plus the additional data about a shortened incubation period for chickenpox in unusual clinical circumstances, which are described further in the text. Taken together, these data strongly suggested a dual viremia model for pathogenesis.

phase must be 4–6 days, which is consistent with shortened incubation period observed under the unusual circumstances of direct VZV injection. The concept that the first phase of pathogenesis is about 4–6 days is supported indirectly by the fact that varicella-zoster immune globulin must be administered within a few days after exposure, in order to prevent symptoms of chickenpox (Abramson, 1944; Gershon et al., 1974). Assuming that the first phase is 4–6 days, the second phase, which represents the interval before appearance of the exanthem, must be 8–10 days. In Figure 5.3, the suggested site of initial infection is either the conjunctivae or the nasal mucosa. The location of the primary cycle of replication is not known but it may occur in the regional cervical lymphatic tissue. This hypothesis is supported by the observation of multinucleated giant cells in the adenoidal and tonsillar tissue of a young girl who by chance underwent adenoidotonsillectomy three days before the onset of chickenpox (Tomlinson, 1939). According to the Fenner–Grose model, replication at the local site is followed by primary viremia and distribution of the virus throughout the body.

The major internal sites of viral replication suggested in the schema are not

known with certainty. However, histologic data collected during autopsies of two infants who died with neonatal chickenpox clearly demonstrated numerous minute white foci scattered throughout the liver and spleen (Oppenheimer, 1944; Ehrlich et al., 1958). Based on microscopic enumeration of intranuclear viral inclusions as an index of viral replication, VZV exhibited a marked predilection for growth in epithelial derivatives, including the squamous epithelium of the skin and esophagus; the columnar epithelium of the trachea, bronchioles, intestines, and bile ducts; the glandular epithelium of liver, pancreas, and adrenals; and the epithelial cells of the renal tubules, pulmonary alveoli, and Hassall's bodies. No abnormalities were found in mesenteric and mediastinal lymph nodes, thyroid, heart and aorta, skeletal muscles, bladder and ureter, uterus and cervix or brain.

After its presumed cycle of replication at internal sites, a secondary viremia occurs during which VZV is again released into the bloodstream and the virus quickly invades the cutaneous tissues. The occurrence of this major viremia has been well documented in both healthy and immunocompromised children (Myers, 1979; Ozaki et al., 1986; Koropchak et al., 1989; Asano et al., 1990). The viremia is detectable from 5 days before appearance of rash and up to 3 days after the appearance of the exanthem. The virus is detected by culture of unfractionated mononuclear cells (Asano et al., 1990), with localization to cells of lymphocytic morphology by in situ hybridization (Koropchak et al., 1989). VZV tropism for CD4 and CD8 T lymphocytes has been demonstrated (Moffat et al., 1998). Experimental evidence is lacking to confirm mechanisms of VZV transport to skin sites. The virus may exit the capillaries while within lymphocytes and enter the epidermal layer, possibly transiting via the melanocytes which reside near the basal layer (Harson & Grose, 1995). Extrapolating from its pattern of infectivity in vitro, each VZV replication cycle lasts 14–18 hours, which suggests that VZV may undergo about 20 replication cycles during the entire incubation period.

The clinical severity of chickenpox, as judged by the number of vesicles in the exanthem, is presumed to correlate with the number of infected lymphocytes in the peripheral blood of a child with chickenpox (Asano et al., 1990). Histologic examination of newly formed vesicles obtained by skin biopsy suggests that infection begins in the dermis and spreads outward to the epidermis, a progression compatible with blood-borne infection (Tyzzer, 1906). The earliest lesions include eosinophilic inclusions in the nuclei of endothelial cells lining the small blood vessels. The first alterations in the epidermis consist of swelling of both the cytoplasm and nuclei of the epithelial cells. The swelling progresses to what has been called ballooning degeneration, a term coined to describe the development of enormous cells with multiple nuclei. The characteristic unilocular vesicles are formed by the exudation of clear fluid at discrete sites of degenerated epithelium. The chamber of each vesicle lies in the middle layer of the epidermis, with a portion of

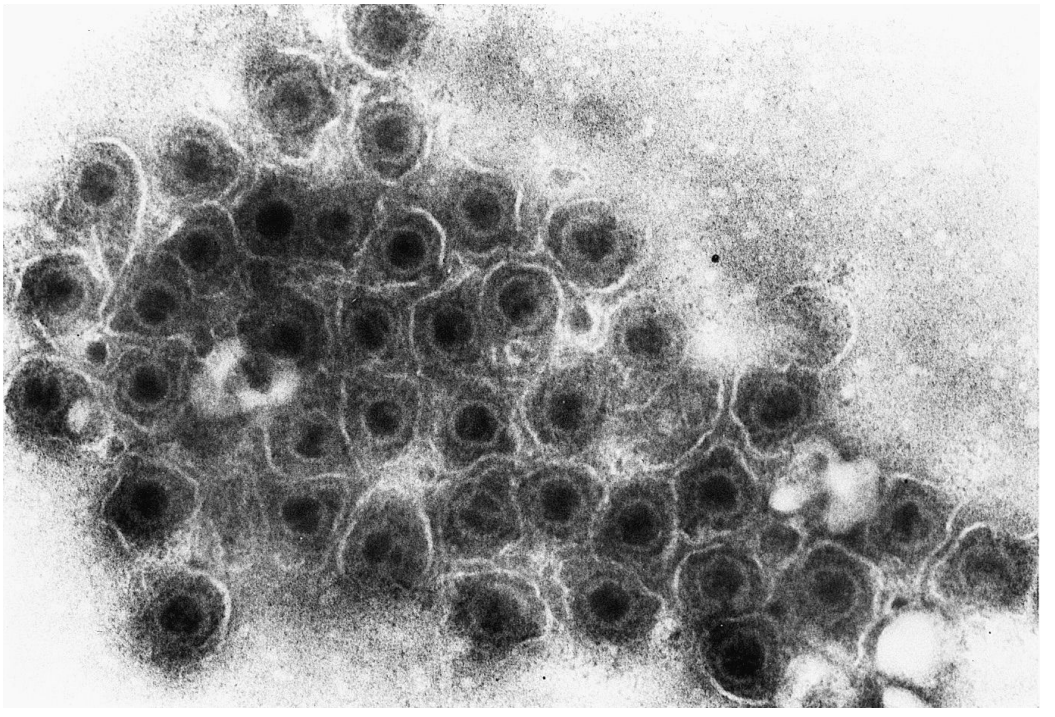

Figure 5.4 Virions in vesicular fluid. Fluid collected from the vesicular fluid of a child with chickenpox was examined by transmission electron microscopy. Numerous prototypic virions were observed in the sample; each virion is approximately 200 nm in diameter.

the stratum spinosum forming the roof, while the floor is made up of large multinucleated epithelial cells. The fluid within the chamber is filled with virions (Figure 5.4). After 3–4 days, the vesicular fluid becomes cloudy because of the increased fibrin content and the entrance of granulocytes and macrophages. The epidermis overlying the vesicle eventually desquamates. The index child in a family develops an average of 207–258 vesicles during the course of chickenpox (Ross, 1962). Secondary household cases tend to have somewhat more severe disease in association with a pox count of 310–510.

Observations on genetic variation and VZV pathogenesis

VZV has been thought to be a remarkably stable virus genetically. Differences in the severity of primary VZV infection have been considered to depend upon interactions between the virus and the host, and possible inoculum effects but not upon genetic variations in the virus. Only one VZV serotype has been recognized, although comprehensive comparisons of VZV strains using panels of monoclonal

antibodies have not been done. Sequencing of the VZV Dumas strain reveals a genome of approximately 69 open reading frames (ORFs) (Davison & Scott, 1986). Full sequence comparisons of multiple strains of VZV have not been reported. However, the first indication that the VZV can undergo genetic changes that result in the loss of an antibody-binding epitope was provided by an analysis of the ORF10 protein (Kinchington & Turse, 1995). The ORF10 protein is a constituent of the amorphous tegument which surrounds the capsid and is not thought to be detectable on the surface of the virion. In order to investigate the antigenicity of this protein, antibodies were prepared against a synthetic peptide MECNLGTEH-PSTDT, a sequence that represents the N-terminal 14 amino acids of ORF10 in the prototypic Dumas strain (Figure 5.5). The antibodies reacted with ten North American and European VZV strains but failed to react with ten Japanese strains, including the Oka vaccine strain. To further investigate the genetic basis for the epitope loss, the ORF10 genes of the Japanese strains were subjected to DNA sequencing. Results of the nucleotide analyses demonstrated that all Japanese strains had a CAT codon rather than a CCT codon; this change resulted in a proline to histidine substitution in the tenth codon (Figure 5.5). Presumably this single amino acid substitution destroyed the ability of the anti-peptide antibody to bind to ORF10 protein from the Japanese VZV strains.

This intriguing loss of antibody recognition within the ORF10 protein was described as a geographical variant because it was found only in ten Japanese strains. One hypothesis to explain this observation is the occurrence of a founder effect, representing an example of non-Darwinian selection which occurs in a isolated population. This term is applied to genetic syndromes found only in people living in small communities isolated by geography or religious beliefs, for example, small farming communities in rural Finland or small Amish villages in the US. When applied to VZV, the concept requires the assumption that VZV was brought to Japan from China during human migrations that occurred in past millennia. If, by chance, the ORF10 mutation occurred in the VZV strain harbored within the original inhabitants living around Osaka, Japan, the variant might have been perpetuated through more recent human generations even if the viral variant exhibited no special adaptive features that enhanced survival.

The second antigenic variant of VZV that has been described is an isolate recovered from a pediatric cancer patient with chickenpox (Santos et al., 1998). This VZV isolate has a mutation in the ectodomain of the gE protein. The gE protein (ORF68) is a typical transmembrane glycoprotein consisting of 623 amino acids with a relatively long ectodomain and a shorter endodomain. This glycoprotein is produced in large amounts in the VZV vesicle in infected children. Immunologic staining of vesicles for gE delineates the extent of virus infection within the skin (Figure 5.6).

ORF 10 (residues 1–14)

Wild type	M	E	C	N	L	G	T	E	H	P	S	T	D	T
Mutant	M	E	C	N	L	G	T	E	H	H	S	T	D	T
										*				

ORF 68 (residues 150–161)

Wild type	D	Q	R	Q	Y	G	D	V	F	K	G	D
Mutant	N	Q	R	Q	Y	G	D	V	F	K	G	D
*												

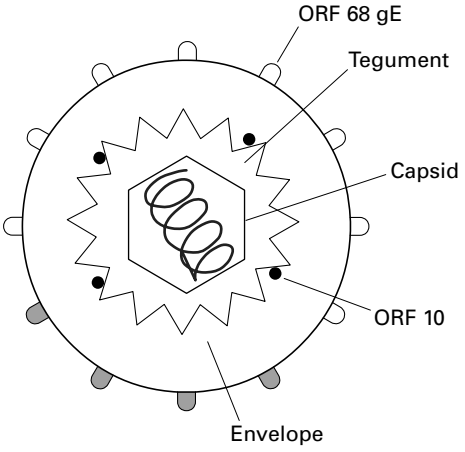

Figure 5.5 Mutations in two VZV proteins. The amino acid sequence in each wild type VZV epitope is compared with the sequence in the mutated epitope no longer recognized by an antibody. The mutated amino acid within each epitope is marked with an asterisk. The diagram of the virion illustrates the location of each protein. ORF68 is also known as gE and was formerly called gpI or gp98. C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; Y, tyrosine.

The newly discovered VZV gE mutation consists of a single nucleotide change in codon 150; in turn, this change leads to a substitution of an aspartic acid residue with an asparagine residue (Figure 5.5). Because of the mutation, the gE molecule is no longer recognized by the murine monoclonal antibody called 3B3. When the sequence of entire gE gene was analyzed, a second nucleotide change was discovered but it was a silent mutation. Even though the ORF10 variant had been described in 1995, the discovery in 1998 of a lost epitope on the preponderant VZV glycoprotein was a completely unexpected finding. Based on immunostaining

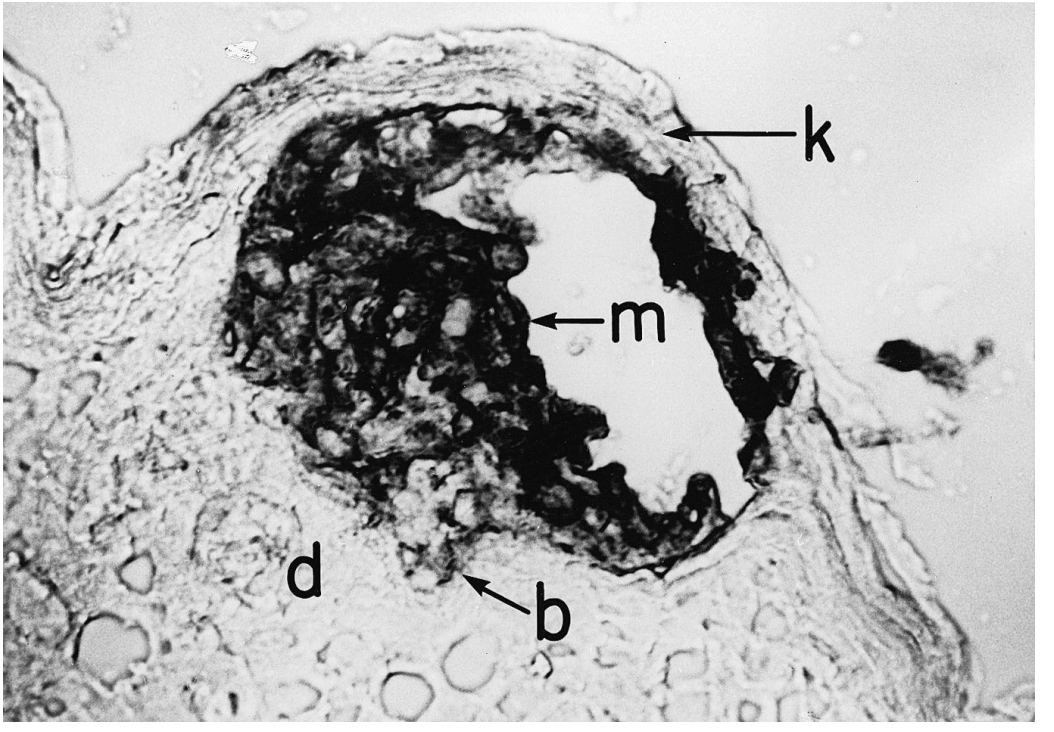

Figure 5.6 Localization of VZV gE in a vesicular lesion. Immunostaining of a chickenpox vesicle with anti-gE antibody (clone 3B3) revealed strong VZV-specific reactivity in the basal layer (b) and throughout the spinous (malpighian) and granular cells of the epidermis (m). The outermost layer of the keratinized cells in the stratum corneum (k) was spared. Likewise, little or no reactivity was detected in the dermis (d).

results, the 3B3 epitope is present not only in wild type strains from the USA examined in this laboratory but also in the Oka vaccine strain (Grose et al., 1983; Grose, 1990; Santos et al., 1998). Based on the published sequence of the prototype European VZV strain analyzed by Davison and Scott (1986), the epitope consists of at least 12 amino acids (150–161): DQRQYGDVFKGD (Figure 5.5). The corresponding sequence in the mutant isolate contains an asparagine residue in position 150; however, this mutation does not introduce a site for N-linked glycosylation (asparagine-x-serine/threonine). In addition to the gE gene (ORF68), the adjacent gI gene (ORF67) has now been sequenced and found to be identical to that of the Dumas strain gI. The new isolate has been designated VZV-MSP.

Whether the mutation in gE had an effect on cytopathology was initially examined by scanning electron microscopy. VZV usually exhibits an unusual pattern of egress called viral highways (Figure 5.7). The viral highways consist of thousands of emerging viral particles that cross the monolayers in linear pathways (Figure

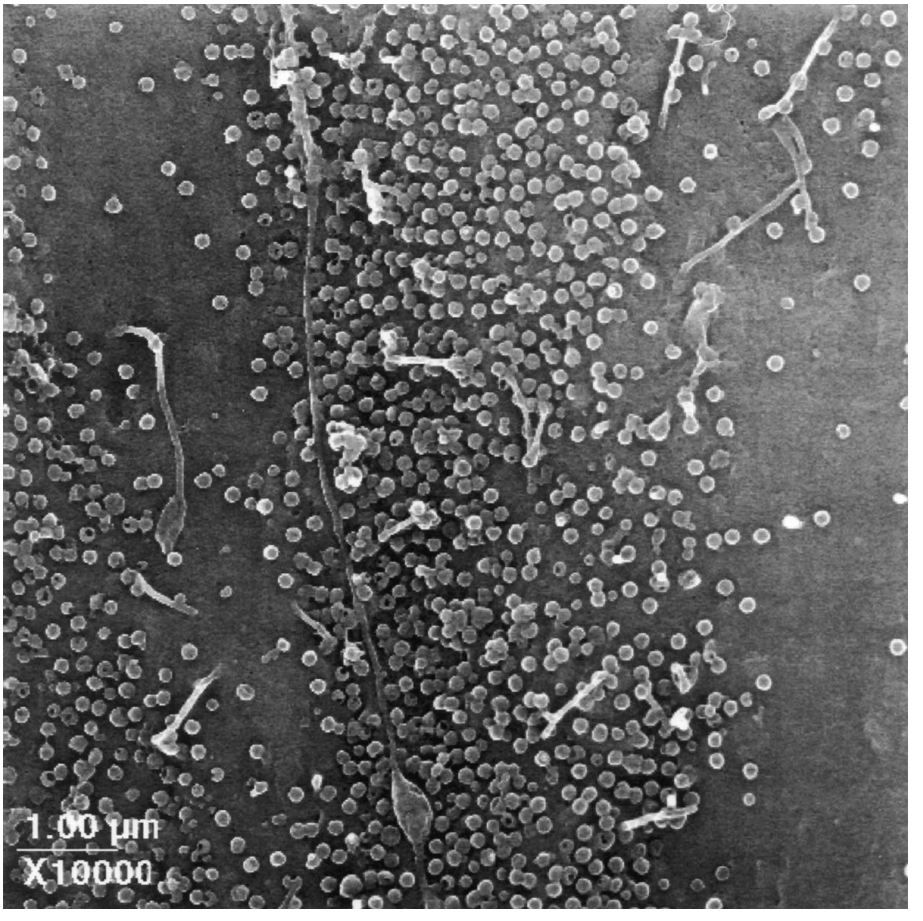

Figure 5.7 Topography of egress of wild-type VZV. When examined by scanning electron microscopy, viral particles in infected cell culture emerge across the cell surface in pathways which have been called viral highways. This pattern is seen in VZV-infected human melanoma cells with wild-type strains but not with a mutant VZV-MSP strain.

5.8). When the VZV-MSP mutant was examined under similar conditions, the pattern of viral particles on the cell surface showed a more uniform distribution rather than the previously recognized viral highways. Since the only mutation known in VZV-MSP involves gE, these differences suggested that gE may play a role in viral egress.

The mutation in VZV-MSP gE resembles those associated with antigenic drift in influenza A virus, in which a single base substitution leads to a single amino acid change which results in loss of an epitope on the major cell surface hemagglutinin (HA) molecule (Burnet, 1955; Smith & Palese, 1989; Wharton et al., 1989). The mutation rate within the HA ectodomain has been estimated at 0.0067 substitutions

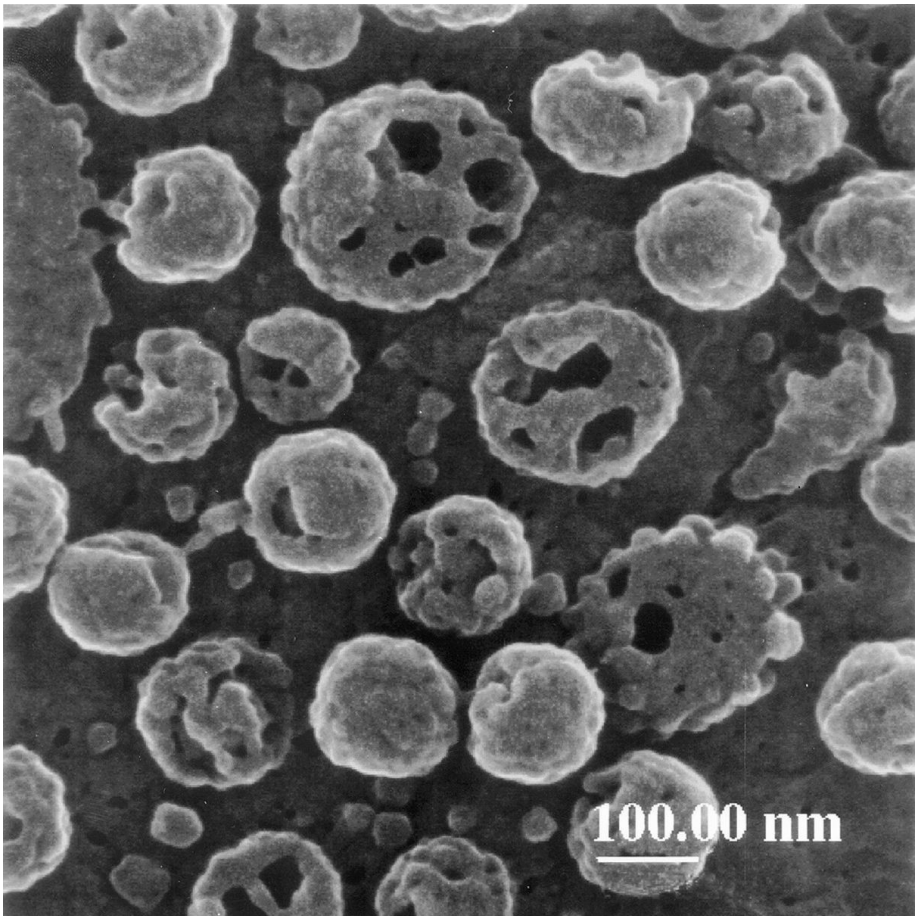

Figure 5.8 Higher magnification of viral highways. Many individual viral particles within the viral highways are aberrant in appearance when examined by scanning electron microscopy at very high magnifications. Some particles appear to represent envelopes without capsids. The aberrant shape is not the result of fixation artifact because herpes simplex viral particles, when examined under similar conditions, are prototypic in appearance.

per site per year. One recognized HA codon change is identical to that seen in VZV gE, namely, a switch from an aspartic acid to an asparagine residue. As part of the intensive analysis of human immunodeficiency virus type 1 (HIV-1) over the past 15 years, mutation rates have been tabulated for the individual viral genes (Coffin, 1995). The nucleotide substitution rate for the HIV-1 envelope gene is estimated to be around 10^{-3} per site per year. The high degree of variability in the influenza HA and HIV glycoprotein ORFs suggest immunological selection directed to a viral surface immunogen (Drake, 1993). In contrast, the mutation rates for eukaryotic DNA genomes have been estimated to be about 10^{-9} nucleotide substitutions per

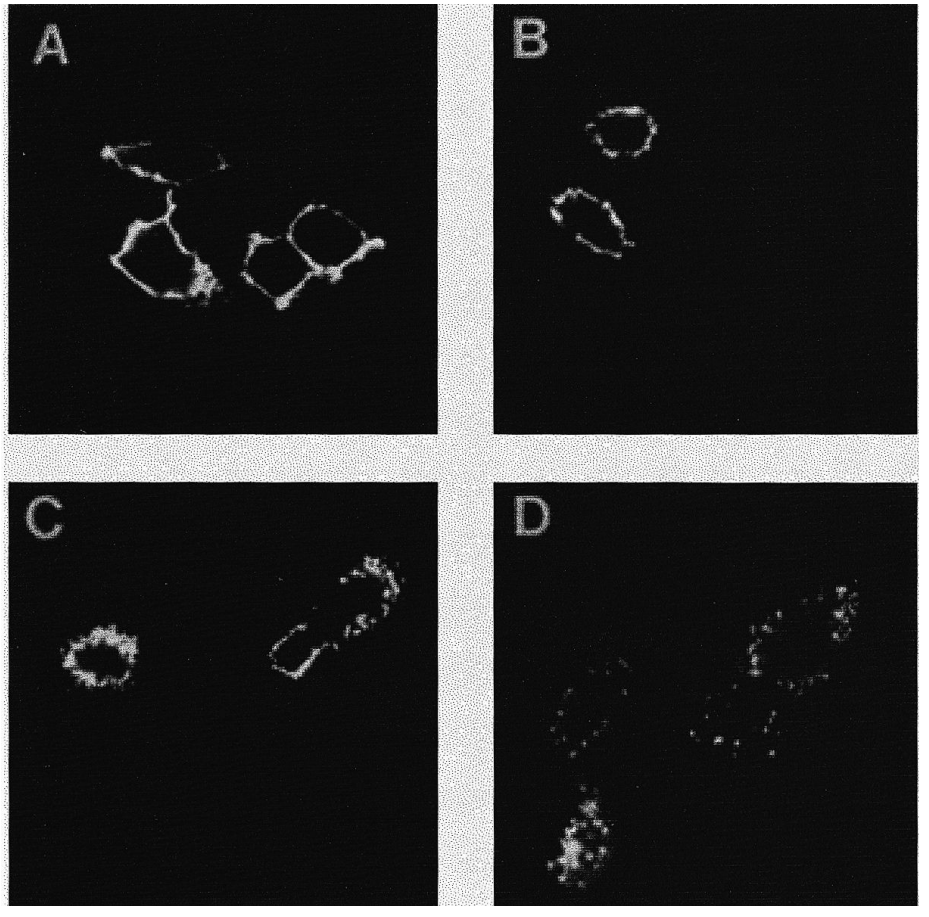

Figure 5.9 Endocytosis of VZV gE. The VZV gE gene has been cloned into a plasmid expression vector. In transient transfection studies, progressive internalization of gE from the cell surface can be documented in a timed experiment. Optical tomography was performed by laser scanning confocal microscopy after immunostaining of gE with monoclonal antibody 3B3. At time 0 (panel A), gE forms a broad fluorescent rim around the surface of each transfected cell. At 15 minute intervals thereafter (panels B–D), internalization of gE in clathrin-coated pits leads to an increasingly large number of gE-filled vesicles in the cytoplasm of each transfected cell (panel D).

site per year (Holland et al., 1982; Gojobori & Yokayama, 1985). After extensive sequence analysis of homologous herpesviral genes by means of both maximum parsimony and distance methods, VZV and other alphaherpesviruses were found to exhibit a nucleotide substitution rate between 10 and 100 times faster than eukaryotic DNA (McGeoch et al., 1995). Although no experimental data exist to support the hypothesis, the relative genetic stability of VZV may reflect a limited

number of replication cycles during chickenpox. If VZV undergoes a total of only 20 replication cycles while the HIV-1 replication cycle repeats itself every 1.5 days throughout the lifetime of an untreated patient, the opportunity for VZV mutation is restricted, whether it is random or mediated by selective immunologic pressure.

The analysis of antigenic variation in the gE protein may have implications for our understanding of the co-evolution of virus and human host and the possible selective advantage of gE mutations in that relationship, if additional mutations are identified. For example, the isolation of a community VZV isolate with a lost B-cell epitope on the structural gE glycoprotein could imply that antibody modulates an early event in VZV pathogenesis. As is true of all human herpesviruses, these questions require further investigation to improve understanding of VZV pathogenesis.

Whether there are significant genetic variations of VZV gE is of particular interest because of the evidence that the gE protein or alternatively the gE/gI complex is strongly associated with the pathogenesis of primary infection with the alphaherpesviruses. Although HSV-1 gE is not involved in adsorption or penetration of input virus, both gE and gI facilitate cell-to-cell spread of virus in cultured cells (Dingwell & Johnson, 1998). In animal models of pseudorabies virus, a swine alphaherpesvirus, gE or gI null mutants are markedly less virulent during initial infection of the central nervous system (Whealy et al., 1993; Tirabassi & Enquist, 1998). Both gE and gI exhibit many structural similarities with mammalian cell surface receptors. The mechanisms by which the gE/gI complexes exert their potential for facilitating cell-to-cell spread and ultimately affect virulence, undoubtedly involve a large number of signaling and sorting motifs, which are found in their cytoplasmic tails. The nature and location of these motifs are illustrated in Figure 5.10. For example, both VZV gE and gI contain endocytosis motifs in their endodomains (Alconada et al., 1996; Zhu et al., 1996; Olson & Grose, 1997, 1998; Olson et al., 1998; Wang et al., 1998). In addition, recent evidence suggests that there is a potential for cross-talk between the cytoplasmic tails because of the different phosphorylation sites. The cytoplasmic tail of gE contains a casein kinase II (CKII) phosphorylation site (Grose et al., 1989); this same site or an overlapping site has been extensively studied and sometimes redesignated as an acidic sequence (Grose, 1990; Yao et al., 1993; Zhu et al., 1995, 1996; Alconada et al., 1996; Olson et al., 1997). The cytoplasmic tail of gI also contains a phosphorylatable serine residue which lies within a consensus site for a cyclin dependent kinase (CDK) (Ye et al., 1999). In turn, CKII is known to be modified by CDK phosphorylation. As illustrated in Figure 5.10, phosphorylation and dephosphorylation activities on the endodomain of one component of the gE/gI complex can potentially modulate the other component and thereby facilitate sorting and trafficking of the viral glycoproteins and ultimately cell-to-cell spread of the virus.

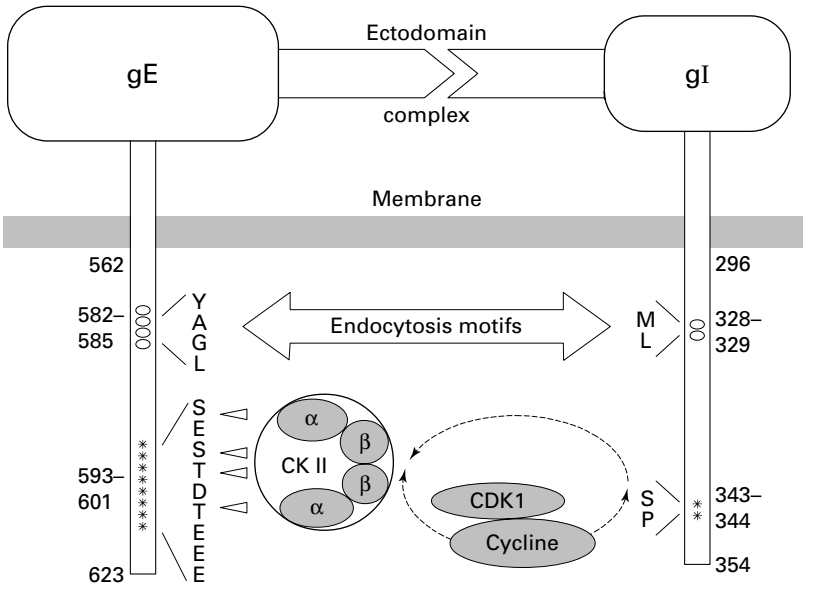

Figure 5.10 Potential mechanisms for cross-talk between the components of the VZV gE/gI complex. The locations of the endocytosis motifs and the phosphorylation consensus sites within the cytoplasmic tails are designated with their respective amino acid sequences. Protein kinases attached to gE can be modified by protein kinases attached to gI. Protein kinases: CKII, casein kinase II with its subunits; CDK, cyclin-dependent kinase.

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6

Pathogenesis of latency and reactivation

Saul Silverstein and Stephen E. Straus

Introduction

Infections with herpesviruses are characterized by intermittent bouts of symptomatic or asymptomatic recrudescence (Straus, 1994; Roizman, 1996). The ability to cycle between productive and latent infection is a hallmark of infections by members of this family of viruses. The alphaherpesviruses, including herpes simplex viruses 1 and 2 (HSV-1; HSV-2) and the varicella-zoster virus (VZV), have evolved genetically defined and effective strategies to infect, persist latently within, and reactivate from neuronal tissues. The aim of this chapter is to summarize the existing body of data regarding VZV latency, how it is established, the specific cell types in which the virus persists, the state of VZV during latency, and factors that might regulate its reactivation.

HSV latency

The mechanism by which HSV-1 and -2 establish and maintain latency is different from that of VZV. The differences are readily evident when considering the clinical patterns of infection with each of these viruses (Meier & Straus, 1992; Straus, 1993). As these differences provide important clues to the individual mechanisms for latency that underlay them, they are worth summarizing here.

HSV reactivates at a surprisingly high frequency. Individuals may experience hundreds of clinically apparent recurrences of genital or labial lesions during their lifetimes (Corey & Spear, 1986; Whitley, 1996). Moreover, numerous episodes arise in which the virus reactivates without causing recognized symptoms. Virus can be recovered from sites of prior infection of individuals lacking such symptoms on 1–3% of days; viral DNA can be detected in swabs of those sites on 5–15% of days using PCR assays (Koelle et al., 1992; Cone et al., 1994; Schacker et al., 1998). HSV infections not only reactivate frequently, but their reactivation may be associated with a variety of provocative stimuli including fever and ultraviolet light exposure.

Much is known about the cellular and molecular biology of HSV-1 and -2 latency

(Wagner & Bloom, 1997). These viruses spread directly from their primary sites of inoculation to the peripheral extensions of sensory and autonomic neurons innervating skin and mucus membranes. Virion cores ascend neuronal axons, and the viral genome establishes lifelong residence in neurons, largely if not entirely as episomes. HSV latency is characterized by the expression of only one family of transcripts, the latency-associated transcripts (LATs) (Croen et al., 1987; Stevens et al., 1987). There is no evidence that these RNAs are translated into protein, and their precise role in HSV latency remains obscure. Nonetheless, a variety of studies involving HSV strains containing targeted mutations in the LAT-encoding region indicate that LAT expression increases, in some manner, the efficiency of virus reactivation (Javier et al., 1988; Krause et al., 1995; Bloom et al., 1996).

In contrast to the clinical features of HSV latency and reactivation, VZV infections reactivate infrequently, usually only once if at all in a lifetime; there is minimal evidence for asymptomatic reactivation of VZV in normal people; and stimuli such as heat, fever, and ultraviolet light are not strongly associated with them (Meier & Straus, 1992). Far less is known about the nature and mechanisms regulating VZV latency, but what is known bespeaks a process that is very different from HSV latency. It is our belief that these differences underlie the abovementioned clinical and epidemiological differences between HSV and VZV reactivation infections.

Establishment of VZV latency

Our current model for VZV pathogenesis is based upon an extension of data derived from classical studies of mousepox (ectromelia), namely, that the virus undergoes two waves of viremia before the exanthem appears (Fenner, 1948; Gilden et al., 1992; Cohen & Straus, 1995). After primary infection, the virus replicates in regional lymphoid tissue or the upper respiratory tract; it exits these tissues and initiates a first wave of viremia, during which it spreads to more distant cells of the reticuloendothelial system. The virus inoculum is amplified further there and is then released as a more substantive secondary viremic phase, during which it spreads to skin, mucus membranes, and viscera. This model suggests two mechanisms by which VZV can access sensory nerve tissues. The first mechanism entails direct hematogenous spread to sensory ganglia; the second possible route to the ganglia is by retrograde axonal transport from infected epidermal and dermal tissues.

There are few data that bear directly on the validity of these competing mechanisms of neural infection by VZV. That viremia occurs is undisputed: it is a cardinal and obligatory feature of classical varicella. There are multiple reports of isolating VZV from the blood of patients with varicella. Isolation rates are greatest in the 4 to 5 days before the onset of the rash, when the patient is most contagious

(Asano et al., 1990; Ozaki et al., 1994). By in situ hybridization and PCR, as few as 1 in 10^5 peripheral blood mononuclear cells were shown to contain the virus (Koropchak et al., 1991). Analyses of ganglia recovered from autopsies of fatal varicella cases also support the possibility of viremic spread (Cheatham et al., 1956). Large percentages of both neurons and satellite cells are virus positive and, in many cases, there are positive satellite cells surrounding a neuron that appears virus negative (Croen et al., 1988). Had the virus ascended sensory neurons and spread through the ganglia to their neighboring satellite cells, there should be some correspondence between positive neurons and non-neuronal cells.

Clinical observations, however, favor most strongly the direct spread of virus by retrograde axonal transport to the ganglia. First, the frequency at which individual dermatomes are involved in zoster resembles the distribution of lesions in varicella (Straus & Oxman, 1999). The highest concentration of varicella lesions is on the face and upper trunk, while zoster most often involves the trigeminal, cervical, and thoracic dermatomes serving these anatomic regions. Second, longitudinal clinical trials revealed that episodes of vaccine-associated zoster often occur at or near the prior site of inoculation, suggesting that latency had been established preferentially within the sensory ganglion serving that single dermatome (Lawrence et al., 1988; Hardy et al., 1991). Had VZV spread to ganglia solely by a hematogenous route, then other body sites should have been well represented among the post-vaccination zoster cases.

Recent immunohistochemical and molecular studies in humans and in experimentally infected animals also bear on these competing hypotheses. Skin-punch biopsies of varicella lesions reveal the presence of VZV DNA in keratinocytes, in intradermal antigen-presenting cells, and in nearby capillary endothelial cells. At issue is the state of the virus in these cells. Immunohistochemical and in situ hybridization studies of the dermis by Nikkels et al. demonstrated virus DNA and the late viral glycoprotein E (gE) in Mac 387+ and CD68+ cells, members of the monocyte/macrophage lineage (Nikkels et al., 1995*a,b*). That gE was expressed in these cells argues that they were lytically infected by VZV. Further support for active infection of tissue antigen-presenting cells was provided by immunohistochemical studies of varicella skin biopsies in which antibodies to members of all three VZV-replicative kinetic classes were employed (Figure 6.1; Annunziato et al., 1999). The results of these investigations are summarized in Table 6.1. Among the seven varicella skin biopsies examined, six were positive for the putative immediate-early product of gene 4 and for the early product of gene 29. Four of the seven specimens were positive for putative immediate early protein encoded by gene 63, while five were positive for glycoprotein C (gC), the late product of gene 14. In the positive tissues, immunostaining was evident over epithelial cells, endothelial cells, antigen-presenting cells, and nerves and their surrounding support

Table 6.1 Detection of VZV proteins in skin biopsies[a]

Protein		IE63			ORF29			gC		
Dx	+/n[b]	EP	EN	N	EP	EN	N	EP	EN	N
CP	6 / 6	4	3	0	6	4	2	4	5	3
Z	8 / 8	5	1	0	8	4	0	5	5	4
G	0 / 5	0	0	0	0	2	0	0	0	3

Notes:

[a] Tissues from patients with clinical and histopathological diagnoses of chickenpox (CP), zoster (Z), or Grover's disease (G) were subjected to immunohistochemical analysis for immediate-early (IE) 63, early, open reading frame (ORF) 29 proteins, and late glycoprotein C (gC) proteins in epithelial cells (EP), endothelial cells (EN), or in dermal nerves (N). The results are expressed as the absolute number of biopsies with detectable protein. Zero indicates absence of detectable protein.

[b] The number of cases positive for any VZV protein/the number of cases examined.

cells. Where it was possible to ascertain the subcellular localization of the viral antigens, they were localized as one would expect in a lytic infection, i.e., the DNA-binding gene 29 protein was observed over nuclei, gC was seen on cell membranes and in the cytoplasm, and IE63 protein was seen in both nuclear and cytoplasmic compartments.

The observation in these studies that mononuclear cells in the dermis were antigen positive led to further immunohistochemical analyses. Using markers specific for distinct lymphocyte lineages revealed that professional antigen-presenting cells such as macrophages, monocytes, and Langerhan's cells were infected (Annunziato et al., 1999). Other cells that stained strongly for VZV antigens included the Schwann cells, which support the neurillema surrounding the nerve axons, and the nerve termini themselves. Infection of both the Schwann cells and the neurons indicates that VZV has ready access to the sensory ganglia through either or both of these cellular conduits.

The cumulative data from the studies of human skin biopsies, then, provide a physical basis for the direct spread of VZV from skin lesions to ganglia. Studies in experimentally infected adult rats provide additional support for this model. Sadzot-Delvaux and her colleagues in Liege, Belgium, injected VZV-infected cells into the flank of rats (Sadzot-Delvaux et al., 1990, 1995). They showed that most if not all VZV DNA persisting in the animal can be found in the dorsal root ganglia subserving the site of injection, also favoring retrograde transport of VZV as the dominant means of accessing the ganglia. More recent studies with neonatal rats, however, showed spread of VZV from the peritoneum to trigeminal ganglia, an

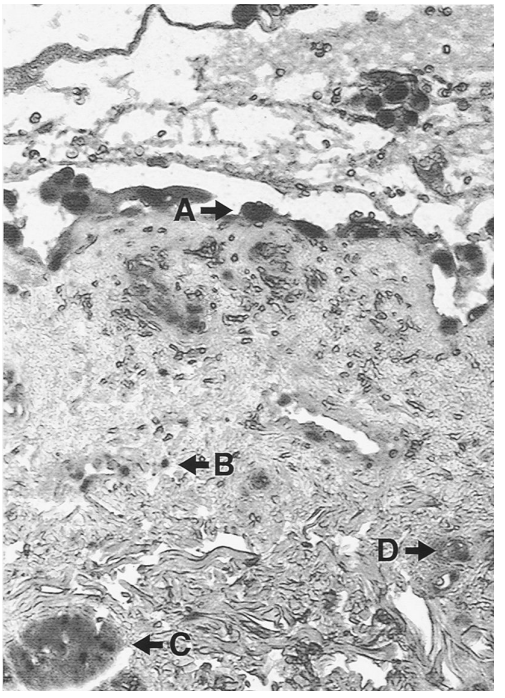
outcome that is certainly best explained by hematogenous spread of virus (Brunell et al., 1999). Thus, data support both possible mechanisms for VZV transport to ganglia.

Once VZV has infected human neural tissues, the virus has a "choice" of undergoing productive replication there or switching to a latent infection. The mechanism by which this might occur is not known, and there are no data from the HSV system that can shed light on this process. Clearly, VZV can replicate productively in human sensory neurons and satellite cells, because infectious virus has been recovered from ganglia during acute infection, and histochemical and molecular analyses verify the expression there of a full complement of viral transcripts and proteins (Cheatham et al., 1956; Taylor-Robinson & Caunt, 1972; Takashima & Becker, 1979; Weller, 1983; Croen et al., 1988). Presumably, some percentage of the cells that become infected with VZV do not support a full replicative cycle, because such replication should kill the infected host cell. In zoster, many infected cells may be supporting VZV replication and die as a consequence of doing so. This is evident from histologic studies showing widespread necrosis of ganglia associated with regional zoster (Head & Campbell, 1900; Watson & Deck, 1993). During varicella, the switch to latency must occur either before many cells become infected, or in a high percentage of those that do, since there is no evidence for the kind of sensory neuropathy in varicella that characterizes zoster. Whatever mechanism affords VZV the opportunity to downregulate expression of viral genes normally engaged in lytic replication, it does so efficiently during varicella infection.

The cellular locus of VZV latency

That latency of VZV occurs largely, if not exclusively, within nerve ganglia was evident from the classical dermatomal distribution of zoster lesions (Head & Campbell, 1900). The question has remained for many years what type or types of cells within the ganglia are hosts to the latent virus. The first descriptions of latent VZV nucleic acids in human ganglia involved in situ hybridization studies. The early studies by Hyman, by Gilden, and by Tenser and their colleagues used tritiated VZV DNA probes and autoradiography to detect VZV DNA in human ganglion sections (Gilden et al., 1983; Hyman et al., 1983; Tenser & Hyman, 1987). All three studies reported radiographic signals over a small percentage of neurons. A subsequent in situ hybridization study by Croen et al. (1988) used ^{35}S-labeled single-stranded VZV or HSV RNA probes to detect latent viral RNA. HSV-specific transcripts were detected in neurons, but VZV signals were only observed over satellite cells (Figure 6.2).

The conflicting results of both these early studies stood unresolved for several years. Vafai et al. (1988), however, reported immunohistochemical evidence of



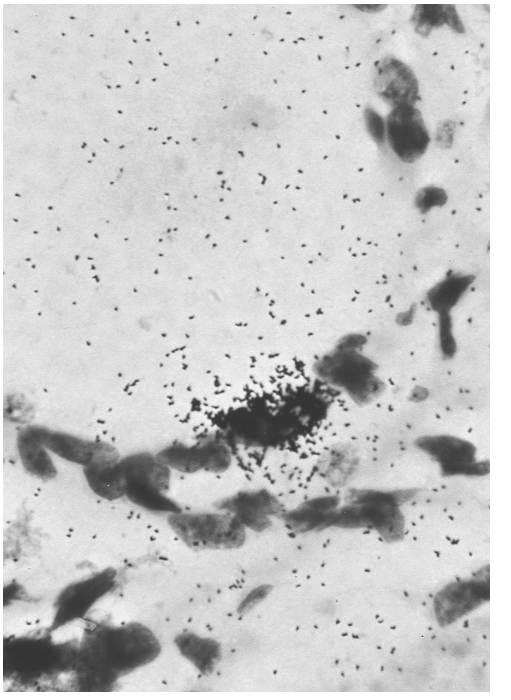


Figure 6.1 (*left*) Immunohistochemical detection of VZV protein in a skin biopsy. A section of a punch biopsy from a patient with chickenpox was analyzed for the presence of ORF29 by reacting the tissue sample with a polyclonal rabbit antiserum prepared against ORF29p. (A) Indicates positive epithelial cells, (B) points to positive inflammatory cells, (C) indicates positive dermal nerves, and (D) shows positive endothelial cells. The products of ORFs 4, 62, 63, 21, and 29 were detected using purified anti-VZV proteins, rabbit antibodies, and an alkaline phosphatase (AP)-conjugated goat anti-rabbit immunoglobulin secondary antibody. Signal was visualized with AP substrate.

Figure 6.2 (*right*) In situ detection of VZV ORF29-encoded RNA in a satellite cell adjacent to a large sensory neuron in human trigeminal ganglia.

VZV protein encoded by gene 63 in neurons of human ganglia that had been incubated for several days. The identical distribution of gene 63 protein was found by Mahalingam et al. (1996) with fresh human ganglia and by Sadzot-Delvaux et al. (1990, 1995) and Debrus et al. (1995) in their studies of regional ganglia of adult rats injected peripherally with VZV-infected cells. Both of these latter studies implicated neurons as the likely, if not only site of latent gene expression.

Within the past few years, renewed efforts to define the site of VZV latency have been facilitated by development of more sensitive in situ hybridization techniques involving enzymatic amplification of bound probes or of proteins coupled to them. Lungu et al. (1995) (Figure 6.3) and Kennedy et al. (1998) independently observed VZV DNA in both neurons and satellite cells.

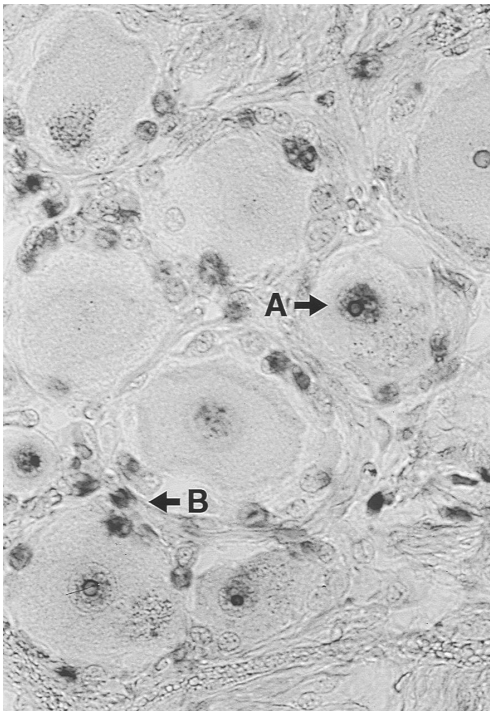


Figure 6.3 In situ detection of VZV DNA in human dorsal root ganglia. VZV DNA was detected using a fluorescein-labeled oligonucleotide probe complementary to 27 bp within ORF54 and an AP-conjugated anti-fluorescein antibody. (A) denotes a positive neuron, and (B) identifies satellite cells harboring VZV DNA.

The question remains why earlier data had been inconsistent and even conflicting on this point. The simplest explanation is that one or more of those early studies were technically flawed or involved assays that were too insensitive. The discrepancy among the studies could be explained if the density of VZV DNA signal is greatest in neurons and that of RNA is greatest in satellite cells. Nonetheless, the current data compel us to conclude that VZV persists in both neuronal and non-neuronal cells.

The state of the latent genome

Life-long persistence of VZV within neural tissues requires that the viral genome coexist with the cellular genome. By analogy with other virus–host cell patterns, there are two obvious mechanisms by which it could do so: either as a stable integrant into the cellular genome or as a stable episomal element. HIV and the adeno-associated virus are examples of human viruses whose genomes integrate into cell DNA. Epstein-Barr virus and human papillomaviruses are known to persist as episomes (Nokoyama & Pagano, 1973; Ikenberg et al., 1990). The evolutionary advantage of becoming integrated is that a viral genome is certain to be retained and replicated every time the host cell genome is replicated. The

advantage to remaining an episome is that numerous copies of the genome can coexist with the cell and multiply any time viral replication genes are expressed. The obvious disadvantage to episomal persistence is that the viral genome will be diluted in number and eventually lost among cellular progeny unless its replication is in some way tied to that of the host cell.

The locus of VZV latency and the pattern of virus reactivation afford some predictions about the manner in which its genome might persist. Because VZV remains poised at all times to reactivate, from a teleological viewpoint it would be best for it to remain episomal. In that neurons do not replicate, there is no risk that the VZV genome would be lost with successive generations of cells. The existing evidence suggests that HSV persists in neurons as a circular episome, so a similar state of persistence for VZV DNA would be reasonable (Mellerick & Fraser, 1987; Deshmane & Fraser, 1989). Latency in satellite cells, however, is another matter. Satellite cells do replicate, so maintenance in an episomal form can be risky for the lifelong carriage of VZV.

Unfortunately, we have little direct data about the status of the VZV genome in latency. The structure of the genome, with its terminal repeat elements, suggests that it should be able to circularize, and early endonuclease analyses of the VZV genome predicted that the encapsidated virion genome is circular (Kinchington et al., 1985). More recent PCR studies provided data to suggest that the genome in human sensory nerve ganglia is also circular (Clarke et al., 1995). Thus, there is at least some evidence that the latent VZV genome pool in human tissues includes a circular and, therefore, episomal form.

Latent viral load

Latent VZV nucleic acid sequences are obviously in low copy number, because routine Southern and Northern blot hybridization analyses never succeeded in detecting them in extracts of human ganglia (Meier et al., 1993). It remained for far more sensitive in situ hybridization and PCR assays to make their detection feasible (Gilden et al., 1983; Hyman et al., 1983; Tenser & Hyman, 1987; Croen et al., 1988; Cohrs et al., 1994, 1995, 1996). Despite the availability of these tools today, there remains some controversy regarding the quantity of latent viral DNA – what is now, by extension from the HIV literature, termed the latent viral load – and the percentage of latently infected cells in a ganglion.

Mahalingam et al. (1993) compared the intensity of hybridized PCR signals from ganglion extracts with signals created by amplification of serial dilutions of known quantities of VZV DNA-containing plasmids. By so doing, they reported that human ganglia contain 6–31 copies of VZV DNA in the total amount of DNA equivalent to that of 10^5 cells. This estimated latent VZV load was far less than that

reported by Efstathiou et al. (1986) for HSV-1 in human ganglia: 10^3–10^4 copies per 10^5 cells. More important, the latent VZV load estimated by Mahalingam et al. is much too low to be compatible with the reported percentages of neurons reported by Lungu et al. (1995) to contain VZV (see below). Nevertheless, it is entirely plausible that there are wide variations in the virus load among individuals.

These considerations prompted recent studies by Pevenstein et al. (1999) using quantitative DNA PCR assays to analyze extracts of human trigeminal ganglia. Three separate assays were developed with concordant results among them, leading to the conclusion that there are about 250 VZV genome equivalents per 10^5 cells. Precise estimates of the number of VZV transcripts in human ganglia do not exist. That routine RT-PCR assays on unselected total RNA from human ganglia were never reported to be positive suggests that the number of viral transcripts in latently infected tissues is low. This contrasts with the data regarding HSV-encoded LATs, which are estimated at 100 or more copies for every latent viral genome (Ramakrishnan et al., 1994). Thus, the quantity of VZV RNA in latently infected human ganglia must be substantially below this level, because HSV LATs are readily detected by Northern blot hybridization and by RT-PCR assays (Stevens et al., 1987; Croen et al., 1988; Wang et al., 1997).

Latent gene transcription

HSV latency is characterized by high-level accumulation of one family of colinear virally encoded, nonpolyadenylated LATs (Croen et al., 1987; Stevens et al., 1987). Existing data suggest that VZV has adopted a markedly different latency paradigm. First, as noted above, it persists in non-neuronal cells. Second, at least four VZV genes are expressed during latency, ones that are actively translated. The following is a synopsis of the data supporting these contentions.

Early in situ hybridization studies using pools of recombinant plasmids containing VZV DNA restriction fragments revealed that only limited regions of the viral genome are expressed in human ganglia (Croen et al., 1988). Subsequent in situ studies using specific riboprobes documented RNAs encoded by VZV genes 29 and 62 but not genes 28 or 61. Northern hybridizations of highly poly-A^+ enriched RNA from pooled human ganglia confirmed the presence of gene 29 and gene 62 message (Meier et al., 1993). These initial findings, in themselves, revealed three major differences between HSV and VZV latency: that VZV-encoded sequences are present in satellite cells; that multiple protein-encoding genes are expressed; and that the viral RNA in ganglia is polyadenylated and thus likely to serve as mRNA.

In other studies, hybridization-selected RNA from human ganglia was reverse transcribed, and the resulting cDNAs were labeled and used as probes of blots

Table 6.2 Expression of VZV genes in latently infected human ganglia

Gene	Function	RNA[a]	Protein
4	IE protein with regulatory activity	−	+
61	IE protein with regulatory activity	−	−
62	IE protein with DNA binding and regulatory activity, tegument associated	+	ND
63	IE protein with possible regulatory activity, tegument associated	+	+
21	E? protein, HSV homolog (UL37) is a virion protein that is also associated with the DNA polymerase	+	+
29	E protein, single-stranded DNA binding protein	+	+
10	L protein, virion-associated transactivator	−	−
14	L protein, glycoprotein	−	−
67	L protein, glycoprotein	−	−

Notes: [a] VZV-specific RNAs were detected by either in situ hybridization, Northern blot hybridization, RT-PCR, or cDNA cloning. −, not detected; +, detected; ND, not done.

containing VZV genomic fragments. A series of reports summarizing the evolution of these experiments detailed the detection in ganglia of RNAs homologous to VZV genes 21, 29, 62, and 63. VZV genes 28, 40, and 61 were not found to encode detectable levels of latent transcripts (Cohrs et al., 1994, 1995, 1996) (Table 6.2).

While the latter work was proceeding, Sadzot-Delvaux et al. (1990, 1995) studied latency in VZV-infected adult rats. The model was largely used to identify latently expressed viral proteins (see below), but it stimulated Brunell and colleagues at UCLA and the NIH to test peritoneal injections in newborn rats, postulating that the immunologic immaturity of the animals would permit higher levels of viral spread and latency (Brunell et al., 1999). While work with this model is still evolving, the initial report emanating from the group documented in neonatal rat trigeminal ganglia the presence of RNA homologous to VZV gene 21, but not gene 40, in accord with the findings in human tissues.

Protein expression

Multiple lines of evidence indicate that, in contrast to HSV latency, VZV proteins are expressed in latently infected human ganglia. As indicated above, the first such data were reported by Vafai et al. (1988), who detected gene 63 protein in extracts of ganglia that had been incubated for several days. At the time of this report, it was unclear whether the protein represented reactivation of VZV or whether the protein would have been detected without incubating the ganglia in vitro. The experiments by Sadzot-Delvaux et al. (1990) with subcutaneous injection of

VZV-infected cells into adult rats implicated gene 63 as the source of a protein expressed during latency.

The presence of gene 63 protein in the cytoplasm of latently infected human neurons provides evidence that this putative regulatory product is translated and that there are differences in the pattern of gene expression when cells are lytically or latently infected with VZV. In lytic infection, the gene 63 protein is also found in the nucleus (Debrus et al., 1995). Thus, the apparent restriction of gene 63 protein to the cytosol of latently infected cells prompted a fuller exploration of VZV protein expression and localization during latency. To this end, Lungu et al. (1998) conducted extensive immunohistochemical analyses of ganglia using polyclonal antisera raised to fusion proteins containing epitopes of VZV proteins coded by genes 4, 21, 29, and 62 (Figure 6.4). They revealed the accumulation in neuronal cytoplasm of all four of these proteins, as opposed to the accumulation of all four of these proteins in nuclei in lytic infections (Table 6.2). Perhaps during latency neurons (and satellite cells as well) express a cellular product that impedes viral protein maturation and/or transport. Although it remains possible that levels of VZV latency proteins in the nucleus were below the limit of detection because VZV expression of genes 21 and 29 is dependent upon production of gene 62 protein and its transport back to the nucleus, these findings do suggest that an obstacle to viral protein transport to the nucleus is associated with failure of VZV replication to progress beyond the early phase.

Regardless of the existence of a problem in viral protein transport in latency, the proportion of cells observed by Lungu et al. (1995) to contain VZV proteins poses an additional conundrum. With immunohistochemical techniques, VZV proteins encoded by genes 4, 21, 29, 62, and 63 were detected in 6–21% of all neurons and satellite cells in the human ganglia. As indicated above, and subject to the caveat enunciated, quantitative measures of the VZV latent viral load in human ganglia were commensurate with far lower proportions of infected cells (Mahalingam et al., 1993; Pevenstein et al., 1999).

Maintenance

The finding that VZV undergoes an aborted replication cycle in human ganglia prompts one to ask: why is the replicative cycle aborted, and what keeps it from switching back to a lytic cycle? Clearly, during varicella, the first rounds of VZV replication are completed in neurons, as late viral proteins and intact viral particles are seen in acutely infected ganglia. At some point during these first days of varicella infection, the replicative program deviates from normal, and only a limited repertoire of immediate early and early genes is expressed. Given the purported role of immediate early gene products in the temporal regulation of VZV gene

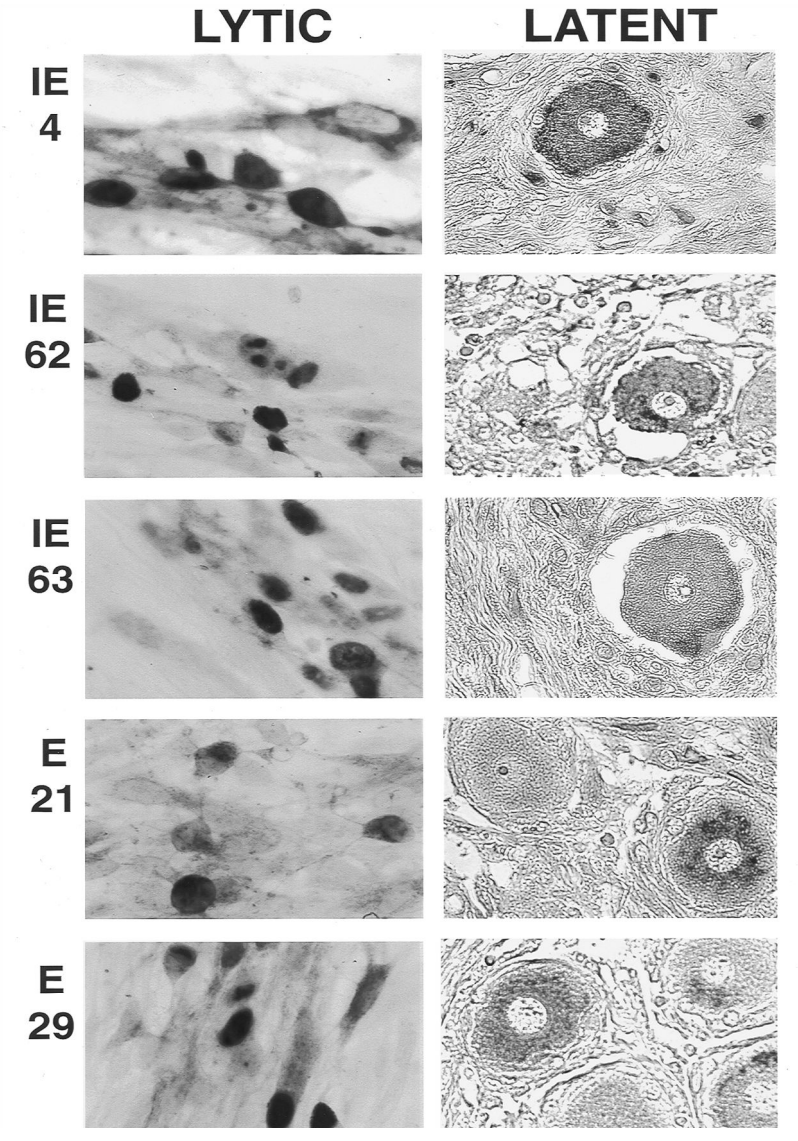


Figure 6.4 Detection of VZV proteins in productively infected cells and in human dorsal root ganglia. Immunohistochemical detection of VZV proteins was performed in fibroblasts (HELF) infected with VZV strain Ellen and in dorsal root ganglia harboring latent virus. The products of ORFs 4, 62, 63, 21, and 29 were detected using purified anti-VZV proteins, rabbit antibodies, and AP-conjugated goat anti-rabbit immunoglobulin secondary antibody. Signal was visualized by developing with AP substrate. The specimens used are identified on the top of the figure, and the individual genes whose products were analyzed are shown on the left. The arrows indicate neurons with positive nuclei. The staining seen near the plasma membrane of some neurons is from coloration of lipofuschin.

expression, the mere production of immediate early transregulatory proteins should be sufficient to drive a full replicative cascade (Ostrove, 1990). It becomes obvious that some viral or host cell factor that participates in the control of the replicative cycle is absent or is withdrawn soon after acute infection. We can only surmise the mechanisms for viral protein transport to the nucleus or the accumulation of nuclear binding factors that activate transcription of all classes of VZV genes are deficient for long periods during which this virus remains dormant. In the latter regard, it is noteworthy that a host cell factor shown to modulate HSV immediate early gene transcription was reported recently to accumulate in the cytoplasm of neurons (Kristie et al., 1999). In response to stimuli that induce reactivation, the host protein translocated to the nucleus. Translocation was independent of protein synthesis, suggesting that it was governed by a signaling event.

Latency, however, involves more than just the silencing of VZV replicative genes. In the grander scheme of things, latency also results from the host's ability to prevent overt infection by removing infectious virus and virus-producing cells before they initiate further rounds of infection. In principle, this defense mechanism must lie with the immune system.

Immune avoidance

Two major host mechanisms rid our bodies of virus-infected cells. Both require immune recognition of viral peptides as foreign antigens (Yewdell & Bennink, 1997). The first entails degradation of viral proteins into peptides and their transport to the cell surface within the binding cleft of major histocompatibility (MHC) proteins. Cells displaying MHC class I proteins bearing foreign peptides are recognized and killed by $CD8^+$ T cells, while cells displaying viral peptides in the context of MHC class II proteins are recognized and killed by $CD4^+$ T cells. Cells that display viral proteins but no MHC proteins are destroyed by natural killer (NK) cells.

Latency is an evolutionary strategy that affords herpesviruses the ability to avoid host immune clearance so that they can persist in humans for life. For HSV, the strategy involves at least four distinct mechanisms of immune avoidance: persistence in neurons, which normally express no MHC proteins; persistence without expressing any immunogenic proteins; HSV-infected cell protein 47 (ICP47)-mediated inhibition of viral peptide expression in the context of MHC class I proteins; and possibly also, the inhibition of infected cell apoptosis (Hill et al., 1995; Galvan et al., 1999). The cumulative effect of these counteroffensive measures is that HSV infections can reactivate hundreds if not thousands of times in the life of the individual without exhausting its neuronal reservoir (Koelle et al., 1992; Schacker et al., 1998).

The above data regarding VZV latency in neuronal and non-neuronal cells – and the expression within them of multiple, immunogenic proteins – represent a true conundrum for which there is no current resolution. VZV persists in non-neuronal cells that do express MHC proteins, so that lymphocyte-mediated defenses can be aroused against them. How is this avoided? There are data to suggest that VZV can suppress MHC protein expression, but we have no knowledge as yet which gene encodes that function and whether it is expressed during latency (Cohen, 1998).

In many regards, the recent discoveries regarding VZV latency challenge the entire concept of viral latency. Once considered a process of genetic inactivity, we now appreciate that latency can be associated with expression of some viral genes, e.g. HSV LATs. Moreover, some herpesvirus latency transcripts can be translated into proteins, as long as cytotoxic T cell-mediated mechanisms are not engaged, e.g. Epstein-Barr virus-encoded nuclear antigen 1 (Levitskaya et al., 1995). By these considerations, we are forced to postulate that neuronal and non-neuronal cells that host VZV latency proteins must not display them, either in the context of MHC or alone. Otherwise, latently infected cells would be cleared by either cytotoxic T cells or NK cells. Ongoing cytolysis of VZV-infected neural cells would not be an acceptable strategy for the virus, as it would expose the host to progressive neuronal loss or demyelination, processes that might restrict opportunities for spread to susceptible humans. More important, these neurologic processes are not subtle, and they would have been appreciated clinically long ago were they to occur with any frequency following varicella.

Reactivation

The molecular mechanisms that permit VZV to persist in neural tissues, without evoking host inflammatory responses against it, cease to operate once productive virus replication is reactivated. The full repertoire of viral genes are expressed, new virions are assembled, and they are transported by anterograde axonal flow to nerve terminals in the dermis. There, progeny virus is released; it spreads to adjacent epithelial cells and evokes the histopathologic reaction manifested clinically as dermatomal zoster.

What unleashes VZV replication is not known. As indicated earlier, abundant data implicate fever, ultraviolet light, local trauma, and hormones as inducing HSV reactivation (Rooney et al., 1992; Halford et al., 1996). In contrast, the vast majority of cases of zoster arise without such inciting events as these. Published clinical anecdotes describe cases in recently X-irradiated dermatomes or at sites of recent surgery, but they are few in number (Guss et al., 1971; Dirbus & Swain, 1990). These considerations reveal that VZV latency is tightly engaged and not casually released.

The very nature of VZV latency appears to dictate this consequence. HSV frequently taunts the host immune system with its hide-and-seek process of latency. The implications of any HSV reactivation event are few for the host: minor mucosal lesions, at worst. Even though HSV recurrences involve small numbers of lesions, it has evolved to ensure its spread by direct contact by reactivating a very large number of times. VZV cannot afford an equally profligate cycle of latency and activation, for each one risks cumulative injury to numerous neurons and their supporting cells. Herpes zoster does afford one glorious conflagration in which vast regions of skin shed new VZV progeny – a rich opportunity for their direct spread to susceptible hosts. Moreover, unlike HSV, VZV need not rely on direct contact to sustain its incidence. It spreads by aerosols during varicella, provoking annual epidemics.

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7

Host response to primary infection

Allison Abendroth and Ann M. Arvin

Introduction

Exposure of a susceptible individual to varicella-zoster virus (VZV) usually results in the clinical manifestations of varicella, in contrast to the other human herpesviruses, which are often acquired asymptomatically. Although symptoms of fever, malaise and vesicular rash occur in most cases, host responses play a critical role in limiting the progression of primary VZV infection. During the interval after mucosal inoculation, through the incubation period, the onset of varicella and the resolution of the acute illness, the virus first evades host clearance mechanisms and is then controlled by the induction of virus-specific immunity. VZV shares the characteristic of infectivity for cells of the host immune system that is common among viral pathogens. Like the human herpesviruses 6 and 7, VZV exhibits a tropism for T lymphocytes which is critical for the pathogenesis of primary infection (Moffat et al., 1995). Following mucosal inoculation, cell-associated viremia is presumed to be required for spread from regional lymph nodes to other sites of early viral replication in the liver, and viremia allows transport of VZV to cutaneous sites of replication during the late incubation period (Grose, 1981; Koropchak et al., 1989; Mainka et al.,1998).

Innate immunity may limit the initial spread of VZV within the host, but these defenses are usually not sufficient to prevent symptoms of illness before VZV-specific adaptive immunity is elicited. Adaptive antiviral immunity consists of the clonal expansion of T lymphocytes and B lymphocytes that have the functional capacity to recognize specific VZV proteins and to interfere with viral replication and transfer of virions from infected to uninfected cells within the host. In the immunocompromised host, life-threatening complications of VZV may result before adaptive immunity is generated. In the otherwise healthy host, this sequence of events favors the virus first, since cutaneous viral replication permits VZV transmission to other susceptible contacts, and then the host, since the acquisition of memory immunity protects against illness upon subsequent exposures to VZV. Although VZV-specific immunity is associated with cessation of active viral

replication, latency is established. This pattern of virus–host interaction has allowed VZV to persist in the evolving human species for an estimated 75 million years.

Innate immune responses to VZV

The initial control of primary VZV infection depends on mechanisms that comprise the innate immune system. The lysis of VZV-infected cells by natural killer (NK) cells and the production of interferon-alpha (IFNα) are prominent among these responses. These early responses are triggered by changes in VZV-infected cells that cause their recognition as "foreign". NK cell function has not been assessed in the course of primary VZV infection but NK effector cells are detected in peripheral blood mononuclear cell (PBMC) populations from healthy, susceptible children. NK cells from non-immune individuals have been shown to lyse VZV infected fibroblasts in vitro (Ihara et al., 1984; Bowden et al., 1985; Ito et al., 1996). Lysis of VZV-infected cells by lymphocytes from susceptible donors was eliminated when NK cells were removed by incubating PBMC with anti-CD16, an NK cell surface marker, in the presence of complement (Ihara et al., 1986, 1989). Nonspecific cytotoxicity can also be enhanced by incubating NK cells with IL-2.

Granulysin is a newly described cytolytic protein which is made by NK cells, as well as by antigen-specific CD8+ T lymphocytes (Pena et al., 1997). Our recent experiments demonstrate that granulysin enhances the death of VZV-infected cells in vitro (Hata et al., 1999). When VZV-infected cells were exposed to granulysin, infectious virus titers were reduced by an order of three logs, with no residual infectivity detected at high concentrations, and cell death was accelerated dramatically. VZV infection of fibroblasts in the absence of granulysin caused down-regulation of the surface expression of the cellular protein, fas, and inhibited susceptibility of infected cells to apoptosis triggered by anti-fas IgM. Granulysin release by NK cells represents an innate immune response that may reverse this virus-induced block of apoptosis, resulting in cell death before large numbers of infectious virions can be synthesized. NK cells have direct cytotoxic effects on VZV-infected targets. In addition, the secretion of granulsyin by NK cells may enhance the early destruction of VZV-infected cells. Both of these NK cell functions act as innate mechanisms for controlling spread of the virus before adaptive immunity is elicited.

PBMC from nonimmune subjects secrete IFNα in response to stimulation with VZV antigen in vitro. IFNα is made by monocytes as well as NK cells and has direct antiviral effects on VZV, inhibiting its replication in vitro. IFNα is made in sufficient quantities to be detected in the serum during acute varicella (Arvin et al., 1986; Wallace et al., 1994). In vivo, administration of exogenous IFNα reduced the severity of varicella in immunocompromised children when it was given by

intramuscular injection within 72 hours after the appearance of the cutaneous lesions (Arvin et al., 1982). Otherwise healthy adults are more susceptible to prolonged, extensive varicella, and have reduced serum IFNα levels compared with children, providing further evidence that IFNα modulates viral replication during primary VZV infection in vivo (Arvin et al., 1986).

Adaptive immunity during primary VZV infection

Early studies of immunocompromised children who were known to be at risk for progressive VZV infection demonstrated that the resolution of primary VZV infection depends upon the induction of antigen-specific, adaptive immune responses to the virus (Gershon & Steinberg, 1979; Patel et al., 1979; Arvin et al., 1986). The unusual syndromes of primary VZV infection that have been observed in children with acquired immunodeficiency disease provide further evidence that acquisiton of adaptive immune responses are required to limit viral replication and establish latency (Gershon et al., 1997). In order to induce adaptive immunity, viral proteins must be processed by dendritic cells or macrophages, which are specialized antigen-presenting cells that mediate the cell surface expression of viral peptides in combination with the class I or class II major histocompatibility complex (MHC) proteins. MHC-restricted antigen presentation creates populations of T lymphocytes within the CD4+ and CD8+ subpopulations that are primed to synthesize cytokines, such as interleukin 2 (IL-2) or interferon gamma (IFNγ), when exposed to the same viral peptide-MHC class I or class II protein complex. Cytokines modulate the inflammatory response, expanding and recruiting antigen-specific, cytotoxic T lymphocytes (CTL) to the site of viral infection. Cytokines made by CD4+ lymphocytes are also required to induce B lymphocytes to produce antibodies of the IgG, IgM and IgA subclasses that can bind to VZV proteins or mediate antibody-dependent cellular cytotoxicity. Effective priming of adaptive T lymphocyte and B lymphocyte responses and the generation of "memory" immunity is associated with protection against symptoms of varicella when the immune host is exposed to the pathogen. The correlation of a higher risk of herpes zoster with waning T lymphocyte recognition of VZV antigen suggests that these responses also restrict the replication of endogenous VZV that persists in neuronal sites of latency, or prevent symptoms when viral reactivation occurs.

Primary cell-mediated immune responses to VZV

Assays of VZV-specific T lymphocyte responses

The assays that are used to measure VZV-specific T lymphocyte-mediated immunity include proliferation, cytokine production and cytotoxicity (Arvin, 1992).

The proliferation assay tests whether the individual has any detectable VZV specific T lymphocytes in circulating PBMC populations. Whole VZV antigen is prepared from extracts of VZV infected tissue culture cells and contains a mixture of VZV gene products, including glycoproteins, regulatory/structural proteins and nonstructural proteins involved in VZV replication (Arvin, 1992). T lymphocyte recognition of viral proteins in this unfractionated VZV antigen is detected by stimulating PBMC for 5–7 days and measuring the incorporation of [^{3}H]-thymidine. The stimulation index (SI) is calculated as the ratio of counts per minute (cpm) between antigen stimulated and uninfected cell control wells. An SI of at least 3.0 is expected for a healthy VZV immune individual. VZV specific T lymphocytes that proliferate under these conditions are predominantly from the CD4+ subpopulation and synthesize Th1 type cytokines, such as IL-2 and IFNγ (Bergen et al., 1991; Zhang et al., 1994). IL-4, which is a characteristic Th2 type cytokine in the murine paradigm of CD4+ helper T lymphocyte responses, is not detected in response to VZV antigen, although IL-10 is produced (Jenkins et al., 1998). The viral protein specificity of CD4+ T lymphocyte recognition is demonstrated by using purified VZV proteins or synthetic peptides to stimulate PBMC in the T lymphocyte proliferation assay (Arvin et al., 1986; Giller et al., 1989; Hayward, 1990; Bergen et al., 1991; Arvin, 1992). Protein-specific responses to VZV glycoproteins and the IE62 tegument/regulatory protein can be demonstrated using reagents made by immunoaffinity purification from VZV infected cells. The quantitation of VZV-specific T lymphocytes can be accomplished by limiting dilution proliferation assay. Recently, a sensitive intracellular cytokine (ICC) assay using flow cytometry has been used to enumerate antigen-specific T lymphocytes, but these methods have not been used to analyze host responses during primary VZV infection (Hayward & Herberger, 1987; Asunama et al., 2000).

Antiviral cytotoxic T lymphocyte responses are demonstrated using effector T lymphocytes generated by secondary in vitro stimulation of PBMC with unfractionated VZV antigen or viral proteins. Cytotoxicity is detected by incubating effector T lymphocytes with target cells, consisting of virus-infected autologous lymphoblastoid cells, which express both MHC class I and class II antigens, or VZV infected MHC matched fibroblasts, which express only MHC class I antigens (Hayward et al., 1986; Hickling et al., 1987; Diaz et al., 1989; Arvin et al., 1991). Antigen specific lysis of target cells is measured by chromium release. The frequency of CTLs and protein specificity of the CTL response to VZV is determined by generating effector T lymphocytes under limiting dilution conditions and infecting targets with vaccinia virus recombinants that express a single VZV protein (Arvin et al., 1991).

Acquisition of VZV-specific cellular immunity

The kinetics with which adaptive immune responses are elicited correlates with the severity of clinical course of varicella (Gershon & Steinberg, 1979; Patel et al., 1979; Arvin et al., 1986; Ihara et al., 1994). Sequential analysis of the induction of antigen-specific CD4+ and CD8+ T lymphocytes demonstrates that the virus usually escapes immune surveillance during the incubation period. In healthy individuals, VZV-specific T lymphocytes begin to be detected 24–72 hours after the onset of varicella. Individuals who have detectable antigen-specific T lymphocyte responses during this period are likely to experience mild primary VZV infection (Asano et al., 1985). The kinetics of the appearance of VZV-specific T lymphocytes within circulating PBMC populations suggests that antigen presentation may require the replication of VZV in skin cells (Arvin, 1998). Delays in acquisition of adaptive immunity are associated with a more prolonged and extensive cutaneous rash, more days of new lesion formation, the persistence of viremia and a higher risk of visceral dissemination. Among immunocompetent individuals tested within 72 hours after the onset of varicella, the mean SI to VZV antigen was 7.5 ± 10.43 standard deviation (SD) in those with <100 lesions/m^2, compared with 1.4 ± 1.85 SD for those with >400 lesions/m^2 ($P<0.05$) (Arvin et al., 1986). In this study, only one (7.7%) of 13 immunocompromised patients had early VZV-specific T-cell proliferation compared with 19 (42%) of 45 healthy subjects ($P<0.05$). These observations are consistent with clinical experiences documenting that varicella is usually more severe in children who have congenital deficiences of cell-mediated immunity or who have been immunosuppressed by treatment for malignancy or transplantation. Among immunodeficient children, an absolute lymphopenia of <500/mm^3 at the onset of varicella is associated with a significant risk of life-threatening varicella (Feldman & Lott, 1987). Without a prompt, effective cell-mediated immune response, the viremia that is characteristic of varicella persists, allowing dissemination of the virus to the lungs and other organs.

Both MHC class I restricted, CD8+ T lymphocytes and MHC class II restricted CD4+ T lymphocytes are sensitized to VZV antigens during primary VZV infection. The early T lymphocyte proliferative response to VZV antigen is mediated by VZV specific CD4+ T lymphocytes and is accompanied by the release of Th1 type cytokines, including IL-2 and IFNγ ((Bergen et al., 1991; Ito et al., 1991; 1992; Zhang et al., 1994). In particular, high levels of IFNγ are made, which enhances the rapid clonal expansion of VZV specific CD4+ and CD8+ T lymphocytes (Arvin et al., 1986; Wallace et al., 1994). PBMC that synthesize IL-12 in response to VZV antigen are detected during the induction of VZV specific immunity (Jenkins et al., 1998). IL-12 is critical for the initial sensitization of T lymphocytes and for further amplification of adaptive immunity to the virus, because IL-12 increases IFNγ production and supports the dominance of Th1-like cytokine responses.

The production of cytokines by VZV-specific CD4+ T lymphocytes that are elicited during primary VZV infection is sufficient to allow their release at concentrations that can be detected in serum. Among adults with primary VZV infection, tumor necrosis factor alpha (TNFα) alpha, IL-2 and IFNγ concentrations did not correlate with the duration or extent of cutaneous lesions, duration of fever, or the occurrence of hepatitis or thrombocytopenia. However, measuring concentrations of these inflammatory mediators in serum may not be a sensitive method to assess their contribution to the control of viral replication at local tissue sites (Wallace et al., 1994). Adults, who are more likely to have prolonged, extensive varicella, are less likely to have detectable serum IFNγ than children, which is consistent with an age-related decrease in the capacity to generate VZV-specific CD4+ T lymphocytes.

T lymphocytes elicited during the initial cell-mediated immune response to VZV infection recognize VZV glycoproteins gC, gE, gH, gI and the IE62 protein (Arvin et al., 1986; Hayward, 1990; Sharp et al., 1992; Weigle & Grose, 1996). The glycoproteins are expected to be important targets of the initial host response because they are virion envelope components and are expressed on the surfaces of VZV-infected cells. In contrast, IE62 is the major tegument protein of VZV virion which is released early after viral entry into target cells and is the primary transactivator of viral gene expression. Since the VZV genome encodes at least 69 distinct gene products, other VZV proteins that have not yet been studied are also likely to be targets of T lymphocytes induced during primary adpative immunity. In our studies, the spectrum of proteins recognized by T lymphocytes during the course of primary VZV infection was variable. Among healthy subjects, 67% had early T lymphocyte proliferation to gE, 71% to gH and 57% to IE62 protein. This variability of the protein specificity of early cellular immunity in individuals with uncomplicated varicella suggests that T lymphocyte recognition of several VZV proteins is equally effective for terminating primary VZV infection (Arvin, 1996).

Antigen-specific cytotoxic T lymphocyte responses to primary VZV infection are likely to be an important component of the primary host response because viral clearance correlates with the induction of T lymphocytes that mediate lysis of virus-infected cells. Although the classic CTL response is mediated by CD8+ T lymphocytes that recognize antigenic peptides in the context of MHC class I molecules, VZV-specific CTL exhibit MHC class II as well as MHC class I restricted killing of infected cells (Hayward et al., 1986; Hickling et al., 1987; Cooper et al., 1988; Diaz et al., 1989; Hayward et al., 1989; Huang et al., 1992; Sharp et al., 1992). When fresh PBMC from five subjects with acute primary VZV infection were tested directly as effector cells, no lysis of VZV-infected autologous target cells was observed (Diaz et al.,1989). However, when T lymphocytes from individuals with acute varicella were expanded by in vitro stimulation with VZV antigen and IL-2, specific killing of

VZV-infected targets was detected. These observations suggest that relatively few CTL are present in PBMC immediately after the onset of the varicella rash. CTL recognition of both IE62 protein and gE was demonstrated by limiting dilution culture of PBMC from individuals who were convalescent from primary VZV infection. The frequencies of CTL that were specific for IE62 protein were 1:69 000–1:173 000 compared with 1:57 000–1:166 000 for gE (Sharp et al., 1992).

Whether the resolution of primary infection and the establishment of latency requires the clonal expansion of T lymphocytes that recognize epitopes from a particular VZV protein or proteins is not known. The VZV proteins that are known to be recognized by CTL include gB, gC, gE and gI as well as regulatory/structural proteins, including IE62, IE63, and the products of ORF4, ORF10 and ORF29. The analysis of the VZV protein targets of CD4+ and CD8+ CTL indicates that individuals of diverse MHC class I and class II phenotypes develop T lymphocytes that can recognize the same major viral proteins, although the specific amino acid sequences that are processed for class I and class II presentation can be expected to be different (Jenkins et al., 1999). The pattern of viral peptide recognition by T lymphocytes that leads to the clearance of VZV viremia is likely to depend upon host genetic factors and may be directed against residues of viral proteins that have not yet been investigated, as well as those known to function as CTL targets.

Humoral immunity during primary VZV infection

Assays of VZV-specific humoral immune responses

Humoral immunity directed against VZV proteins is elicited during primary VZV infection. VZV specific antibodies of the three immunoglobulin classes, IgG, IgM and IgA, are stimulated rapidly and simultaneously during primary VZV infection (Brunell et al., 1975, 1987; Levy et al., 1983; Bogger-Goren et al., 1984; Palumbo et al., 1984; Dubey et al., 1988; Hammerschlag et al., 1989; Gershon et al., 1991; Takahashi et al., 1992). Sensitive techniques that are useful for measuring antibodies to VZV include the fluorescent antibody-membrane antigen assay (FAMA), enzyme linked immunosorbent assay (ELISA), glycoprotein ELISA (gpELISA), and latex agglutination assays (Gershon et al., 1991). Antibody recognition of specific viral proteins is demonstrated by immunoprecipitation or immunoblot or by using purified proteins in ELISA assays (Grose & Friedrichs, 1982; Keller et al., 1984; Palumbo et al., 1984; Grose & Litwin, 1988; Bergen et al., 1991).

IgG, IgM and IgA antibodies that bind to VZV proteins can mediate viral neutralization either directly, or in the presence of complement (Schmidt & Lennette, 1975; Zweernik & Neff, 1981). VZV antibodies also have the capacity to mediate the lysis of infected cells by antibody-dependent cell mediated cytotoxicity (ADCC) (Ihara et al., 1984).

Acquisition of VZV-specific humoral immunity

In most healthy individuals, VZV specific antibodies begin to be detected in the serum from 1 to 3 days after the appearance of skin lesions. Antibodies induced during primary VZV infection recognize viral proteins that are made at early stages of VZV replication in the infected cell, such as nucleocapsid proteins, as well as the late glycoproteins (Schmidt & Gallo, 1984). Neutralizing antibodies in children with acute varicella are found predominantly in the IgM fraction, but IgG antibodies with neutralizing activity are also produced and persist after resolution of primary VZV infection. IgA antibodies to VZV appear in nasopharyneal secretions during the first week in parallel with their detection in serum and increase until three to four weeks after the onset of symptoms. By immunoblot analysis, IgM antibodies specific for an average of seven polypeptides were detected in sera obtained from children within the first week after the onset of varicella, while early IgG and IgA antibodies were detected against an average of only three VZV infected cell proteins (Palumbo et al., 1984). IgG antibodies to 14–18 prominent VZV polypeptides can be detected by early convalescence using immunoprecipitation (Weigle & Grose, 1996). Among healthy individuals with varicella, the specificity of IgG and IgM antibodies for binding to particular VZV proteins was variable in convalescent sera. Varicella was uncomplicated in all cases, suggesting that the production of antibody to all major viral proteins is not essential for recovery.

Distinguishing the contributions of humoral and cellular immunity to the control of primary VZV infection is difficult because both types of adaptive responses are induced early in the clinical course of varicella. Nevertheless, the evidence suggests that VZV-specific antibody responses are less important than cell-mediated immunity for restricting VZV replication and spread during acute infection. Firstly, children with agammaglobulinemia were described as having uncomplicated varicella before passive antibody preparations were available to compensate for their deficiencies of humoral immunity whereas those with deficiencies of cellular immunity were recognized as being at high risk for progressive varicella (Arvin, 1987). Secondly, the presence of high titer IgG and IgM antibodies within the first 72 hours after the appearance of the cutaneous rash did not correlate with a milder clinical course of varicella (Arvin et al., 1986). Thirdly, transfer of high titer anti-VZV antibodies to children with immunodeficiences after the appearance of cutaneous varicella lesions does not influence VZV replication (Camitta et al., 1994). Nonetheless, there is some evidence that the failure to produce antibodies to specific VZV proteins may influence the pathogenesis of primary VZV infection, since some immunocompromised children with progressive varicella infection lacked IgG antibodies to major VZV proteins (Weigle & Grose, 1996). Since antigen-specific CD4+ T lymphocytes are required for the induction of B lymphocyte responses to VZV, any limitations of adaptive

cell-mediated immunity can be anticipated to restrict humoral immune responses to the virus during primary infection.

Although active antibody responses may have limited impact on the outcome of primary VZV infection, passively acquired VZV-specific antibodies that are present at the time of exposure can interfere with the initial stages of VZV replication in vivo, reducing the capacity of the virus to infect the susceptible host (Zaia et al., 1983; Miller et al., 1989). VZV IgG antibodies acquired transplacentally can protect infants from developing primary VZV infection or modify the severity of varicella during the first six months of life. Even if infection is not prevented, the administration of VZV immune globulin to immunocompromised children or newborns within 72 hours after exposure reduces the severity of varicella. Passive antibodies are presumed to block VZV infection at the initial site of inoculation by mechanisms of viral neutralization, mediated particularly by antibodies that bind to the viral envelope glycoproteins (Arvin, 1995). The observation that disease severity may be reduced suggests that there are further effects of VZV-specific antibodies on viral spread after inoculation. The mechanisms by which passive antibodies may block the progression of VZV infection in the susceptible host are not known. VZV-infected T lymphocytes release intact, enveloped virions which may be accessible for direct neutralization (Moffat et al., 1995). At cutaneous sites, VZV infection of epithelial and dermal cells progresses by cell fusion and spread of the virus to adjacent, uninfected cells. Under these circumstances, antibodies to the VZV glycoprotein gH may restrict replication, based upon the observation that a monoclonal antibody to gH restricts cell-to-cell spread of the virus in vitro (Rodriguez et al., 1993).

Mechanisms of VZV immune evasion

Identifying mechanisms by which viral pathogens interfere with components of the host response has become an important issue for understanding virus–host interactions during primary infection. The capacity for direct infection of cells of the immune system is one mechanism of interference with antiviral immunity which VZV shares with other viruses. Lymphopenia is common among healthy children who are evaluated immediately after the onset of the varicella rash and one of the important risk factors for progressive primary VZV infection in immunocompromised children is an absolute lymphocyte count of fewer than 500 cells (Feldman & Lott, 1987). VZV tropism for T lymphocytes is not likely to be a major direct cause of these effects because the frequency of infected cells is only about 1:50 000–1:100 000 PBMC (Koropchak et al., 1989). However, VZV encodes viral gene products that mediate the downregulation of MHC class I expression on VZV-infected T lymphocytes, an effect which may facilitate the transport of VZV to cuta-

neous sites of replication during the incubation period, without evoking adaptive immune responses (Abendroth et al., 1999). MHC class I molecules are also decreased on fibroblasts infected with VZV (Cohen, 1998; Abendroth et al., 1999). VZV acts to retain MHC class I molecules in the Golgi compartment of infected cells. Since MHC class I molecules present peptides to CD8+ T lymphocytes, interference with their transport to the cell surface makes the infected cells' expression invisible to this component of the antiviral immune response.

In addition to interference with MHC class I expression, VZV-infected cells resist the upregulation of MHC class II expression that is triggered by IFNγ (Abendroth et al., 2000). MHC class II molecules present peptides to elicit CD4+ T lymphocytes and to support their clonal expansion. Although the constitutive expression of MHC class II proteins is restricted primarily to B cells, monocytes and thymic epithelium, IFNγ can induce MHC class II expression on many cell types, including fibroblasts. VZV acts to block IFNγ-induced upregulation of MHC class II expression at the level of host cell gene transcription. Examination of skin biopsies taken early in the course of varicella or herpes zoster lesion formation demonstrate the inhibition of MHC class II expression on VZV-infected cells in vivo, suggesting that this effect may transiently protect VZV-infected cells from immune surveillance by CD4+ T lymphocytes.

Although VZV inhibits the IFNγ -mediated induction of MHC class II cell-surface expression, the virus does not downregulate MHC class II expression on cells that are exposed to IFNγ before infection (Abendroth et al., 2000). Thus, after the induction of VZV specific CD4+ T lymphocytes that produce IFNγ, their trafficking to the site of VZV replication should control the spread of the virus. Even though VZV spreads to adjacent uninfected cells, local release of IFNγ should act to make these secondarily infected cells "visible" to immune surveillance.

Summary

Knowledge about the host response to primary VZV infection has improved in parallel with advances in understanding antiviral immunity and with the progress made toward understanding the molecular biology of VZV. From the perspective of achieving viral persistence in the human population over time, the immunomodulatory effects of VZV function optimally if they are transient and limited during primary infection, so that the host recovers and latency, with the potential for later reactivation, is established. Although the virus has mechanims for immune evasion, the innate and adaptive immune responses act to terminate VZV replication within a few days after the appearance of the cutaneous lesions in the otherwise healthy host.

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8

Host response during latency and reactivation

Anthony R. Hayward

Studies of immunity to varicella-zoster virus (VZV) after primary infections are predicated largely on the view that cell-mediated immune responses contribute to the prevention of reactivation from latency. The evidence supporting this comes from the increased frequency of zoster following immunosuppressive treatments, particularly total lymphoid irradiation and cancer chemotherapy. The principal difficulty in examining immunity to VZV is the lack of an animal model supporting primary disease and, later, emergence of the virus from latency. Animal models of cell-mediated immunity to other viruses and to HSV and CMV in particular, have recently advanced enormously. These animal data are summarized first in the following paragraphs, so as to provide a background against which the more limited human studies of VZV-specific immunity can be placed.

Immunity to viruses

Once a primary infection has been controlled, the importance of antibody for neutralizing viruses before they infect susceptible cells remains unchallenged. The memory cells, which maintain immunity to viruses including VZV, are selected from lymphocytes that participated first in the primary immune response – so the course and kinetics of primary immunity are key to understanding the host response during latency. The past few years have seen advances in our understanding of the interactions between viruses and the cells they infect and of the interactions between antigen-presenting cells and the CD4 and CD8 T cells that respond.

Lytic viruses such as VZV destroy cells whose debris is phagocytosed mostly by neutrophils, macrophages and dendritic cells. When the phagocytic vacuoles and lysosomes fuse the lysosomal proteases activate and cleave off an invariant chain from class II major histocompatibility (MHC) molecules, preparing them for antigen binding. One of these proteases, an asparaginyl endopeptidase, initiates the digestion of the virus-encoded proteins and polypeptides that ends with the production of peptides capable of binding to the antigen-presenting groove in the

MHC molecule (Manoury et al., 1998). MHC molecules are membrane anchored so when the digested contents of the phago-lysosome are discharged outside the phagocyte the peptides that have complexed with MHC are retained and displayed on the cell surface. These peptide–MHC complexes are accessible for binding by the antigen receptors of complementary specificity on the T cell's surface. Dendritic cells, with processed antigen on their surface, commonly detach from the area of antigen uptake and travel to the local lymph node where binding to the antigen-specific T cells takes place. It is CD4 T cells that recognize and respond to peptides held in class II MHC molecules while peptides complexed with class I MHC molecules are bound by the antigen receptors of CD8 T cells.

The role of CD4 cells in "helping" responses by CD8 cells is becoming clearer. Antigen presented in class I MHC on dendritic cells to CD8 cells may fail to stimulate a response unless the dendritic cell can provide an additional "help" signal. This help takes the form of B-7 on the antigen-presenting cell binding to CD28 on the T cell (Rock et al., 1999). The availability of B-7 on the antigen presenting cell is regulated through multiple pathways and it is increased if the cell encounters microbial products such as lipopolysaccharide. Another important source of help for the production of CD8 cytotoxic T lymphocytes (CTL) comes from dendritic cells that present antigen. The ability of these dendritic cells to stimulate long-lived CD8 CTL is increased if the CD40 on the dendritic cell surface is first bound by CD154 on the surface of an activated CD4 T cell (Ridge et al., 1998).

Synthetic antigens in which peptides complexed with soluble class MHC molecules are polymerized with a fluorescent label have very recently made it possible to follow the kinetics of primary and secondary immune responses. This approach is giving new insights into the process of T cell priming and memory cell production. Fewer than 1 in 100 000 CD4 cells of a nonimmune mouse will bind to a peptide complex with class II MHC and this number rises to greater than 1:10 000 in the responding lymph node in a period of 10 days following immunization (Crawford et al., 1998). The frequency of antigen-binding T cells in the blood remains low, at least during a primary immune response, perhaps because those that leave the lymph node localize to the area of infection. Even after an infection the number of responder T cells in the blood does not rise more than 2–10 fold, so it seems likely that many of the effector cells that are produced during an immune response fail to survive. The mechanism by which those that do not survive are removed appears to be programmed cell death. The factors determining which cells survive as memory cells and which die are not yet fully defined: in mice, signaling through CD28 (Liu et al., 1997) and cytokines contribute to the decision (Vella et al., 1998). In humans the half-life of CD4 and CD8 T cells has been estimated to be 75–85 days (Hellerstein et al., 1999) though no distinction between memory and naïve T cells was made. The thymus of healthy individuals up to the age of at least

70 years has been shown to export new populations of naïve cells (Poulin et al., 1999) and these could presumably be selected by antigen.

CD8 responses are paramount in immunity to respiratory viruses (influenza and respiratory syncytial virus) but, as discussed below, they may be much less important for VZV-specific immunity. The peptide–class I MHC complex recognized by CD8 T cells is mostly assembled in the endoplasmic reticulum of infected cells and has to be transported to the cell surface through the Golgi apparatus. Studies with synthetic MHC–peptide tetramers show that about two lymph node cells per million from a nonimmune mouse have specificity for an influenza epitope and that influenza infection stimulates these cells to divide very rapidly – up to three times daily. Influenza viruses are eliminated in 3–5 days and the frequency of influenza-specific CD8 cells can reach 50% of the cells in the responding lymph node (Flynn et al., 1998). With lymphocytic chorio-meningitis virus the frequency of responding CD8 cells reaches 80% in the spleen (Butz & Bevan, 1998; Murali-Krishna et al., 1998). By 2 months after infection the frequency of influenza epitope specific cells falls to about 1:3000 in spleen and it is these cells that confer long-term specific memory. Comparable data from humans are becoming available. Ogg et al. (1998), for example, sorted 0.04% of $CD8^+$ T cells from an HLA A*0201 subject with specificity for an influenza A matrix peptide GILGFVFTL (residues 58 to 66) from blood. All these cells responded to antigen specific stimulation with γ-IFN production.

Direct studies of antigen binding require the synthesis of a peptide–MHC complex – but this has been achieved for only a limited number of antigens and MHC molecules. Another approach to quantitating T cell responses uses a brief period of stimulation with antigen in tissue culture followed by measurement of the number of CD4 or CD8 cells with intracellular cytokine. At present it appears that the number of responder cells detected in this assay (which is technically fairly simple) is close to that detected by the antigen binding assays.

Immunity and reactivation model herpesviruses in animals

Because there is no animal model for VZV latency in dorsal root ganglia immunologic studies of response during latency have to be modeled on events that follow herpes simplex virus (HSV) infection. HSV infects mice readily enough (Yu et al., 1996) and stimulates antibody production and specific T cell responses. Of the various patterns of infection (for example, corneal, encephalitic, pulmonary) the zosteriform is the closest model to events following VZV infection. Zosteriform HSV (Simmonds & Nash, 1984) occurs in mice following intracutaneous infection with virus. There is local replication at the site of infection followed by spread of the virus through sensory nerves to the dorsal root ganglia, where further replication

occurs. The virus then disseminates through sensory nerves back to the skin in a dermatomal distribution – justifying the term "zosteriform'. Immunity to zosteriform HSV is transferred to naïve recipients by adoptive transfer of CD4 T cells but not CD8 T cells. Establishing the mechanism of action of these CD4 cells might be very helpful in understanding responses to VZV-infected neurons. Production of γ-IFN by the CD4 cells does not seem to be critical because cells with disrupted genes for this cytokine are still able to mediate resistance (Yu et al., 1996). Perhaps cell associated T-cell molecules are important: the CD154 which is transiently expressed on activated T cells might, for example, bind to CD40 positive cells of the mononuclear phagocyte series and stimulate them to make cytokines.

Three days after acute HSV infection some of the cells surrounding infected neurons in the trigeminal ganglion make TNFα and/or IL-6. The TNF-making cells include satellite and Schwann cells and small numbers of γ-IFN-making cells also appear. Lymphocytes making IL-2 and/or IL-4 appear later, when viral antigens have almost cleared (Shimeld et al., 1997). TNFα was the predominant cytokine in HSV-infected ganglia and the dorsal root throughout acute and latent infection and even by day 30, numbers of satellite cells expressing this cytokine were three times higher than those in normal ganglia. The actions of TNFα include stimulating antigen presentation by increasing the number of MHC class II molecules on the cell surface and by increasing IL12 production (Reisner et al., 1997). The mechanism of anti-HSV action of the CD4 cells probably depends on the target of response. CD4 T cells can kill cells with which they have contact, using perforin. They also release TNFβ which in turn triggers apoptosis in target cells, and fragments their DNA (Yasukawa et al., 1996). Other effector mechanisms such as FasÑFas ligand interactions or CD154 binding to CD40 are also likely to trigger apoptosis in susceptible cells.

The requirement for CD4 cells for protection from zosteriform HSV is perhaps the best clue we have to the type of cell-mediated immunity that may be important during latency. That it is a CD4 rather than a CD8 cell that is required may stem from the ability of HSV 1 & 2 gene products to interfere with the class I pathway for antigen presentation to CD8 cells. The mechanism by which HSV blocks class I antigen presentation is at the level of transport of HLA class I–peptide dimers (which are synthesized in normal amounts) from the ER to the Golgi (Hill et al., 1994) and results from interference with specific transporters (Hill et al., 1995). Other herpesviruses have alternative mechanisms for minimizing class I associated antigen presentation. These include the US2 and US11 gene products of CMV which dislocate newly synthesized class I molecules from the endoplasmic reticulum, resulting in their catabolism. The EBV genome has Gly-Ala repeats that generate a signal interfering with antigen processing (Levitskaya et al., 1995). Interference with class I antigen presentation would be expected to reduce the

immune stimulus for a CD8 T cell response to infection. In turn, a reduced CD8 response would make it more difficult to detect a class I restricted HSV-specific cytotoxicity in immune subjects. Whether reducing a class I restricted response gives HSV or other herpesviruses a significant survival advantage is not known. Cytotoxicity, an effector mechanism traditionally linked with CD8 cell responses, is also a property of CD4 cells using both direct (class II restricted) and Fas-ligand mediated killing and cytokine production. There are no data on the relative importance of different types of cytotoxicity in the CNS, nor even on the relative importance of specific killing versus cytokine-mediated responses.

Suppression of apoptosis is an additional area in which HSV research has advanced further than our knowledge of VZV. This is important in the context of immunity during latency because any mechanism that prevents infected cells from disintegrating is likely to reduce the amount of stimulus to antigen-presenting cells. This reduction in turn is likely to lessen the amount of viral antigen that is presented to lymphocytes. The product of the HSV g134.5 gene can prevent the activation of an RNA-dependent kinase, PKR, which is essential for progression through apoptosis (Kieff & Shenk, 1998). Although VZV is reported to promote apoptosis in semi-permissive Vero cells, these are not the cells in which the virus normally remains latent. It remains to be seen what effect VZV transcripts made in neurons during latency have on neuronal susceptibility to apoptosis. The finding that VZV has far fewer latency associated transcripts than HSV may mean that it is less able to suppress apoptosis. VZV is also likely to differ from HSV in the amount and/or diversity of antigenic material that might be synthesized during latency (Wagner & Bloom, 1997).

VZV antigens

The large genome of VZV encodes many polypeptides, each of which may have many regions recognizable by lymphocytes (epitopes). The three-dimensional structure of intact molecules is recognized by B lymphocytes and is a necessary prelude to antibody production. The ability of immunity to an individual epitope to confer protection from infection depends, in the context of antibody, on whether the epitope in question has an important function. Antibodies to gI, for example, can neutralize the infectivity of VZV for cell lines in tissue culture. In general the requirements for immune recognition by T lymphocytes are that the polypeptide can be cleaved into a fragment of 8–16 amino acids which (a) binds to a class I or class II MHC molecule and (b) does not resemble a self peptide to which tolerance has been established. Subjects who have recovered from a primary VZV infection have T cells which recognize gE, gB, gH, the IE62 protein and IE63 (Sadzot-Delvaux et al 1998, Arvin et al., 1986). At least some IE62 peptides are

sufficiently immunogenic to elicit primary responses in vitro (Jenkins et al., 1999). Individuals vary in the rate at which responses to individual VZV proteins develop. In vitro stimulation of blood lymphocytes by synthetic peptides allow responses to putative T cell epitopes to be identified but it is worth remembering that the actual peptide–MHC complex being recognized will differ between individuals of different HLA types. It is the heterogeneity of class II MHC antigens in man that has generally discouraged searches for a hypothetical dominant epitopes for complex viruses.

Class I MHC antigens are a little easier to study than class II antigens because some, such as HLA A2, occur with quite high frequencies in Caucasian populations. It should therefore be possible to quantitate CD8 responses by the same approaches used with influenza. Studies to date show that VZV polypeptides from IE62, gE, gI and gC can be recognized by CD8 cells in tests of cytotoxicity (Arvin, 1992).

Herpesviruses use "assembly proteins" as the scaffolding on which viral capsids are assembled in an infected cell. One such protein has now been cloned from the ORF33 of the VZV genome and shown to stimulate antibody production and a T cell response in laboratory animals (Garcia-Valcarcel et al., 1997). How much of the human T cell response is directed to relatively late-produced proteins like assemblins remains to be determined.

VZV latency and maintenance of immunity

VZV antigens stimulate CD4 blood lymphocytes from immune individuals to proliferate in culture. Studies in which the number of lymphocytes in each culture is progressively reduced in "limiting dilution" format suggest that about 1:20 000 blood lymphocytes is a "responder" (Figure 8.1). This is fewer lymphocytes than respond to pathogens causing chronic infections, such as mycobacteria (Van Oers et al., 1978) and is in the same range as respond to tetanus toxoid after a booster immunization. The fact that the frequency is not lower suggests that at least some stimulation of VZV-specific T cells continues after recovery from acute infection. Most of the responder cells isolated from blood also make IFNγ (Zhang et al., 1994) and a small number make IL-4. Immunization stimulates at least transient production of IL-10 and IL-12 by blood mononuclear cells (Jenkins et al., 1998). Our own results point to a gradual decline in the number of VZV-responder cells with aging (see Chapter 25 in this volume) and, curiously, the frequency of responders in limiting dilution cultures is lower under about 12 years of age. Extensive studies by Arvin and co-workers document the persistence of VZV-specific CD4 and CD8 T cells for 20 years following a primary infection. The frequency ranges from 1:15 000 to $<$1:200 000 for IE62-specific CD8 cells and responses in these orders of magnitude have been observed for CD4 and CD8 cells with specificity for gE, gI and gC

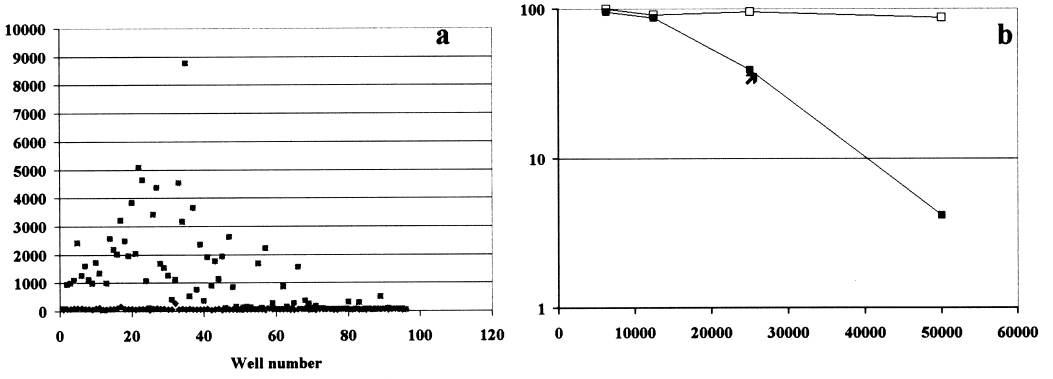

Figure 8.1 Estimation of responder-cell frequency by limiting dilution culture. (a) Shows thymidine uptake on the *x*-axis as counts per minute for wells 1–24, which contain 50 000 cells/well. Wells 25–48 contain 25 000 cells; wells 49–72 contain 12 500 cells and wells 73–96 contain 6520 cells. The fraction of wells positive for thymidine uptake falls as the cell number per well falls. In (b) the percentage of wells negative for thymidine uptake is plotted on the *x*- axis against the number of cells per well for a VZV immune (-□-) and nonimmune (-■-) individual. The responder cell frequency is derived by interpolating at the 37% nonresponder frequency (arrowed).

(Arvin, 1996). There are, nevertheless, many uncertainties in measurements of cell mediated immunity to VZV during latency. Limiting dilution cultures, for example, only measure those cells that can respond to stimulation by sustained proliferation and there may well be a class of memory effector cells without this potential for clonal expansion.

Recent studies in immunized mice suggest that the frequency of T cells stained by peptide-MHC tetramers approximates to the frequency identified by virtue of intracellular cytokine synthesis. Studies directed primarily to enumerating cells that respond to VZV antigens by IFNγ production are not yet available but it has been used as a control for the response to CMV (Waldrop et al., 1998). The frequency of blood CD4 lymphocytes making IFNγ in response to VZV is in the 0.2–2% range we observed (Figure 8.2). If this estimate for VZV-responsive cells is correct and VZV is a representative herpesvirus, then perhaps as many as 10% of blood lymphocytes have specificity for a herpesvirus. This seems surprisingly high in relation to the numbers of pathogens that can cause disease in man. Perhaps a high frequency of responses to herpesviruses is a consequence of their capacity for persistence, and it is antigens produced during latency that stimulate memory T cells to remain at a high level. The possibility that latency is responsible for a high frequency of VZV-specific T cells is compatible with results in mice. For example, the frequency of CD4 cells that make IFNγ in response to the mouse gammaherpesvirus 68 (which remains latent in B cells) remains at about 1:1000 in the spleen

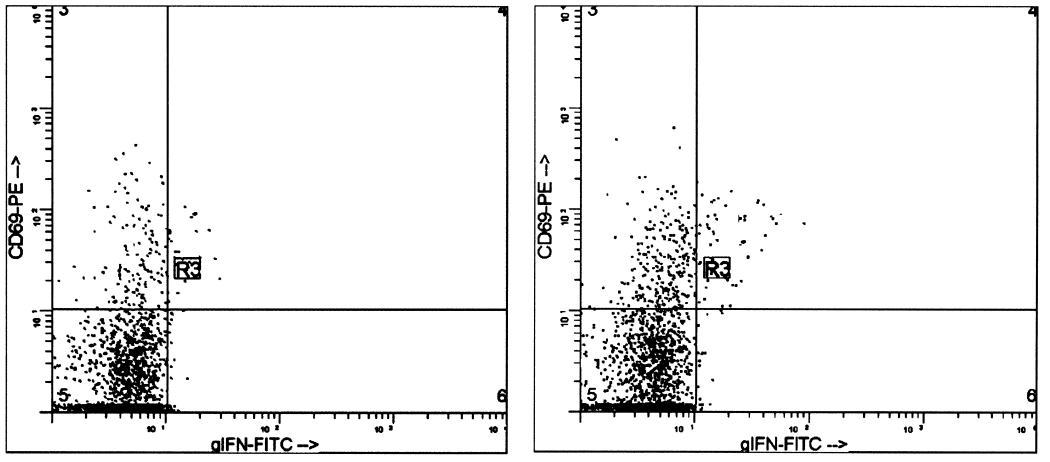

Figure 8.2 Blood lymphocytes from a VZV-immune donor were cultured overnight with control antigen (left) or VZV antigen (right) and then stained for CD69 (*x*-axis) and for γIFN (*y*-axis). The cytofluorograph profiles show more cells in the upper right quadrant (staining for CD69 and γIFN) for the VZV-stimulated than the control cultures.

or mediastinal lymph node for 100 days after infection (Stevenson & Doherty., 1998).

Cellular source of VZV antigens during latency

The neuronal nuclei of sensory ganglia are an important site for VZV latency (Gilden et al., 1987; Kennedy et al., 1998) and there is also evidence for VZV gene expression during latency in the satellite cells that surround neurons (Croen et al., 1988). The presence of at least some VZV encoded proteins in the cytoplasm of neurons during latency (Lungu et al., 1998) makes them potential contributors to persisting T cell responses. The identity of the cell(s) in which VZV remains latent is important because it will determine what co-stimulatory molecules are available for T cell stimulation. Resting hippocampal neurons do not express MHC molecules on the cell surface because they do not transcribe β_2-microglobulin or make the TAP proteins required for antigen transport (Neumann et al., 1997). Sustained exposure to γ-IFN induces class I expression on neurons, which is yet further increased if their function is impaired, but there is no class II expression. The recognition by CD4 cells of any VZV antigens associated with neurons would therefore require processing through antigen-presenting cells. Satellite cells, which surround the neuronal bodies in ganglia, express both class I and class II MHC antigens, and may be able to present antigen directly to T cells. Presentation alone, though, is unlikely to suffice to stimulate resting CD4 cells to proliferate because additional molecules such as B7 and CD40 are required. The antigens available for

presentation are limited: the VZV genome remains in circular form during latency and only genes 4, 21, 29, 62, and 63 are transcribed. Of these, the immediate-early genes 62 and 63 encode regulators of virus gene transcription, and VZV gene 29 encodes a major DNA-binding protein (Cohrs et al., 1998).

VZV gene effects on MHC expression

Whether class I MHC antigens are actually an important route for presentation of VZV antigens to T cells is uncertain. Cohen (1998) has recently reported that VZV, like HSV, interferes with class I associated antigen presentation on the surface of infected cells. The mechanism may well involve interference with the export of class I complexes with antigen to the cell surface, as radiolabelling did not suggest that the overall amount of class I synthesis was immediately reduced by VZV infection. Abendroth et al. have found that class I molecules are retained in the Golgi of infected cells (Chapter 7). It seems unlikely that VZV interference with class I MHC trafficking is sufficient to abolish all the priming or expansion of VZV-specific CD8+ T cells because at least some CD8 responses are detectable in healthy subjects following infection or vaccination (Hayward et al., 1996). Perhaps class I restricted responses are only impaired by VZV effects on antigen presentation, and would be much greater if the pathway to class I presentation were fully functional.

VZV immunity boosting during zoster

In principle one might learn which VZV antigens were expressed as the virus emerges from latency by studying responses to specific proteins during herpes zoster. The sporadic occurrence and low incidence of zoster make a prospective study of T cell responses in subjects with ostensibly normal immune responses almost impossible. Prospective studies might, on the other hand, be possible in HIV+ children, whose rate of zoster can reach 70% (Gershon et al.,1997; Derryck et al., 1998). Retrospective studies of subjects in the years following zoster show a boost in antibody response and of T cell proliferative response (Hayward et al., 1991). Second attacks of zoster are rare in subjects who are not immunosuppressed – suggesting that the immune stimulation that follows an attack is sufficient to maintain latency. Immunity can also be boosted during latency by immunization, as described in Chapter 25.

Conclusions

It seems likely that VZV immunity during latency is based on the repertoire that is established during primary infection and is modulated or expanded by antigens

that reach antigen-presenting cells during latency. Virus-specific factors that might contribute to successful latency would include evasion of an immune response that had the potential to eliminate the virus. Strategies known to be used by other herpesviruses include a substantial inhibition of the viral genes that are transcribed during latency and, in the case of HSV, an ability to prevent viral peptides from entering the class I antigen processing pathway. The neuronal–satellite cell complex in which VZV is normally latent, and from which reactivation occurs, has the potential to be immunologically silent while shielded from IFNγ. Major advances remain to be made in our understanding of antigen presentation pathways in posterior root ganglia and the mechanisms by which immune responses might contribute to the maintenance of latency.

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9

Animal models of Infection

Catherine Sadzot-Delvaux and Bernard Rentier

Immunopathological studies of varicella-zoster virus (VZV) have long been hampered by the lack of a suitable experimental model that could mimic the clinical events observed during human infection. The virus is highly species-specific and does not replicate efficiently in nonhuman cells. In addition, VZV produced in vitro, even in human cells remains cell-associated and it is difficult to obtain the high titers of virus required to inoculate animals. Although many efforts have been made to infect laboratory animals belonging to various species, including non-human primates, human disease has never been reproduced in all of its manifestations. As a consequence, most investigations on the biology and the pathophysiology of the VZV, especially aspects concerning latency, had to be deduced from studies on herpes simplex virus (HSV), another human herpesvirus that shares with VZV the capacity to remain persistent in the nervous system and to reactivate. However, it is now obvious that the molecular mechanisms involved in VZV neurotropism and latency are different from those of the other alphaherpesviruses.

Although none of the animal models described so far is satisfactory to evaluate all aspects of VZV pathophysiology in humans, many of them are useful to investigate acute infection, the immune response or latency. Different models help to answer different questions.

Models of acute infection and the immune response

As early as 1926, although the varicella-zoster virus had not yet been isolated, Rivers reported a limited VZV infection after inoculation of emulsified vesicles and papules collected from a human chickenpox lesion into testes of green monkeys (Rivers 1926, 1927). Other nonhuman primates, such as patas monkeys (Felsenfeld & Schmidt, 1970), Macaque monkeys (Blakely et al., 1973), and pygmy marmosets (Asano et al., 1983) were infected with VZV, but even if a seroconversion was induced, none of these animals developed disease and no virus was recovered. More recently, gorillas were shown to become naturally infected with VZV and to have

clinical signs of varicella that appear similar to human infection (Myers et al., 1987). Viral replication in the lung of young adult common marmosets inoculated with wild-type or vaccine VZV strains was suggested by both the presence of viral antigens and signs of pneumonia. The virus was isolated from infected lung, was cultured in vitro and was transmissible in vivo (Provost et al., 1987). Finally, two chimpanzees were inoculated subcutaneously on the breast with wild-type Oka VZV that had not been extensively passaged in culture (Cohen et al., 1996). Viral DNA was detected in peripheral mononuclear cells (PBMC) 1, 6, or 11 days after inoculation. Both animals developed leucocytosis and fever, and 10 days after inoculation, a bilateral rash was observed. However, the rash lasted only 24 to 48 hours and was restricted to the area involving dermatomes T4 and T5, located near the inoculation site. The lesions were erythematous and papular but not vesicular or pustular and resembled the mild eruption, usually located at the site of inoculation, observed in 4% of vaccinated children. There was no evidence of viral genome in DRG to indicate that the virus established latency in chimpanzees.

While VZV appears to infect nonhuman primates, which have some merit as models of varicella, limitations on their use in laboratory experimentation make it difficult or even impossible to use these animals for extensive analysis. These limitations have forced the researchers to focus their efforts on smaller animal models. Since its first description in 1980 by Yamanishi, the guinea pig model of VZV infection has been used to investigate pathogenesis and the immune response. Guinea pigs were first inoculated by subcutaneous injections of either the Oka vaccine strain or two wild-type (wt) strains. Surprisingly, only the Oka vaccine strain, previously attenuated by repeated passages in guinea pig cells, elicited the production of neutralizing and complement-fixing anti-VZV antibodies, while little or no immune response was observed using two wt strains (Yamanishi et al., 1980). The efficiency of this model was improved by using virus previously adapted to guinea pig cells (Matsunaga et al., 1982; Myers et al., 1980, 1985; Walz-Cicconi et al., 1986). Inoculation of a guinea pig-adapted virus to strain 2 or Hartley guinea pigs was followed by a viremia as shown by animal-to-animal transmission or by explantation of various organs including DRGs, spinal cord and cerebral cortex, and recovery of the virus. This viremia was detectable about 3 weeks after inoculation. After this interval, the virus could no longer be recovered by explantation. However, infection in guinea pigs was minimally symptomatic, probably because of the basal body temperature (39.3 °C), which corresponds approximately to the shut-off temperature for this VZV strain (39 °C). Repeated depilation of newborn guinea pigs or use of euthymic hairless guinea pigs, which are immunologically intact but congenitally hairless, causes animals to have a body temperature about 1 °C below normal. Under these conditions, 88% of the animals inoculated with guinea pig adapted VZV were infected and developed a papular exanthem. The rash did not resemble

the vesicular rash usually seen in varicella but was like the lesions sometimes seen among vaccinated patients (Myers et al., 1990). In addition, using highly sensitive techniques such as in situ hybridization or polymerase chain reaction (PCR), viral genome was detected in PBMCs soon after intramuscular or subcutaneous inoculation (Myers et al. 1990, Arvin et al. 1987) and in DRGs after intraocular inoculation (Tenser & Hyman, 1987). The VZV genome was detected in trigeminal ganglia and in thoracic root ganglia of 63% and 75% of infected guinea pigs respectively, 20 to 80 days after subcutaneous inoculation (Lowry et al., 1992a).

The combined use of guinea pigs and of guinea pig-adapted virus constitutes a good model where the virus replicates, infects PBMCs (a critical step for viral dissemination), reaches the skin and causes lesions if the body temperature is kept lower than the viral replication shut-off temperature. The virus even reaches the DRGs, where it remains detectable for up to 80 days. Unfortunately, neurotropism and latency have not been investigated further in this model.

The guinea pig model has also been used to evaluate humoral or cellular immune responses to VZV. Infected animals produce neutralizing or complement-fixing antibodies to viral proteins and develop a delayed-type hypersensitivity to viral antigens (Matsunaga et al., 1982). Activation of a cellular immune response has been confirmed by the detection of specific helper and cytotoxic T cells (Arvin et al., 1987; Hayward et al., 1991). In parallel, the guinea pig model was used to compare the immunogenicity of viral proteins such as gE, gI, gC and IE62 (Lowry et al., 1992b). Finally, protection experiments in which animals were immunized with viral protein prior to being infected by guinea pig-adapted VZV indicate that IE62, an immediate–early protein found in the viral tegument, was highly protective while ORF29p, a nonstructural early (major DNA-binding) protein, was not (Sabella et al., 1993).

Recently, severe combined immunodeficient (SCID) mice, which can receive successfully grafts of various human tissues, have been used to study VZV pathogenesis (Moffat et al., 1995, 1998a, 1998b). In these mice, implanted cells differentiate and reconstitute tissues containing the human cell population (McCune et al., 1988). This model provides the first opportunity to study VZV tropism for a variety of human cell types in vivo in the absence of control by the host immune system, as described earlier for other human viruses such as HIV and CMV (Stanley et al., 1993; Mocarski et al., 1993). Such a model can also sustain the comparison of wt or Oka vaccine strains and of cosmid-generated VZV mutants (Moffat et al., 1998a,b; Cohen & Seidel, 1993).

Mononuclear cells are infected at a very early stage in natural infection and play a critical role in viral dissemination. The T-cell tropism of VZV has been demonstrated in SCID-hu mice with both human fetal thymus and liver tissues implanted together under the kidney capsule. Thymus–liver (thy/liv) implants

were inoculated with wild-type or Oka vaccine strains and were analyzed during 21 days for their ability to support VZV infection. Both viral strains replicated efficiently in the thy/liv tissue, the Oka vaccine strain growing at a slightly slower rate. On day 21, an extensive CD4+ and CD8+ lymphocyte depletion was observed in the thymus, as verified by histological and FACS analysis (Moffat et al., 1995). Viral protein expression was demonstrated throughout the lymphoid lobes of the implants, while only trace amounts of viral antigens were detected in the thymic stroma cells or in the capsule. It was not clear whether macrophages became infected or whether they appeared stained because they had phagocytosed VZV-infected T cells. FACS analysis showed that 10 to 30% of cells from all T-cell subpopulations, including CD4+, CD8+ and CD4+/CD8+ cells, expressed VZV antigens, after inoculation with either the wt or the vaccine Oka strain. VZV-infected T cells released intact, infectious virions.

The role of two viral kinases (ORF47p and ORF66p) in viral tropism has been evaluated in vivo in the SCID-hu thy/liv model. Recombinant viruses lacking either ORF47p or ORF66p have been produced using cosmids derived from the Oka vaccine strain (ROka) and these deletion mutants were called ROka 47S and ROka 66S, respectively. These deletion mutants grew as well as ROka in tissue culture, indicating that both kinases are dispensable for the replication of these mutants in vitro. In contrast, ROka47S mutants did not grow in T cells of the SCID-hu thy/liv implants. ROka66S mutants replicated in T cells of the SCID-hu thy/liv implants but with a lower efficiency than ROka. The ORF47 kinase thus appears to be required for viral replication in human T cells and the ORF66 kinase enhances viral infectivity in T cells. This is the first demonstration of different viral phenotypes among VZV strains that appear to be similar in vitro (Table 9.1).

The relationship between VZV and skin cells was evaluated with SCID-hu in which human fetal skin was introduced subcutaneously (Moffat et al., 1995, 1998a). Seven days after inoculation of VZV into the skin implants, multinucleated cells and mononuclear cells infiltrated the epidermis. Later, lesions were associated with disruption of the keratinized outer layer of the skin and spread of the virus in the dermis. Electron microscopic analysis showed virions carried to the cell surface in cytoplasmic vacuoles, egressing through the cell membrane and dispersing from the cell surfaces (Moffat et al., 1998a). This observation correlates with the concept that the strong cell-association of VZV in tissue culture does not reflect the in vivo behavior of the virus. Although lesions observed in skin implants are smaller than those observed clinically, this experimental system provides a good model of natural VZV infection of the skin tissues.

This model has been used to evaluate various viral strains: the pathogenicity of the Oka vaccine strain (V-Oka) was compared with that of its parent (P-Oka), a

Table 9.1 Characterization of the virulence of VZV strains and mutant VZV viruses evaluated in SCID mice implanted with human fetal skin or human fetal thymus/liver cells

	In vitro	Skin implant	Thy/liv implant
Wild type	+	+++	++
Ellen	+	+	N.D.
Parent-Oka strain	+	+++	N.D.
Vaccine-Oka strain	+	Attenuated	++
gC minus	+	Does not grow (gC is essential)	N.D. N.D.
ROka	+	Attenuated	++
ROka66S	+	Attenuated (= ROka)	Grows slowly (ORF66p is nonessential but enhances virulence)
ROka47S	+	Does not grow (ORF47p is essential)	Does not grow (ORF47p is essential)

Note: After Moffat et al., 1998a, b.

low-passage wt virus and the Ellen strain often used as the reference laboratory strain, passaged many times in tissue culture. All these viruses replicated with the same efficiency in tissue culture but, in skin implants, only the low-passaged clinical isolates and the parent Oka strain were fully virulent. Repeated passages in tissue culture diminished dramatically the in vivo virulence: V-Oka inoculated in skin implants was less effective than P-Oka or low-passaged clinical samples as measured by the yield of infectious virus obtained from infected implants or by viral antigen detection in each sample (Figure 9.1). VZV-Ellen, intensively passaged in human tissue cultures, was also significantly less virulent in skin implants than low-passage clinical isolates. Repeated passages were also associated with a decreased viral protein synthesis in human skin in vivo (Figure 9.2).

The role of gC was also tested in this model: gC minus variants obtained from V-Oka were isolated and mutants grew as well as other virus strains in tissue culture, but their capacity to replicate in vivo in human skin was diminished compared to V-Oka. gC minus infected only cells of the superficial keratinocyte layer and produced only small epidermal lesions compared to the large vesicular area of necrosis extending deep into the dermis in skin implants infected with V-Oka or with P-Oka (Moffat et al., 1998a).

The role of the two viral kinases (ORF47 kinase and ORF66 kinase) previously evaluated in the SCID-hu thy/liv system has been studied in the SCID-hu skin model: ROka66S showed no more attenuation in skin than ROka and caused large necrotic lesions resembling those produced by the vaccine. In contrast, ROka47S

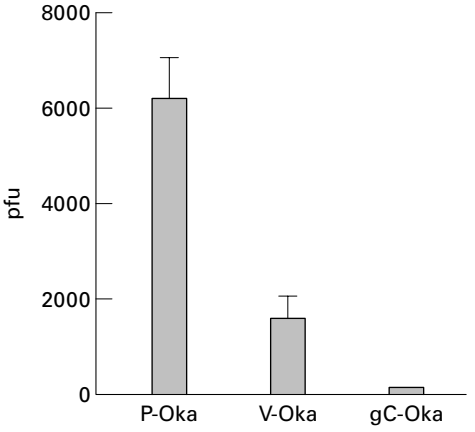

Figure 9.1 Cell-associated infectious virus in SCID-hu skin implants inoculated with P-Oka, V-Oka or gC minus-Oka. PFUs (plaque-forming units) were measured by infectious focus assay 21 days after inoculation (after Moffat et al., 1998a).

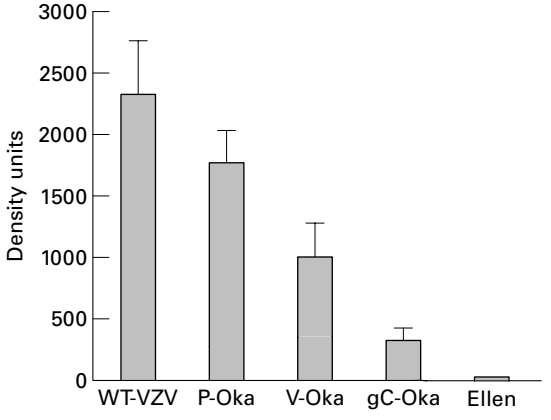

Figure 9.2 VZV protein synthesis in SCID-hu skin implants inoculated with a low passage VZV clinical isolate (WT-VZV), P-Oka, V-Oka, gC minus-Oka or Ellen, a laboratory reference strain. The viral proteins synthesized were measured 21 days after infection by western blot analysis. The concentration of VZV proteins in the range of 70 to 120 kDa was measured after immunological ECL detection, analyzed on a Phosphorimager and reported as density units (after Moffat et al., 1998a).

did not replicate in skin implants and neither viral antigens nor viral genomes were detected, suggesting that this viral kinase is required for the human skin tropism of the virus (Moffat et al., 1998b).

SCID-hu skin implants have offered the first opportunity to suggest that extensive passages of VZV in human cultured tissue result in a drastic loss of virulence

in human skin in vivo, and that the viral kinase encoded by ORF47 and the viral glycoprotein C, both dispensable for replication in tissue culture, play a critical role in the virulence of VZV in human skin (Table 9.1).

In conclusion, the SCID-hu model combined with the use of recombinant viruses now available through the cosmid technology constitute a very interesting model to address the questions of VZV tropism towards PBMCs or skin cells, two major VZV target tissues during acute natural infection.

Models of ocular pathology

VZV is responsible for noncutaneous complications, especially in immunodeficient patients. Ocular pathology due to VZV can result in keratitis, uveitis or acute retinal necrosis. Different small animal models, including guinea pigs (Matsunaga et al., 1982; Pavan-Langston & Dunkel, 1989; Cohen et al., 1997), rabbits (Dunkel et al., 1995) or mice (Wroblewska et al., 1993) have been used to reproduce the clinical manifestations of these ocular pathologies.

In 1982, Matsunaga and colleagues described the production of specific antibodies after ocular inoculation of vaccine Oka strain, though they showed no evidence of ocular disease. However, other studies reported that corneal inoculation of guinea pigs resulted in a punctate keratitis with apparent spread of virus to the trigeminal ganglion, midbrain and cerebellum (Pavan-Langston & Dunkel, 1989). The virus was detected 9 days after intraocular inoculation, but was no longer detected after 15 days when clinical signs of infection had completely disappeared. More recently, Cohen and colleagues (1997) have shown that intravitreal inoculation to guinea pigs of a recombinant VZV expressing the *E. coli* β-galactosidase resulted in a chronic uveitis characterized by a mononuclear cell infiltrate in the posterior segment of the eye. The inflammatory response markedly increased in animals inoculated with infectious virus compared to animals infected with inactivated virus. The use of a recombinant virus expressing a bacterial enzyme was convenient to detect the virus in the eye for at least 4 months after inoculation: virus was detected in the iris and in the ciliary body two weeks after intravitreal inoculation and in the retina after one month. However, while inflammation was present in the vitreous adjacent to the retina and β-galactosidase activity was detectable in the retina itself, no acute necrosis was apparent, probably because of the poor vascularization of the guinea pig retina. The fact that virus was recovered in the pigmented epithelial cells of the retina is interesting because these cells play an important role in maintaining physiological and structural integrity of the retina and in presenting foreign antigens to T cells. This model could prove very useful to study more closely the interactions between infected cells and the immune system.

Similarly, intrastromal inoculation of VZV into rabbit eyes resulted in keratitis (Dunkel et al., 1995). Viral DNA was detected in trigeminal ganglia and all clinical signs of infection had disappeared two weeks after inoculation. In mice, after corneal scarification with VZV, viral DNA was found in various organs such as trigeminal ganglia, kidney, spleen, liver or brain, but neither inflammation nor other clinical signs were observed (Wroblewska et al., 1993).

Models of latency and reactivation

Little information exists on the molecular mechanisms involved in VZV latency and reactivation. A precise identification of the neuronal or non-neuronal cell populations infected by VZV, a better characterization of the viral or cellular events leading to the quiescence of the virus and an evaluation of the modifications (inflammatory process, cytokine expression, etc.) observed in infected ganglia are critical to understand not only latency itself, but also postherpetic neuralgia (PHN) that often follows viral reactivation.

Although the latent virus can be detected in human DRGs, the difficulties encountered in obtaining ganglia from autopsy explain the slow progression of knowledge. To circumvent this limitation, it is necessary to reproduce experimentally the events observed during human latency, a difficult endeavor when working with a virus known to be species-specific and to remain cell-associated in vitro. Many attempts have been made to induce VZV latency either in vitro by infection of dissociated DRG cells from human or nonhuman origin, or in vivo, using mice, rats or guinea pigs.

In vitro infections of human fetal dorsal root ganglia cells have been described (Assouline et al., 1990; Somekh et al., 1992; Somekh & Levin, 1993). It is obvious that working with human cells constitutes an advantage when studying a species-specific virus, but using fetal neural cells is probably not totally adequate since during normal natural infection, the nervous system is no longer fetal.

The first in vitro study of VZV–neuron interaction reporting acute infection of human fetal DRGs was described by Wigdalh et al. in 1986. Dissociated neural cells were obtained from aborted fetuses (10 to 14 weeks) and treated with antimitotic agents prior to viral inoculation, to minimize proliferation of dividing cells. Infection of these neuron-enriched (70–85%) cultures resulted in the development of virus-specific cytopathic effects, as evidenced by neuronal enlargement and nuclear granulation of some of the neuron-like cells, viral antigen expression and production of virus particles. However, viral antigen expression and cytopathic damage occurred later in the neuronal than in the non-neuronal cell population, suggesting that neurons are less susceptible to virus-induced damage than other ganglionic cell types. Transmission electron

microscopy indicated that morphogenesis of VZV in human DRG neurons was similar to that in fibroblasts but with a lower efficacy since fewer virus particles were observed in neurons.

These results were confirmed by other studies focused on the characterization of the relative ability of neural cell types to support VZV replication or latency in vitro (Assouline et al., 1990). Cultures obtained from human fetuses were enriched for astrocytes, Schwann cells or neurons and were infected with cell-free virus. The time course of viral gene expression was compared in each cell type. Even if the kinetics of viral protein expression, accumulation and localization were different in these cells and in fibroblasts, each of the three neural cell types evaluated was readily infected with VZV. They supported virus replication and subsequent progressive lytic infection. In addition, the ability of different VZV strains to infect human neurons has been evaluated in this in vitro model showing that a clinical isolate could infect human fetal neurons with a seven-fold better efficiency than the live attenuated vaccine strain. This may be an additional explanation of the low incidence of herpes zoster in vaccinees (Somekh & Levin, 1993).

Lytic infection of DRG-derived cells was completely prevented when the antiviral agent BVaraU was added (Somekh et al., 1992). However, neither spontaneous viral reactivation nor viral antigen expression was observed after removal of BVaraU, and virus was recovered only when neural cells were trypsinized and cocultivated on permissive human fibroblasts. However, although the virus infected satellite cells as well as neurons, reactivation was observed only from mixed cultures containing both cell types and not from trypsinized cultures enriched in either cell type. These data suggest that an interaction between neuronal and non-neuronal cells is required to establish and/or to maintain latency.

The lytic infection of fetal neural cells is in contrast with the data published by Merville-Louis et al. in 1989, who reported the first in vitro model of a persistent infection in neurons, using dissociated adult rat dorsal root ganglia cells infected by wild-type VZV without any antiviral or cytokine treatment. Viral nucleic acids were detected in cells identified as neurons by immunodetection of neurofilament proteins. Roughly 15–20 % of the neurons carried the viral genome 1 day after infection and this proportion had increased to 50% on day 6, remaining stable until the end of the experiment (10 days). However, no cytopathic effect was observed and cells survived the infection. Expression of viral antigens was observed by immunohistochemistry only in very few neurons (1%) within 1 or 2 days after infection and disappeared completely by day 5. No infectious virus was released in the culture medium. Neuron-specific infection of adult rat DRGs appeared to be persistent and this was not due to the species barrier, since rat fibroblasts had been shown to support productive VZV infection (personal observation). Although presenting the major disadvantage of crossing the species barrier by using cells of

non-human origin, this in vitro model using dissociated rat dorsal root ganglia cells was the first model involving an *adult* peripheral nervous system.

In parallel, several attempts have been made to set up in vivo models of viral persistence and/or latency. As previously mentioned, although the presence of viral nucleic acids in DRGs was documented in the guinea pig, this model was mainly used to study viremia and immune response to VZV infection rather than persistence. A persistent VZV infection of the adult peripheral nervous system resulted from subcutaneous injections of VZV-infected cells along the spine or in the footpad of rats (Sadzot-Delvaux et al., 1990; Annunziato et al., 1998). The virus had not been adapted to rat cells before inoculation, as opposed to the guinea pig model. None of the inoculated animals showed any clinical sign of infection and no rash was observed, whereas specific antibodies were detected up to the end of the experiment (9 months). Viral genomic sequences were found by PCR in DRGs corresponding to the inoculation site (L4, 5 and 6 after footpad inoculation) as soon as 2 days post infection in about 75% of the animals and remained detectable up to 9 months later. Virus seemed to reach the ganglia by the retrograde axonal flow, since after inoculation on one flank, viral genome was not detected in contralateral ganglia. Moreover, when dorsal roots were sectioned prior to inoculation, VZV genome found by PCR was barely detectable in radicotomized ganglia. In situ hybridization was performed on ganglia sections to identify the nature of infected cells: the viral genome was present in 80% of the neurons (Figure 9.3a) but in some cases, early infection was observed in non-neuronal cells surrounding the neurons (Figure 9.3b) (Sadzot-Delvaux et al., 1995). It has not been clarified whether this cellular tropism resulted from a variation of the inoculum or whether the early events of infection led to viral replication in both cell types. These results do not put an end to the current debate concerning the nature of cells hosting VZV latency in human, a debate fueled by evidence that viral genomes are detected in neuronal and/or non-neuronal cells. Spontaneous viral reactivation did not occur in explants of infected ganglia and no infectious virus was released from neural cells. However, Somekh et al. (1992) have shown that repeated trypsinization of human neural cells can lead to reactivation, although this was quite a rare event.

The rat model has been used extensively to characterize VZV transcription and protein expression during latency. Northern blot analysis and RT-PCR were performed on infected DRGs, at least one month after inoculation, and transcripts of ORF 4, 62, 63 and 29 (Major DNA binding protein) were found whereas transcripts of the TK gene and of gE, a late protein, were not detected. Based on the transcription pattern, protein expression was evaluated in rat DRG sections. This work allowed the first demonstration that during persistence, a VZV protein, the immediate–early protein encoded by ORF63, is highly expressed, while gE was undetectable. Protein IE63 was mainly expressed in neuronal cytoplasm, but was also detected in

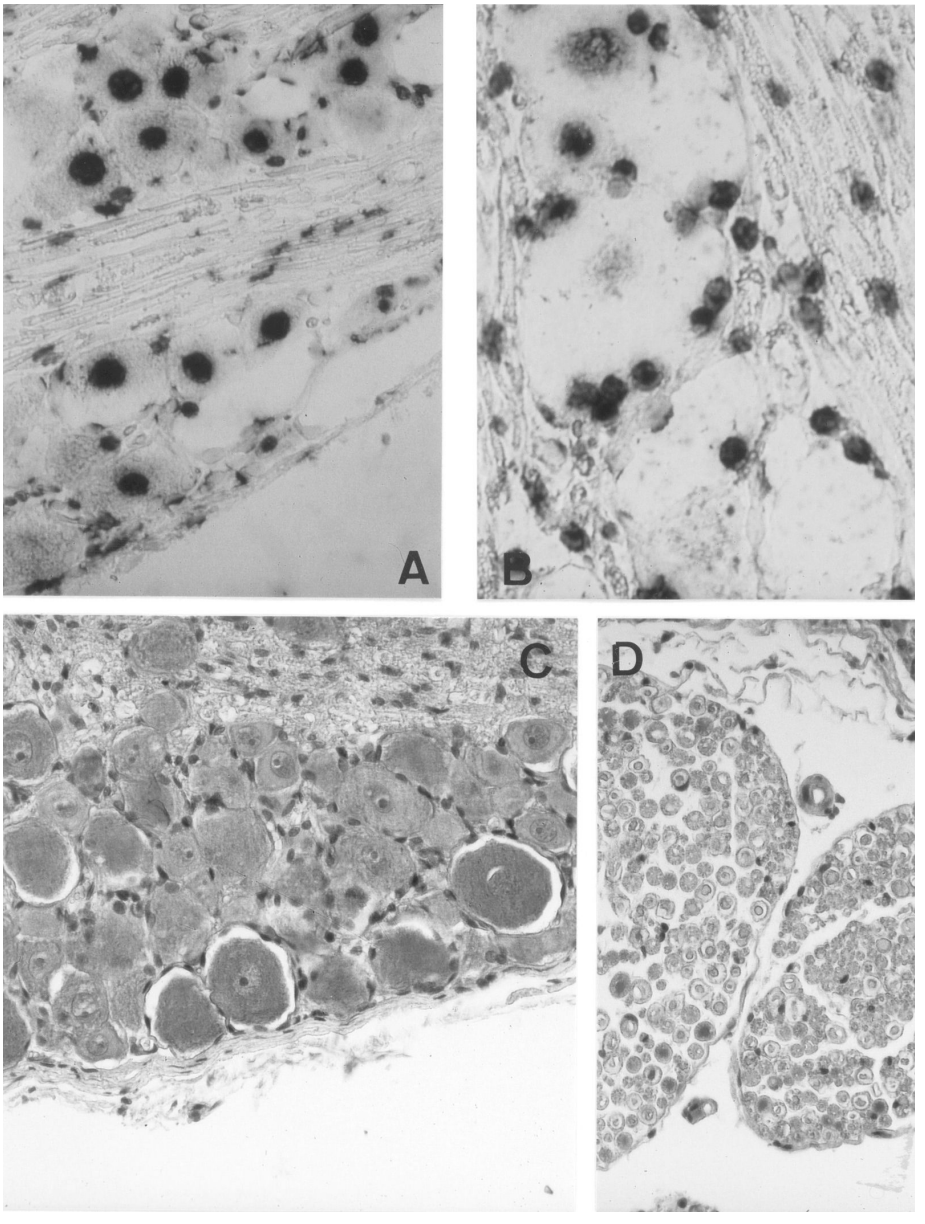

Figure 9.3 (a) and (b) Detection by in situ hybridization of varicella-zoster virus nucleic acids in DRG sections of an infected adult rat, 7 days after infection, using digoxigenin-labeled mapping about 50% of the viral genome. Depending on the animal, viral genome is detected mostly in nuclei of neurons (a) or in both non-neuronal cells and neurons (b). Immunohistochemical detection of VZV IE63 protein in a lumbar ganglion of an infected adult rat, 6 weeks after inoculation (c), and in the corresponding root (d) indicating that the protein can accumulate in axons.

axons in the root corresponding to the infected ganglion (Figures 9.3c and d). Rare non-neuronal cells were found to express IE63 in their cytoplasm (Debrus et al., 1995). This first demonstration of the expression, during VZV persistence, of a viral protein also expressed during the lytic cycle, was followed by the detection of other viral proteins in latently infected human ganglia (Mahalingam et al., 1996; Lungu et al., 1998), which suggest other hypotheses about the molecular mechanisms controlling VZV persistence and reactivation. Some preliminary experiments indicate that viral inoculation in the footpad could also be correlated with an increased sensitivity to sensory stimuli, in rats (Fleetwood-Walker et al., 1999).

Based on the fact that humans exposed to VZV in utero are at increased risk to develop zoster early in life, Brunell et al. suggested that newborn rats could be more susceptible than adults to VZV infection. Newborn rats were inoculated intraperitoneally with wild type virus and some viral transcripts were detected in trigeminal ganglia in about 50% of infected animals. However, no extensive comparison of adults and newborns allowed a conclusion that the susceptibility decreased with age (Brunell et al., 1999).

Because of its similarity with VZV, simian varicella virus (SVV) has been described as an appropriate model for VZV pathogenesis. This primate herpesvirus is the causative agent of a primate disease that appears to be the simian counterpart of human chicken pox (Wenner et al., 1977; Roberts et al., 1984). SVV is antigenically related to human VZV (Felsenfeld & Schmidt, 1970; Fletcher & Gray, 1992) and by Southern blot analysis, it seems more related to VZV than to other primate herpesviruses (Gray & Gusick, 1996). Many studies indicate that genes of SVV and VZV genomes are colinear (White et al., 1997). Seven to 10 days after intratracheal SVV inoculation of nine African green Monkeys, all but two monkeys developed a varicella rash and neutralizing antibodies were detected in all animals. Six months to 4 years after the inoculation, virus genome was recovered by PCR in trigeminal or thoracic ganglia of seven monkeys and in cells of the adrenal medulla, in one animal (Mahalingam et al., 1991, 1992). More recently, transcripts of the SVV homolog to VZV gene 21 has been detected in monkey ganglia latently infected with SVV (Clarke et al., 1996). SVV has been proposed as an appropriate model for the studies of immunopathology of ocular varicella virus infection. Clinical signs of ocular infection and inflammation were observed 12 to 14 days after intrastromal and subconjunctival inoculation of SVV to animals treated with methylprednisolone (Metcalf et al., 1995). Finally, efficacy of antiviral compounds used in therapy of varicella-zoster infections in humans have been evaluated using this model (Soike et al., 1993; 1994; Tino et al., 1993). This primate herpesvirus may, to some extent, constitute an interesting alternative to identify parallels in pathogenesis of these related viruses and for pharmacokinetics analyses for which it has been used in the past.

In conclusion, many animal models have been described with the aim of mimicking natural VZV infection. None of these models reproduces VZV pathogenesis completely, but all of them can help answer some of the questions addressed and can be used to investigate particular aspects of this very complicated human-specific virus. Further work is needed since VZV reactivation remains so far not reproducible in vitro or in vivo. A better understanding of the mechanisms involved in latency, whether cellular or viral proteins, or environmental factors, will probably emerge as better animal models of VZV infection are developed.

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Part III

Epidemiology and Clinical Manifestations

10

Epidemiology of varicella

Jane Seward, Karin Galil, and Melinda Wharton

Although varicella occurs worldwide, the epidemiology of the disease differs in temperate compared with tropical climates. Moreover, as vaccine use increases in countries such as the United States, in which the vaccine is recommended as part of the routine childhood immunization schedule (Committee of Infectious Diseases, 1995; CDC, 1996), the epidemiology of the disease will change. This chapter summarizes methodological issues that must be considered when comparing results of varicella studies; reviews varicella epidemiology in the pre-vaccine era for temperate and tropical regions; and outlines the likely changes in the epidemiology of disease following widespread use of vaccine.

Methodological issues

Reports of varicella incidence differ in the methods used to determine the number of cases; the method used impacts completeness of ascertainment and, therefore, estimates of age-specific incidence and age distribution of cases. Because varicella is easily diagnosed by the lay public, information on disease incidence can be collected from household-based surveys (Guess et al., 1986; Finger et al., 1994; Yawn et al., 1997). Data collected from such surveys with a defined recall period, optimally one year or less, are more complete than data obtained from medical record review or passive reporting (surveillance) systems. In surveillance systems, completeness of ascertainment may vary by age, reflecting age- related differences, such as the proportion of patients seeking health care. Variations in completeness of reporting by age have been documented by comparing varicella cases reported via a reportable communicable disease system with cases detected by a household survey over the same time period (Sydenstricker & Hedrick, 1929). Reporting was more complete for adults (50%) than for school-aged children (25%) and was lowest for preschool children (12%). Data from medical records from a health maintenance have demonstrated 20–50% lower varicella incidence among children under 15 years than national estimates, probably reflecting the fact that not all children seek medical attention for varicella illness (Choo et al., 1995). In addition,

patterns of health-seeking behavior, access to health care and family characteristics (such as number of children in a family) may change over time. These differences in methodology and completeness of ascertainment should be considered when comparing varicella age distribution or incidence within or between countries.

Comparisons of seroprevalence studies within and between countries are complicated by differences in methods or the method for sample selection (nationally or regionally representative, convenience sampling) and laboratory methods, which have improved over the 30-year period spanned by these studies.

Methodological issues should also be considered when comparing hospitalizations and deaths from varicella. The validity of varicella as coded on hospital discharge or death records may vary from country to country. Methods for assessing hospitalizations due to varicella may also vary between studies. The true burden of hospitalizations may be overestimated by counting all cases of varicella from discharge records rather than those cases in which varicella was the primary cause for admission. Additionally, comparisons of varicella hospitalizations and mortality data may be problematic due to differences in hospital admission criteria, differences in availability of treatments for varicella and its complications and the fact that standards of treatment may change over time; for example, it has become standard practice in developed countries to admit persons with immunocompromising conditions to hospital for treatment with intravenous acyclovir. This may result in a higher rate of varicella hospitalizations than in the past, which is not necessarily indicative of an increase in the rate of hospitalization. Conversely, a decline in the hospitalization rate may reflect changes in hospital admission policies.

Epidemiology in temperate climates in the absence of vaccination

Seasonality and periodicity

Chickenpox has a striking seasonal pattern with peak incidence most commonly reported in the winter and spring (March–May in the northern hemisphere) (Figure 10.1) (Preblud, 1978; Varughese, 1988; Fornaro et al., 1999; Paul & Thiel, 1996; Wharton, 1996; Chant et al., 1998; Tobias et al., 1998) but a later peak in June in France and the UK (Fairley & Miller, 1996; Degeun et al., 1998), perhaps because the school year ends later in these countries. Periodic epidemic years are followed by years with lower incidence (Gordon, 1962; Joseph & Noah, 1988). The United Kingdom Royal College of General Practitioners' reporting system for communicable diseases, between 1967 and 1985, reported an annual incidence for varicella that varied from 243 to 878 consultations per 100 000 population (Joseph & Noah, 1988); epidemic years were followed by 2 to 3 years with steeply falling reported incidence. Year-to-year variations in incidence of 36% to more than 100% have also

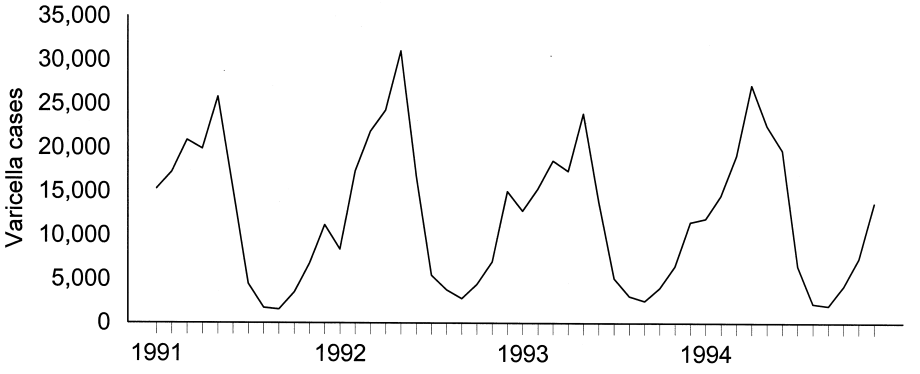


Figure 10.1 Varicella cases from the National Notifiable Disease Surveillance System by month, United States, 1991–1994.

been reported in the US and localities (Gordon, 1962; Choo et al., 1995), as well as one Canadian province and two European countries (Trlifajova et al., 1980; Degeun et al., 1998; Law et al., 1998).

Communicability

Varicella is a highly infectious disease, with reported household secondary attack rates among children <15 years of age from large studies in the UK and the US ranging from 61% (Hope-Simpson, 1952) to 87% (Ross, 1962). Smaller studies examining effectiveness of vaccination following exposure in household settings have reported household secondary attack rates ranging from 92% (Arbeter et al., 1986) to 100% (Asano et al., 1977). In community settings, transmission is lower. In an outbreak in a child care center, the cumulative attack rate among susceptibles was 88% but the outbreak lasted 15 weeks with a much lower secondary attack rate than that described within families (Izurieta et al., 1997).

Incidence

Age

In temperate climates, varicella is a disease of childhood, with approximately 90% of cases occurring by 10–14 years of age (Sydenstricker & Hedrich, 1929; Trlifajova et al., 1980; Guess et al., 1986; Paul & Thiel, 1996; Degeun et al., 1998) and the mean age of infection ranging from 6.7 to 10.6 years (Fales, 1928; Hope-Simpson, 1952; Wharton et al., 1990; Garnett et al., 1993) (Table 10.1). Since the vast majority of cases occur among children, this age group bears the brunt of the health burden from varicella. In temperate climates, virtually all persons are infected by the time they reach adulthood. Thus, on average, the number of infections that occur annually approximates the number in the birth cohort.

In the US, national varicella incidence data are available from the National

Table 10.1 Varicella epidemiology in temperate and tropical climates

	Temperate climates	Tropical climates
Seasonality	Yes (USA, UK, France, Czech., Canada, Italy, Australia, New Zealand, Germany)[1]	Yes (India, Nigeria, Sri Lanka, Guatemala)[14] No (Singapore)[15]
Mean age of infection	6.7 years (urban Maryland)[2] 8.6 years (rural Maryland)[2] 6.7 years (semi-rural England)[3] 10.6 years (West Germany)[4]	6.9 years (India)[16] 12.3 years (India)[17] 23.4 years (India)[18] 38.3 years (St. Lucia, West Indies)[4]
% cases among children	>95% cases among children <15 years, US,1929[5] 92% cases among children <15 years, US, 1980–90[2] 92% cases by age 14 years, France[6] 90% cases by 10 years and 8 months, UK[3]	30–100% (Singapore, India, Guatemala)[19]
VZV seroprevalence young adults (approx. 15–24 years)	90% among 12 year-olds (Czechoslovakia)[7] 91–96% among military recruits 18–25 years (US)[8] 95% among 20–29-year-olds (US, NHANES)[9] 95.4% among persons 20–24 years (Japan)[10]	10–20% (St. Lucia, West Indies)[4] 30–40% (India)[20] 40–50%(Singapore, rural India, Malaysia)[21] 50–60% (Philippines, Puerto Rican army recruits)[22] 60–70% (Micronesian army recruits)[23] 70–80% (India, Thailand [Bangkok])[24] 80–100% (Taiwan, urban India)[25]
Secondary household attack rates among susceptible children	61% UK[3] 87%[11] and 92% US[12] 100% Japan[13]	26%[18] & 76%[26] (India)

Notes:

[1] Fornaro et al., 1999; Gordon, 1962; Preblud, 1979; Joseph & Noah, 1988; Varughese, 1988; Fairley & Miller, 1996; Degeun et al., 1998; Paul & Thiel, 1996; Wharton, 1996; Chant et al., 1998; Law et al., 1998 Tobias et al., 1998. [2] Fales, 1928; Wharton et al., 1990. [3] Hope-Simpson, 1952. [4] Garnett et al., 1993. [5] Sydenstricker & Hedrick, 1929. [6] Degeunet et al., 1998. [7] Trlifajova et al., 1980. [8] Kelley et al., 1991; Streuwing et al., 1993; Jerant et al., 1998. [9] Kilgore et al., 1997. [10] Taylor-Wiedeman et al., 1989. [11] Ross, 1962. [12] Arbeter et al., 1986. [13] Asano et al., 1977. [14] Maretic & Cooray, 1963; Salomon et al., 1966; Sinha, 1976; White, 1978; Venkitaraman & John, 1984; Iyun, 1986; Balraj & John, 1994. [15] Ooi et al., 1992. [16] Balraj & John, 1994. [17] Venkitaraman et al., 1986. [18] Sinha, 1976. [19] Salomon et al., 1966; Sinha, 1976; Ooi et al., 1992; Balraj & John, 1994. [20] Venkitaraman et al., 1984. [21] Ooi et al., 1992; Lee, 1998; Mandal et al., 1998. [22] Barzaga et al., 1994; Longfield et al., 1990. [23] Withers et al., 1994. [24] Bhattarakosol et al., 1996; Lin et al., 1996; Migasena et al., 1997. [25] Yin et al., 1996; Mandal et al., 1998. [26] Balraj & John 1994.

Table 10.2 Varicella incidence by country and study method

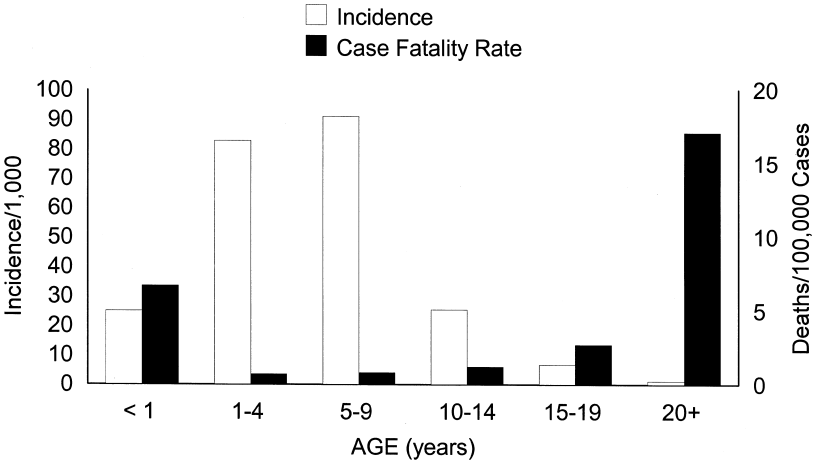
Country	Study method	Years	Annual incidence/1000 population
United States[1]	National survey	1970–78	16.0
United States[2]	National survey	1980–90	15.0
France[3]	Sentinel physician reporting	1991–5	10.0–13.5
United States	Health maintenance	July 1990–June 1991	7.6
(Boston)[4]	organization records	July 1991–June 1992	12.0
England and Wales[5]	Sentinel physician reporting	1967–85	2.4–8.8
		1989–90	6.3
Scotland[5]	Sentinel physician reporting	1989–90	6.2
Australia (Sydney)[6]	Local survey	1995	33.9

Notes:

[1] Guess et al., 1986.
[2] Wharton et al., 1990.
[3] Degeun et al., 1998.
[4] Choo et al., 1995.
[5] Fairley & Miller, 1996.
[6] Chant et al., 1998.

Health Interview Survey (NHIS) and are reported as annual averages for multi-year periods (Table 10.2). Average annual incidence per 1000 persons in two periods, 1972–1978 and 1980–1990, are remarkably consistent at 16.0 and 15.0 respectively (Wharton et al., 1990; Wharton, 1996). Data from medical records and sentinel physician reporting systems in the US, UK and France yield lower annual estimates (Choo et al., 1995; Fairley & Miller, 1996; Degeun et al., 1998). The higher reported incidence from a sentinel physician surveillance system in France compared with a similar surveillance system in England, Wales and Scotland may reflect higher consultation rates for varicella in France and/or more complete reporting. A survey in Australia in 1995 reported a high annual incidence of 33.9/1000 persons, which may reflect an epidemic year of disease (Chant et al., 1998).

Varicella incidence varies markedly by age. In both the 1970s and 1980s, NHIS data showed that US children aged 5–9 years had the highest annual incidence rate (89–90 cases per 1000) followed by children aged 1–4 years (79–82/1000) (Figure 10.2). The age groupings used to describe age-specific incidence from NHIS are <1 year, 1–4 years, 5–9 years, 10–14 years, 15–19 years and 20 years and older. Other survey data collected 60 to 70 years earlier in 1922 and 1923 in Hagerstown, Maryland, reported data using different age groupings for children (Sydenstricker & Hedrich, 1929). In this household-based survey, the highest age-specific varicella incidence occurred at 6 and 7 years (67.5/1000); however, incidence dropped dramatically to 14.2/1000 for children ages 8 and 9 years. Thus, larger age



*Cases: 1980-1990 National Health Interview Survey estimates applied to 1980 US population.
Deaths: 1980-1990 National Center for Health Statistics multiple cause mortality data.

Figure 10.2 Varicella age-specific incidence and case fatality rates, United States, 1980–1990. Cases are the 1980–1990 National Health Interview Survey estimates applied to the US population of 1980. Deaths are the 1980–1990 National Center for Health Statistics multiple cause mortality data.

groupings such as 5–9 years or even larger groupings such as 5–14 years may mask substantial differences in incidence within these age groups and may not be optimal for accurately describing the age-specific incidence of varicella.

In the 1990s, data from state and local surveys in the US show the highest varicella incidence among preschool rather than school-aged children, indicating that the disease may be shifting to younger children (Finger et al., 1994; Yawn et al., 1997). This may be due to earlier and more frequent attendance at preschools and child care centers. In Olmstead County, Minnesota in 1994–1995, varicella incidence was 154.0/1000 for children <5 years compared with 86.0 for children 5–9 years (Yawn et al., 1997). The highest age-specific incidence was at 2 years of age, considerably younger than previously reported. In this population, more than 80% of mothers worked outside the home. The shift to younger ages was also evident among infants; the two state and local surveys reported twice the incidence among infants <12 months (55.0/1000 (Yawn et al., 1997) and 45.3/1000 (Finger et al., 1994)) in the 1990s than was reported from the NHIS in the 1970s and 1980s.

The increasing proportion of cases among younger children has also been documented in the UK. Physician reports show a marked increase in the proportion of consultations for varicella in children <5 years from approximately 18% of all varicella consultations in 1977 to approximately 50% in 1993 (Fairley & Miller, 1996).

Although this shift to younger ages may be due to changes in consultation practices, it coincided with a significant increase in the proportion of children <5 years who attended nursery or day care centers (10% in 1970 versus 50% in 1992) and is thus likely to reflect a true shift in age-specific incidence. Fairley calculated age-specific incidence using serological survey data collected in 1991 in the UK and reported a similar varicella incidence rate for children 0–4 years (140/1000) (Fairley & Miller, 1996) as was reported from Minnesota in 1994–95 (Yawn et al., 1997). Sentinel physician data from France and Italy also confirm the highest age-specific incidence in pre-school-aged children in the 1990s (Fornaro et al., 199?; Degeun et al., 1998).

Although several studies are suggestive that varicella incidence is increasing among adults, interpreting these studies highlights the need to consider methodological differences and potential differences in study populations when comparing study results. For example, two studies conducted in populations served by health maintenance organizations in the US reported higher varicella incidence rates among adolescents 15–19 years in 1995 (Coplan & Guess, 1997) and among adults in 1990–1992 (Choo et al., 1995) compared with NHIS data from 1972–1978. Since data were collected over short time periods (1 and 2 years respectively), the higher rates could have been due to yearly variation in disease incidence. Other explanations include a higher proportion of the health maintenance organizations' populations originating from countries with lower childhood rates of infection compared with the US population as a whole, a real increase in varicella rates over time, or chance.

Evidence for an increase in adult incidence has been noted from the UK, where the proportion of adult (15–44 years) consultations for varicella has increased from 6.7% of all varicella consultations in 1967 to 20% in 1995 (Fairley & Miller, 1996). This trend may be attributable to a change in age-specific consultation patterns over time; however, as suggested by the authors, the change is more likely to reflect a true increase in incidence since varicella mortality rates in adults also rose over the same time period. Although the increase in US hospital admissions among military personnel in the 1980s (Gray et al., 1990) has been widely quoted as evidence of an increase in incidence among adults, rates declined significantly in the 1990s in the absence of an intervention (Herrin & Gray, 1996). Further studies are needed to determine whether varicella incidence rates in adults are increasing. Ideally such studies should measure incidence from household surveys and compare surveys with similar methods over time.

Child care

Varicella is one of the most common communicable diseases in child care centers (Jorm & Capon, 1994; MacDonald et al., 1997). Attendance in child care or preschools provides the opportunity for exposure to the varicella zoster virus (VZV)

at younger ages. Studies demonstrate a higher incidence or prevalence of varicella among children attending day care (Hurwitz et al., 1991; Jorm & Capon, 1994; Eaton Jones et al., 1995; MacDonald et al., 1997). Studies have consistently noted an increased risk of exposure to varicella with increasing size of the center (Hurwitz et al., 1991; Jorm & Capon, 1994; Eaton Jones et al., 1995).

Urban–rural

In 1928, Fales used surveillance data to compare the mean age of varicella infection between urban (6.7 years) and rural children (8.6 years) in Maryland in 1913–1917 (Fales, 1928). Gordon, in reviewing reported cases of varicella in Massachusetts from 1942–1961, speculated that the shift in cases to younger ages was due to increasing urbanization in the state (Gordon, 1962). Few studies, especially household-based surveys, of urban–rural differences in age of varicella infection are available to determine whether observed differences still exist, are reproducible and can be explained by different risks of exposure.

Seroprevalence

Age

Seroprevalence studies reflect varicella age-specific incidence. In the US, nationally representative population-based data on VZV seroprevalence ($n = 18\,000$) available from the National Health and Nutrition Examination Survey III (1988–1994) indicate a high VZV seroprevalence in the US population. Seroprevalence was 66% for 4–5 year olds and increased to 86% for 6–11 year olds, 93% for 12–19 year olds, 95.5 % for 20–29 year olds, and reached 99.8% for adults 50–59 years (Van Loon et al., 1993; Kilgore et al., 1997). Other studies among adults in the US have demonstrated low levels of susceptibility (Gershon et al., 1976); among military recruits aged 18–25 years, susceptibility has ranged between 4–8% (Kelley et al., 1991; Struewing et al., 1993; Jerant et al., 1998). A similar pattern of high seroprevalence is described in other countries with temperate climates (Table 10.1) (Trlifajova et al., 1980; Taylor-Wiedeman et al., 1989; Fairley & Miller, 1996).

Among adults, a positive varicella history is highly predictive of varicella immunity (97–99%) but a negative history is poorly predictive of lack of immunity (Alter et al., 1986; McKinney et al., 1989; Jerant et al., 1998). Approximately 70–90% of adults with a negative or uncertain history of varicella have VZV antibodies when tested, indicating poor recall of history or subclinical infection (Alter et al., 1986; Jerant et al., 1998). Among children, a negative varicella history is a more reliable indicator of susceptibility than in adults. Ross's data aggregating household secondary attack rates among children < 15 years demonstrated that after a primary case in a family, 96% of exposed children who were susceptible by history developed varicella as secondary or tertiary cases (Hope-Simpson, 1952). These data

indicated that, at most, only 4% of children who were susceptible by history may have been immune. In a more recent large study of 937 US children 7–12 years of age with a negative or uncertain varicella history, Lieu demonstrated that varicella seroprevalence increased with age from 13% in 7 year olds to 38% in 12 year olds (Lieu et al., 1998). In a smaller study from the UK that included younger children with a negative or uncertain varicella history, Evans reported that 0/5 children between 3 months and 1 year of age had antibody compared with 6/54 (11%) of children 1–5 years, 9/23 (39%) of children 6–10 years and 6/17 (35%) of children 11–15 years (Evans et al., 1980).

Race, ethnicity, gender and number of siblings in the household

National serology data from the US as well as studies conducted among US Army and Navy recruits have described differences in varicella susceptibility according to race, with higher susceptibility among African Americans compared with whites (Kelley et al., 1991; Struewing et al., 1993; Kilgore et al., 1997; Jerant et al., 1998). In the national data, these differences narrow with increasing age and are not apparent after approximately age 40. No differences were seen by gender or ethnicity. Studies in subpopulations in the US have described differences by country of origin (Gershon et al., 1976; Kelley et al., 1991; Struewing et al., 1993) and number of siblings in the household during childhood (Jerant et al., 1998). Differences by race and siblings in the household are likely to reflect likelihood of exposure and therefore age at primary infection. Since the risk of herpes zoster has been demonstrated to vary by race (Schmader et al., 1995), studies of varicella race and age-specific incidence are indicated. Differences in seroprevalence by country of origin will be discussed under tropical epidemiology.

Factors affecting disease severity

Age, immune status and pregnancy have been described as risk factors for severity of varicella. (See Chapter 16 and Hospitalizations and Mortality, below).

Hospitalizations

Hospitalizations for varicella provide an estimate of the burden of severe disease. These data are important for describing morbidity and for calculating the economic and social costs of varicella infection. An accurate estimate depends on identifying hospitalizations due to varicella and its sequelae while excluding hospitalizations in which varicella was coincidental (e.g. a child with varicella with a fractured arm). Estimates of annual hospitalizations for varicella in the US during the 1970s and 1980s ranged from approximately 4000 to 9000 depending on the dates of the study, the population studied and the definition of varicella hospitalization used (Guess et al., 1986; Wharton, 1996; Wharton et al., 1990). More recent

estimates indicate annual hospitalizations of approximately 11 000 per year in the US (Center for Disease Control, unpublished data).

In contrast to numbers of hospitalizations, hospitalization rates (e.g. number of hospitalizations per 100 000 persons) remain comparable over time – and from country to country – even as populations increase and populations change in age distribution. The risk of being hospitalized for cases of varicella, especially for different age groups, is best described by rates of hospitalization according to the number of varicella cases (e.g. number of hospitalizations per 1000 varicella cases).To express risks of hospitalization for varicella cases, studies rely on age-specific incidence rates from survey or surveillance data applied to the particular population under study. Although this approach has limitations since year to year variability in varicella incidence may occur at the local and state level, local varicella incidence data are rarely available to use for this purpose. For all ages combined, in the US, rates of hospital admission per 1000 varicella cases range from 2.2 to 4.3 per 1000 cases in the 1970s and 1980s (Guess et al., 1986; Wharton et al., 1990; Choo et al., 1995). In the 1990s, among children <13 with varicella, a hospitalization rate of 5.5 per 1000 cases was reported from Minnesota (Yawn et al., 1997).

The risk of hospitalization varies by age. Age-specific patterns for hospitalizations are evident in the US and in other countries where admission practices may differ from those in the US, including Canada, England and Wales (Varughese, 1988; Fairley & Miller, 1996; Wharton, 1996). Although most hospitalizations occur among children (Varughese, 1988; Wharton et al., 1990; Choo et al., 1995; Fairley & Miller, 1996), who also have the highest disease incidence, both infants and adults are at significantly increased risk of severe disease and hospitalization. Studies consistently report the highest risk of hospitalization in adults (6–15 times higher risk), an increased risk in infants (1.3–8 times higher risk) and the lowest risk in children 1–4 years or 5–9 years of age (Guess et al., 1986; Wharton et al., 1990; Fairley & Miller, 1996; Wharton, 1996).

Length of hospitalization varies between sites and over time as a reflection of local practices and policies. The average length of hospitalization ranges from 3 to 6 days; however, adults may be hospitalized for longer time periods, reflecting the increased severity of disease in this age group (Guess et al., 1986; Fairley & Miller, 1996; Chant et al., 1998).

Mortality

In the US, national mortality data reporting varicella as the underlying cause of death averaged approximately 100 deaths per year in the 1970s and declined to a low of 47 deaths in 1986 coincident with the availability of acyclovir, varicella-zoster immune globulin and decreasing use of aspirin once it was linked to the

occurrence of Reye's syndrome. However, in the late 1980s deaths increased again to previous levels (Wharton, 1996). Examining mortality rates eliminates the effects of changing population size and enables comparisons between countries. In the US, crude varicella mortality rates declined from 0.65 per million population in 1973 to 0.02 in 1986 and then increased to average 0.04 per million population from 1990–94 (Seward et al., 1998). Similar mortality rates are reported from Australia with a decline in mortality from the 1970s through the 1980s, which was followed by an increase in mortality from 1991–1993 (Chant et al., 1998). In the UK, an increase in mortality among persons >14 years of age has been reported from 0.02 per 100 000 population in 1967–1970 to 0.06 in 1991–1994; this has paralleled the increase in adult consultations for varicella (Fairley & Miller, 1996).

Case fatality rates (CFR) measure the risk of dying from varicella. Accurate case fatality rates are dependent on accurate mortality and incidence data. In the US, data from the 1970s, 1980s and 1990s show higher risk of dying (most commonly expressed as case fatality rates) in infants <1 year and adults ≥20 years of age compared with children 1–4 and 5–9 years (Preblud, 1981; Wharton et al., 1990; Seward et al., 1998). In the 1980s, infants (CRF 6.7/100 000 cases) and adults (CRF 17.1/100 000 cases) had a 10 and 24 times greater risk of dying from varicella, respectively, than children 1–4 years of age, whose risk was 0.7/100 000 cases (Figure 10.2). Although different age groupings were used, these case fatality rates are similar to those reported from England and Wales from 1988–1992 (0.7 for 0–4 years, 1.4 for 5–14 years and 20 for persons 15–44 years of age) (Fairley & Miller, 1996). Though an extremely high CFR (471/100 000) is reported for adults ≥65 years in the UK, there is a high possibility of misclassification of varicella with herpes zoster in this age group, as documented in US data by Choo (Choo et al., 1995).

Epidemiology in tropical climates in the absence of vaccination

The epidemiology of varicella in tropical regions differs dramatically from that in temperate climates, for reasons that are poorly understood. Differences in age distribution and seroprevalence have been consistently described, with a higher proportion of cases and higher susceptibility among adults. Tropical regions are those located geographically between the Tropics of Capricorn and Cancer; within these latitudes, climates may vary considerably due to altitude and geography (desert or coastal), from hot and humid to dry and cool. Varicella data from tropical and semi-tropical climates is summarized here.

Seasonality

Seasonality has been described from studies in Nigeria, Guatemala, Sri Lanka and many different parts of India, with the highest disease incidence in the driest,

coolest months (Maretic & Cooray, 1963; Salomon et al., 1966; Sinha, 1976; White, 1978; Venkitaraman & John, 1984; Iyun, 1986; Balraj & John, 1994). In Nigeria, in years with low rates of hospitalization for varicella, no seasonality was apparent, in contrast to epidemic years when such seasonality was marked (Iyun, 1986). Seasonality is not apparent, however, in Singapore data reported in the weekly reporting system for communicable diseases (Ooi et al., 1992).

Explanations for lack of seasonality in Singapore are unclear but may include incomplete reporting, description of data during nonepidemic years or absence of seasonality in this climate. As in temperate climates, epidemic years are followed by years with lower incidence (Salomon et al., 1966; Sinha, 1976; Balraj & John, 1994).

Secondary household attack rates

Two household-based studies in India have reported widely variant secondary household attack rates; however, the studies also differed in the age distribution of varicella cases described. A secondary attack rate within families of 76% was reported from a study of an epidemic in Vellore, India, in 1987 (Balraj & John, 1994). In this community, the mean age of varicella infection was 6.9 years and 24% of cases occurred in persons 16 years of age or older. In contrast, in West Bengal, India, Sinha reported very low secondary family attack rates, of 26% for children <8 years (Sinha, 1976). In this study, the mean age of varicella infection was 23.4 years and 63% of cases occurred among persons 15 years of age and older. The secondary attack rate among children varied according to which family member was the index case. The attack rate was only 11% if the father was affected, 27% if the mother was affected and 85% if both parents were affected. Transmission between siblings was low. These findings suggest social customs that limited transmission. In this community, social isolation practices still occurred that had been in place for smallpox such as placing infected persons in another house after the appearance of rash. This may have contributed to the low transmission within households. Further studies of secondary transmission within families and social customs surrounding management of varicella cases in the household are needed.

Age-specific incidence and seroprevalence

The most striking finding in data reported from tropical countries is the older age of infection, although epidemics primarily affecting children are also described. Because of this older age of infection and because varicella infection is more severe in adults than in children, tropical countries may experience greater morbidity and mortality from the disease and its complications (including congenital varicella syndrome). Although studies report increased severity of disease among adults in tropical climates compared with temperate climates (Maretic & Cooray, 1963), such comparisons are difficult to interpret due to differences in access to health

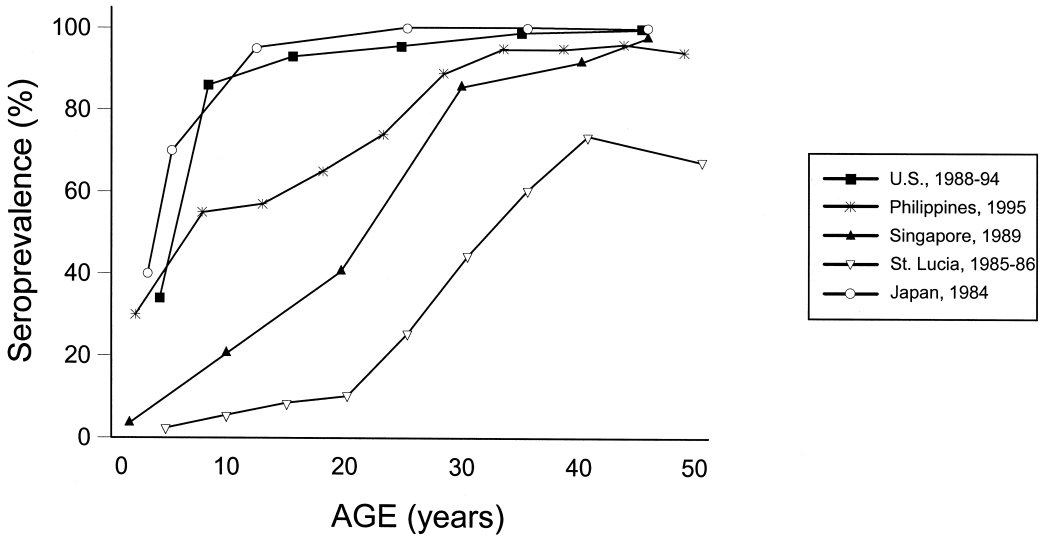


Figure 10.3 Age-related VZV seroprevalence in five countries.

care, antiviral therapy and treatment for varicella complications. Data from tropical climates have been reported from a variety of sources, with limitations similar to those described in temperate countries.

The majority of studies report a higher mean age of infection and a higher proportion of varicella cases and higher VZV susceptibility among adults compared with temperate climates (Table 10.1) (Barzaga et al., 1994; Venkitaraman & John, 1984; Venkitaraman et al., 1984; Nassar & Touma, 1986; Longfield et al., 1990; Ooi et al., 1992; Garnett et al., 1993; Withers et al., 1994; Lin et al., 1996; Migasena et al., 1997; Lee, 1998; Mandal et al., 1998). The exception to this pattern is a varicella epidemic reported from a rural highland village in Guatemala (Salomon et al., 1966). Eighty-five percent of the cases occurred among children under 6 years with the highest age-specific incidence among 3-year-olds. The oldest case was 13 years of age, suggesting that VZV immunity among adults in this community was high. Climate in the highlands of Guatemala, although tropical geographically, may be relatively cool, more similar to temperate climates.

As in temperate climates, VZV seroprevalence data confirm age-specific incidence. VZV seroprevalence from a variety of tropical countries (St. Lucia, West Indies, Singapore, the Philippines, Malaysia, Thailand [Bangkok], Taiwan [semitropical] and Micronesia) almost uniformly confirm higher adult susceptibility compared with adults in the US and other temperate countries (Table 10.1)) (Barzaga et al., 1994; Venkitaraman et al., 1984; Nassar & Touma, 1986; Venkitaraman et al., 1986; Longfield et al., 1990; Ooi et al., 1992; Withers et al., 1994; Bhattarakosol et al., 1996; Lin et al., 1996; Migasena et al., 1997; Lee, 1998;

Mandal et al., 1998). Children acquire varicella at older ages and a higher proportion of young adults are still VZV susceptible (Figure 10.3). The lowest population seroprevalence has been reported from St. Lucia, West Indies (Garnett et al., 1993) with intermediate levels of seroprevalence in Singapore (Ooi et al., 1992) and higher levels noted from large community surveys conducted during the early 1990s in Malaysia (Lee, 1998), Bangkok, Thailand (Migasena et al., 1997) and Manila, Philippines (Barzaga et al., 1994). Of these three surveys, seroprevalence was highest in Bangkok, with 70% of persons having antibodies by 10–14 years of age, 96% by 30–39 years and 100% by 40–49 years (Migasena et al., 1997).

Outbreaks of varicella in temperate countries have confirmed high VZV susceptibility among adults (Tamil refugees, Puerto Rican military recruits, nurses from Guyana, Sri Lanka and the Philippines) raised in tropical countries who develop varicella after relocation in countries with temperate climates (Hastie, 1980; Nassar & Touma, 1986; Kjeresem & Jepsen, 1990; Longfield et al., 1990). In the US, outbreaks of varicella among military recruits from Puerto Rico have highlighted the increased susceptibility of this adult population. Forty-two percent of 810 recruits from Puerto Rico tested after one outbreak were seronegative, a much higher percentage than in US-born recruits (Longfield et al., 1990).

The reasons for the differences in varicella age-specific incidence and VZV seroprevalence observed in tropical compared to temperate climates are unclear. Suggested reasons include differences in population size, population density, an "island" phenomenon comprised of decreased likelihood of exposure, lack of exposure to the infectious agent in confined spaces during the winter months and higher ambient temperatures and humidity, resulting in decreased transmission in the tropics. Because such differences are not observed for other highly contagious diseases such as measles, and varicella-zoster virus is known to be heat labile, the most plausible explanation is that heat diminishes the ability of the virus to survive in the environment and thereby decreases transmission. To address the effect of population density on disease transmission in tropical climates, Mandel compared rates of VZV seropositivity between urban and rural Bengalese populations living in identical climatic conditions. Only 3.4% of the urban adults aged >25 years were seronegative compared with 31% of the rural adults (Mandal et al., 1998). Ninety-six percent of urban adults were immune by age 25 compared with 42% of rural adults. These data suggest that the higher susceptibility seen in the tropics is a rural phenomenon and is due to reduced transmission. Crowding in densely populated urban cities may overcome VZV's diminished ability to spread in warm climates. Further studies of urban–rural differences including secondary transmission within families in tropical climates are needed.

Hospitalizations and mortality

Studies of varicella hospitalizations in tropical climates describe a high proportion of hospitalized cases among adults and also males, which may, in part, reflect hospital admission practices. A comparison of hospital admissions to the Fever Hospital in Angoda, Ceylon, between 1955 and 1959 with those to an infectious diseases ward in Czechoslovakia (1955–1961) found a much higher proportion of admissions and deaths among adults in Angoda (Maretic & Cooray, 1963). Seventy-two percent of the hospitalizations were adults $\geq$20 years of age and 74% were male. Similarly, in Ibadan City, Nigeria, a review of admissions to a communicable disease hospital between 1970 and 1980 showed the majority of hospitalizations occurred in persons $>$15 years (65%) and 75% of the admissions occurred among males (Iyun, 1986).

Although case reports indicate serious disease among adults from tropical climates, no population-based mortality rates or case-fatality rates are available for comparison with data from temperate climates. Additionally, if such data were available, differences in availability and use of antiviral therapies and passive immunization with VZIG would make interpretation of such comparisons difficult.

Changes in epidemiology with vaccination

Programs for universal varicella vaccination of infants are expected to result in changes in the epidemiology of varicella. For programs that achieve high vaccination coverage, the most dramatic effect will be a marked reduction in varicella cases, severe complications and hospitalizations among vaccinated children. Among the remaining greatly reduced number of varicella cases, a higher proportion is expected to occur among older persons, as was seen following the introduction of vaccines against measles, mumps and rubella. The shift in the proportion of cases to older persons will be minimized by catch-up vaccination of adolescents and adults (Halloran et al., 1994). Routine childhood vaccination programs with high coverage will be the most effective strategy for interrupting disease transmission and reducing varicella mortality and morbidity in both temperate and tropical climates. Achieving high vaccination coverage among children will provide the additional benefits of herd immunity with protection of susceptible adults, infants and other persons at high risk for severe varicella disease who are not eligible for vaccination. In contrast, vaccination programs targeting only adolescents and adults will have little impact on the epidemiology of varicella in temperate climates. In the US such a targeted program, assuming that all susceptible adults could be vaccinated, would be expected to result in only a 5–10% decline in cases, a 33% decline in hospitalizations and a 50% decline in deaths (CDC, unpublished data).

In tropical countries, providing vaccine for susceptible adolescents and adults in addition to infants and children will be more important because a higher proportion of adults are likely to be susceptible. Surveillance for varicella will be essential for monitoring the impact of varicella vaccination programs and for adjusting vaccination strategies and policies as needed.

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11

Clinical manifestations of varicella

Philip LaRussa

Introduction

Varicella is a highly contagious disease of childhood. Virus is spread by the airborne route from the skin lesions and oropharynx of infected individuals to susceptible contacts (Thomson, 1919; Leclair et al., 1980; Gustafson et al., 1982). Secondary clinical attack rates in susceptible siblings after a household exposure to varicella are as high as 90% (Ross et al., 1962) and may also be high in closed populations of children such as schools (Wells & Holla, 1950). Humans are the only natural host for this virus, although there is one report of a gorilla in captivity contracting varicella from a handler with lesions (Myers et al., 1987). This chapter reviews the clinical manifestations of varicella in healthy and immunocompromised patients.

Pathogenesis

After contact with the mucosa of the upper respiratory tract or the conjunctiva, the virus invades and is thought to replicate in the local lymphoid tissue. Four to 6 days later, a small primary viremia results in spread to reticuloendothelial cells in the liver, spleen and other organs, where there is further replication until 10 to 12 days after exposure (1±2 days before development of rash). At that time, a more significant secondary viremia results in spread of the virus to the skin. Varicella-zoster virus (VZV) has been isolated from human mononuclear cells up to 5 days prior to onset of rash (Asano et al., 1985a,b). It is during this secondary viremic phase that the prodromal symptoms of fever, malaise, and irritability are seen and herald the onset of rash. These prodromal symptoms are more pronounced in adolescents and adults than in children, who may have no prodrome.

Healthy children and adults

After an average incubation period of 14 to 16 days (range 10 to 20 days) following infection, lesions develop in three or more successive waves for 3 to 7 days (Gordon

& Meader, 1929; Hope-Simpson, 1952; Preblud et al., 1984). Development of new lesions for more than 7 days should provoke consideration of an underlying immunodeficiency. Lesions progress through macular, papular, vesicular, and pustular stages, but not all lesions progress to the vesicular stage and beyond. Vesicles may dry from the center outwards, resulting in an umbilicated appearance. Eventually scabs form, which usually heal without permanent scarring unless secondary bacterial infection occurs. Vesicles also may develop on mucous membranes (oropharynx, conjunctiva, trachea, vagina and rectum) but rapidly rupture to form shallow ulcers that heal without forming scabs. Rash is often more severe and lesions become confluent in areas of skin where there is local irritation (e.g. diaper rash, under an adhesive bandage).

Skin lesions appear on the trunk, face, scalp, and extremities, with the greatest concentration on the trunk. On the extremities, lesions are more concentrated proximally compared to distally. A hallmark of varicella is the presence of lesions in all stages of development at the same time. Healthy children develop an average of approximately 300 skin lesions. (Ross et al., 1962) but the range may vary from as few as five to 10 to over 1000. Secondary cases after a household exposure are usually more severe than primary cases (Ross et al., 1962; Balfour et al., 1990). This probably reflects the greater intensity of exposure in this setting compared to more casual contact outside the home. Older adolescents and adults are likely to have more severe cutaneous disease than children.

Fever often accompanies the rash. Body temperature usually parallels the severity of rash, and may range from normal to greater than 105°F (40.5°C). As the appearance of new lesions slows, fever begins to decline. Other common symptoms include pruritus that may be severe when skin lesions are extensive, headache, malaise, and anorexia.

Clinical manifestations due to involvement of other organs are limited, but otherwise asymptomatic elevations of hepatic transaminases have been reported (Pitel et al., 1980; Myers, 1982). Dehydration secondary to poor oral intake and vomiting occasionally are severe enough to warrant hospitalization (Peterson et al., 1996a).

Based on studies with varicella vaccine (Tsolia et al., 1995), it seems likely that children with extensive rashes are more likely to infect others than those with mild rash. In a household setting where prolonged close exposure may be the rule, however, transmission can occur even with mild rash. Children are usually considered infectious to others for the first 5 days of rash, but also for as long as new lesions continue to appear and while lesions are moist. Transmission may also occur in the 24 to 48 hour period prior to the appreciation of rash.(Gordon & Meader, 1929; Evans, 1940; Brunell, 1989) This phenomenon is probably due to spread of virus in large droplets from the oropharynx prior to development of skin

lesions, but attempts to isolate virus from the oropharynx at this time have been unsuccessful (Nelson & St Geme, 1966; Trlifajova et al., 1984; Brunell, 1989; Ozaki et al., 1989). Isolation of virus from the oropharynx has been demonstrated on rare occasions within the first few days after onset of rash (Gold, 1966; Cesario et al., 1971; Myers, 1979; Ozaki et al., 1989). It is unclear whether this difficulty is due to a paucity of virus in the oropharynx or to inhibition of viral growth by local factors.

Second attacks of varicella are reported but rarely documented. They are usually milder than the primary illness. Subclinical reinfections have been documented in varicella-immune individuals after exposure to VZV (Luby et al., 1977; Arvin et al., 1983; Gershon et al., 1984a, 1988, 1989) and chickenpox has occurred in individuals with VZV-specific antibodies prior to the onset of clinical illness (Weller, 1983; Zaia et al., 1983; Gershon et al., 1984b, 1988, 1989 ; Junker et al., 1991; Junker and Tilley, 1994). Although second attacks are more common in immunocompromised children, they have also been seen in healthy children and adults.

Skin and soft tissue complications

The most frequent complications of varicella in healthy children are secondary bacterial infections of skin lesions, usually due to *Staphylococcus* or *Streptococcus*. These can range in severity from impetiginized lesions and bullous impetigo to cellulitis and erysipelas. Scalded skin and toxic shock syndromes secondary to chickenpox have also been reported (Melish, 1973; Wald et al., 1973; Bradley et al., 1991). Septicemia, pneumonia, empyema, osteomyelitis, fasciitis, and supporative arthritis have also been described. In the pre-vaccine era, over 90% of cases of varicella occurred in children under the age of 10 years. Many complications also occur at an early age. In one study, the mean age of children hospitalized with complications of varicella was 3.8 years (Peterson et al., 1996a). Universal immunization of children at 12 to 18 months of age will prevent most cases of varicella and therefore should greatly reduce the burden of complications. Selective immunization of older adolescents and adults would be expected to have much less of an impact on complications of varicella.

Approximately one-third of invasive Group A streptococcal (GAS) infections in children are temporally associated with varicella (Kiska et al., 1997; Givner, 1998). M1 and M3 serotypes predominate (Vugia et al., 1996; Kiska et al., 1997; Givner, 1998). The risk for invasive disease is highest in the 2 week period following onset of chickenpox (Davies et al., 1996). In a series of 24 cases, invasive GAS disease occurred within 3 to 6 days after onset of varicella (Vugia et al., 1996). The most common presentation was cellulitis, followed by pneumonia, necrotizing fasciitis, and bacteremia without an obvious source. Four of the 24 children died, either at home or on presentation to the hospital. Necrotizing fasciitis associated with vari-

cella often requires surgical debridement, fasciotomy, and systemic antibiotic therapy (Brogan et al., 1995; Wilson et al., 1995).

It has been suggested that the risk of invasive GAS in children with chickenpox is increased by the use of ibuprofen, although an association has not been proven (Peterson et al., 1996b; Zerr et al., 1999). Other nonsteroidal anti-inflammatory drugs have been shown to adversely affect leukocyte function in vitro (MacGregor et al., 1974; Solberg, 1974; Kjosen et al., 1976; Solberg et al., 1978). It has also been proposed that ibuprofen increases levels of cytokines (IL-1, IL-6 and TNF-α) that are associated with an increased risk of death in patients with sepsis (Zerr et al., 1999).

While complications due to secondary skin infections are more common in children, varicella pneumonia is a more common complication in adults (Choo et al., 1995; Peterson et al., 1996a). It is estimated that pneumonia accounts for hospitalization in one in every 400 cases of varicella in adults (Guess et al., 1986). Pneumonia usually presents 1 to 6 days after onset of rash. In one study, the relative risk of pneumonia was 2.3 in adults 35 years of age and older, compared with younger adults. Smoking may further increase the risk of pneumonia (Ellis et al., 1987; Pugh et al., 1998).

In a study of military recruits with varicella, 16% had evidence of pneumonia on chest x-ray, usually a patchy or diffuse bilateral nodular infiltrate (Triebwasser et al., 1967). Only one-quarter of those with abnormal x-rays had clinical symptoms. Pneumonia usually presents with fever, cough, and dyspnea. Physical signs include tachypnea, bronchial breath sounds, and wheezing. Laboratory abnormalities include leukocytosis, hypoxia, and thrombocytopenia. The usual radiographic pattern is consistent with a diffuse viral pneumonia, but bacterial pulmonary superinfection may also occur, which may have a different radiological appearance. While the availability of effective antiviral therapy probably reduces the risk of hospitalization and death from varicella pneumonia, failure to recognize the potential for severe disease has led to delays in instituting therapy and deaths (Anonymous, 1997). Mortality of varicella primary pneumonia has been reported to range from 10 to 30% in healthy adults (Triebwasser et al., 1967; Anonymous, 1984; Ellis et al., 1987; Pugh et al., 1998) to as high as 40% in pregnant women (Harris & Rhoades, 1965). Bacterial superinfection also increases the risk of mortality.

Neurologic complications

These are divided into three categories: Reye s syndrome, cerebellar ataxia, and encephalitis not presenting as Reye s syndrome or ataxia.

In 1984, Preblud estimated that the risks of encephalitis and Reye s syndrome

were 1.7 and 3.2 respectively per 100 000 cases per year in normal children 1 to 14 years of age (Preblud et al., 1984). Before the causal association between salicylate use in viral illnesses and Reye s syndrome was appreciated, there were approximately 350 cases of Reye s syndrome reported annually in the United States, 20 to 30% of which were associated with varicella (Nelson et al., 1979; Hurwitz et al., 1982). There is some confusion in the older literature about the distinction between Reye s syndrome and primary varicella encephalitis, and sometimes these terms are used interchangeably. Some reports of varicella encephalitis were, in retrospect, actually Reye s syndrome (Johnson & Milbourn, 1970). Reye s syndrome is rare today; in a recent review of complications of varicella, no cases were reported (Choo et al., 1995). Unfortunately, some children still receive aspirin during varicella, resulting in rare, but preventable cases of Reye s syndrome (Peterson et al., 1996a).

Cerebellar ataxia is the most common neurologic complication of varicella in children (Applebaum et al., 1953; Peters et al., 1978), occurring in 1 out of 4000 cases (Guess et al., 1986). Symptoms usually develop between 3 and 8 days after the onset of rash, although rarely symptoms develop a few days before the onset of rash (Wagner et al., 1998). Ataxia may be associated with nystagmus, headache, nausea, vomiting, and nuchal rigidity. The course is self-limited and recovery is complete.

Encephalitis due to VZV is more rare in children (1 in 33 000 cases) and may present with depressed levels of consciousness, seizures, headache, and vomiting (Guess et al., 1986). Signs of meningial irritation, cerebral edema, and focal abnormalities such as hemiparesis and aphasia may develop. Variation in reported mortality rates range from 0 to 35%, and may reflect inclusion of cases of Reye s syndrome in this group. Neurologic sequelae, such as paresis, mental retardation, and development of a seizure disorder, may be seen in up to 15% of the survivors (Echevarria et al., 1997).

Other neurologic complications include transverse myelitis (White, 1962; McCarthy & Amer, 1978; Rosenfeld et al., 1993; Gilden et al., 1994), aseptic meningitis, Guillian-Barrè syndrome, optic neuritis and vasculitis, and stroke (Eda et al., 1983; Kamholz & Tremlay, 1985; Hosseinipour et al., 1998; Ichiyama et al., 1990) Febrile seizures, without other neurologic manifestations, have also been reported (Peterson et al., 1996a).

Neurologic complications in adults are more likely to present as encephalitis with altered sensorium, seizures, and focal neurologic signs, with mortality as high as 35% (Straus et al., 1988).

The pathogenesis of central nervous system (CNS) disease associated with varicella is not well defined. While it is extremely difficult to isolate VZV from the cerebrospinal fluid, it is often possible to demonstrate VZV DNA and specific antibodies (Gershon et al., 1980; LaRussa et al., 1995). Cerebellar ataxia develops

at a mean of 5.5 days after the onset of rash, just about the time that an effective immune response occurs (Johnson & Milbourn, 1970). It is attractive to speculate that manifestations such as cerebellar ataxia are a result of a specific postinfectious immune response to the presence of virus in the CNS. In rare instances, neurologic symptoms develop prior to onset of rash, implying that the virus may also be capable of causing direct damage, independent of the immune response to VZV (Underwood, 1935; Goldston et al., 1963; Jackson et al., 1992; Liu & Urion, 1992; Dangond et al., 1993; Wagner et al., 1998).

Other complications

These include nephritis, uveitis, arthritis, carditis, conjunctivitis, orchitis and inappropriate antidiuretic hormone secretion syndrome. Hemorrhagic varicella with multi-organ system failure is rare in healthy children, but its outcome is usually fatal (Anonymous, 1998, 1999)

Mortality

In recent years, varicella has been responsible for about 50 annual deaths in children 15 years of age and younger (Anonymous, 1998). With approximately 4 million annual cases, this is a low case fatality rate. While adults are responsible for less than 5% of varicella cases, over half of the deaths due to varicella each year occur in adults (Anonymous, 1997).

Differential diagnosis

Impetigo, insect bites, scabies, rickettsialpox, eczema herpeticum, Stevens± Johnson syndrome, hand±foot and mouth disease, disseminated zoster, and disseminated herpes simplex infection should be considered in the differential diagnosis of vesicular rash in children.

Immunocompromised patients

Varicella in the immunocompromised host is more likely to be a severe disease with multi-organ system involvement than in otherwise healthy persons. Feldman described varicella in 77 children with cancer (Feldman et al., 1975). There were no complications in the 17 children who had completed chemotherapy at least 2 months prior to onset of chickenpox. However, of 60 children who were still receiving chemotherapy when varicella developed, 19 had disseminated disease (32%) and four (7%) died. All four children who died had pneumonia and two also had encephalitis. The risk of disseminated disease increased with absolute lymphocyte counts less than 500 per cubic millimeter (Feldman, 1986). Pathologic findings at autopsy of fatal disseminated disease included interstitial pneumonia, hepatitis, splenic involvement, lymphadenitis, enterocolitis, and pancreatitis (Miliausakas &

Webber, 1984). In contrast to healthy children, immunocompromised children with varicella are more likely to require hospital admission and antiviral therapy for pneumonia than for skin and soft tissue infections (Peterson et al., 1996a). An unusual presentation of varicella in immunocompromised children and adults is severe abdominal and back pain that precedes the onset of rash and disseminated disease (Morgan & Smalley, 1983; Milone et al., 1992). The pathogenesis of this pain is not clear.

Initial reports of varicella in HIV-infected children described a high rate of severe, disseminated disease. In addition to being retrospective, most of these reports also involved only patients requiring hospitalization, leading to a skewed picture of morbidity and mortality. In one early study, new skin lesions often appeared for up to 6 weeks after onset of rash and recurred despite antiviral therapy (Jura et al., 1989). Children with more than 400 skin lesions at presentation and absolute CD4 counts of less than 200 per cubic millimeter were more likely to have prolonged or recurrent disease. Up to 40% developed complications, usually either an invasive bacterial infection or pneumonia. One of nine children in one study (Jura et al., 1989) and two of 38 in another, died (Leibowitz et al., 1993). A subsequent study showed that more than half of HIV-infected children with varicella developed a recurrence of either varicella or zoster, most within 24 months of onset of primary infection (von Seidlein et al., 1996).

Subsequent prospective studies in HIV-infected children revealed that while the illness was more extensive than in healthy children, fatalities and severe complications from varicella were unusual. These same studies showed that the relative immunocompetence of HIV-infected children at the time of varicella correlated strongly with the subsequent risk of developing zoster. In one study, 70% of HIV-infected children with less than 15% CD4+ lymphocytes at the time of varicella developed zoster (Gershon et al., 1997). A subsequent study in a larger group of children confirmed these findings (Derryck et al., 1998). The incidence of zoster in this group of children was even higher than that seen in HIV-infected adults with prior varicella and less than 200 CD4+ lymphocytes (Gershon et al., 1997). Since most individuals develop varicella by the age of 10 years, primary varicella in HIV-infected adults is rare. In the few reported cases, the clinical presentation and outcome mirror that seen in HIV-infected children (Baran et al., 1997).

Other groups of immunocompromised patients at risk for severe varicella include those who have undergone bone marrow, renal (Lynfield et al., 1992; Kitai et al., 1993; Furth et al., 1997; Kashtan et al., 1997) and liver (Breinig et al., 1987; Alonso et al., 1989; McGregor et al., 1989; Kitai et al., 1993) transplantation. Children receiving high dose corticosteroid therapy (≥ 2 mg/kg/day or ≥ 20 mg/day in children ≥ 10 kg, of prednisone or the equivalent, for more than 14 days) may also be at risk for severe varicella (Committee on Infectious Diseases, 1997).

There is conflicting evidence as to whether children with reactive airway disease (RAD) are at higher than normal risk for severe varicella. Numerous case reports describe severe or fatal varicella in children with asthma (Haggerty & Eley, 1956; Silk et al., 1988; Lantner et al., 1990) A number of small series, however, describe patients with RAD and varicella who were not unusually ill (Grater, 1963; Weinberg & Turchinda, 1973). In one case-control study, children with severe varicella were not more likely to have either a diagnosis of asthma or to have received corticosteroid therapy within the 30-day period prior to onset of rash than control children with uncomplicated varicella (Patel et al., 1996). However, in another case-control study, children with varicella and invasive Group A streptococcal disease were 6.2 times more likely to have asthma than controls with uncomplicated varicella (6/25 vs. 3/62; odds ratio 6.21; 95% confidence interval, 1.16±41.03) (Peterson et al., 1996b). In a retrospective cohort study of vaccine effectiveness during a varicella outbreak in a day care center, varicella occurred more frequently in unvaccinated children with RAD compared to those without RAD (8/8 vs. 64/74; OR 1.2; 95% CI, 1.1±1.3) (Izurieta et al., 1997).

It is also unclear whether corticosteroids, especially at the doses and duration of treatment used in children with RAD, increase the risk of severe varicella. Case reports, retrospective studies, and reviews have suggested that children receiving more than 1 mg/kg/day of systemic corticosteroids are at risk for severe or fatal varicella (Reiches & Jones, 1993). A case-control study suggested that children with severe varicella were more likely to have received systemic corticosteroids within a period of 30 days before to 7 days after onset of rash than a group of control children. When the analysis was restricted to nonimmunocompromised children, the odds ratio for steroid use was 178 in the children with severe varicella compared to the control group (Dowell & Bresee, 1993). The validity of this study was questioned, however, because of concerns about the appropriateness of the control group. Interpretation of these studies is complicated by the observation that even healthy children occasionally develop severe or fatal varicella.

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12

Epidemiology of herpes zoster

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Introduction

Dr. Edgar Hope-Simpson, a premier herpes zoster epidemiologist, fittingly described the allure and difficulties of herpes zoster epidemiology when he noted that "herpes zoster is fascinating because it arrives unpredictably . . . and is difficult to explain" (Hope-Simpson, 1965). Epidemiology seeks to explain and comprehend diseases by studying the characteristics of diseases in populations. Those characteristics include morbidity, mortality, incidence and prevalence rates, and risk factors. The objective of this chapter is to summarize these descriptive and analytical data on the epidemiology of herpes zoster to help the reader better understand this fascinating disorder.

The sources of zoster epidemiological data include cohort studies, case-control studies, large case series and case reports. Cohort studies have the strongest research design and will be emphasized, where available, in the presentation of descriptive and analytical information about zoster. Large, multi-year cohort studies of zoster in the general population are summarized in Table 12.1. The populations in these studies were mostly composed of immunocompetent individuals but did include a small percentage of immunosuppressed individuals. All studies used medical records in one fashion or another to ascertain cases of zoster and all zoster cases were drawn from a larger, well-defined, community-dwelling population. However, the methods of case ascertainment and the populations differed among studies. Hope-Simpson (1965) kept careful practice records on all patients who developed zoster over a 15 year period in the population of Cirencester, England. Ragozzino et al. (1982a) collected data on zoster from the central index data system and medical records of all of the residents of Rochester, Minnesota, US for a 15 year period. Guess et al. (1985) employed the same database to report on zoster in children and adolescents over a 21 year period. Donahue et al. (1995) searched the automated medical record system and computerized claims files of a large health maintenance organization for cases of zoster over a 5 year period in Boston, Massachusetts, US. Schmader et al. (1995) used self-report

Table 12.1 Large cohort studies of herpes zoster

Author & date	Setting & time	Population			No. cases	Zoster incidence per 1000 person-years		
		No.	Person-years	Age		All ages	Elderley (age)	Children (age)
Hope-Simpson, 1965	Cirencester, England 1947–62	3534	53010	0–99	192	3.4	7.8 (60–99)	0.74 (0–9)
Ragozzino et al., 1982a	Rochester, Minnesota, US 1945–59	31544	473170	0–75+	590	1.25	3.9 (65–75+)	0.4 (<14)
Guess et al., 1985	Rochester, Minnesota, US 1960–81	19535	410244	0–19	173	–	–	0.42 (0–19)
Donahue et al., 1995	Boston, US 1990–92	250204	500408	0–75+	1075	2.15	11.8 (65–75+)	0.47 (<14)
Schmader et al., 1995	North Carolina, US 1987–90	3206	9618	65–104	69	–	7.1 (65–104)	–
Helgason et al., 1996	Iceland 1990–95	45909	229547	0–80+	457	2.0	4.6 (60–80+)	–

on a standardized interview and medical records to ascertain zoster over a 3 year period in the Duke Established Populations for Epidemiological Studies of the Elderly (EPESE), a random sample of elderly individuals residing in a five-county area in North Carolina, US. Helgason et al. (1996) utilized the computerized medical record system from 44% of all general practitioners to ascertain zoster in nearly one-third of the population of Iceland over a 5 year period. Not listed in the table, Cooper (1987) reported zoster incidence rates for 1967–1983 from the weekly returns service of the Royal College of General Practitioners Research Unit, which by 1983 covered a population of over 200 000 in practices throughout the United Kingdom (UK). However, data were not provided on the number of cases, person-years of observation, or basic demographics of the population. Several smaller out-patient practice studies have also contributed useful information about zoster (Burgoon & Burgoon, 1957; McGregor, 1957; Hellgren & Hersle, 1966; Molin, 1969; Brown, 1976; Christensen & Norrelund, 1985; Wilson, 1986; Trollor, 1987; Glynn et al., 1990; Paul & Thiel, 1996; Richards, 1996; Goh & Khoo, 1997; Torrens et al., 1998).

Incidence and prevalence

The incidence of herpes zoster in community dwelling populations ranges from 1.2 to 3.4 per 1000 person-years (Table 12.1). Using these figures, investigators estimate that as many as 600 000 of cases of zoster occur in the US each year and that 120 000 cases occur in the UK each year. In the elderly (above 65 years old), investigators have reported an incidence of zoster that ranges from 3.9 to 11.8 per 1000 person-years (Table 12.1). The reason for the nearly three-fold difference in the incidence of zoster between studies in all ages and the elderly is not clear but it is probably secondary to differences in study methodology as much as an indication of true population differences. The incidence of zoster in populations limited to immunosuppressed patients is substantially higher (discussed below). All zoster studies are subject to bias in incidence rate calculations because of under-ascertainment of cases (some cases may not have sought medical care or may have visited a doctor outside the system of data collection), over-ascertainment (a small percentage of clinically diagnosed zoster cases are due to herpes simplex or other causes) and imprecise denominators (movement of people in and out of a population). However, these biases are likely to be small and the above cohort studies represent the best available estimates of zoster incidence. The likelihood of recurrent zoster was 4.1% (8 patients with recurrence out of 192 zoster cases), 5.2% (31 of 590), and 1.7% (18 of 1075) in the studies of Hope-Simpson (1965), Ragozzino et al. (1982a) and Donahue et al. (1995), respectively. The prevalence rate of zoster has not been reported because zoster is an acute, transient illness and the data are difficult to obtain.

Morbidity and mortality

The morbidity of zoster includes acute and chronic pain, neurological complications, ocular events, cutaneous problems and visceral involvement. Investigators have documented the frequency of these events in some of the above cohort studies and in zoster patients from single outpatient practices. Pain lasting one month or more after rash onset has been reported in 8–14% of zoster patients of all ages (Hope-Simpson, 1975; Ragozzino et al., 1982a; Helgason et al., 1996; Choo et al., 1997). In general population cohort studies, the type and estimated frequency of neurological complications include motor neuropathy (1%), cranial polyneuritis (less than 1%), transverse myelitis (less than 1%), meningoencephalitis (less than 1%), and cerebral angiitis and stroke after ophthalmic zoster (less than 1%) (Ragozzino et al., 1982a; Galil et al., 1997). Ocular complications, including keratitis, uveitis, iridocyclitis, panophthalmitis, and glaucoma, have been reported in 2–6% of cases (Burgoon & Burgoon 1957; Ragozzino et al., 1982a; Galil et al., 1997). Serious cutaneous problems, such as bacterial infection and herpes gangrenosum, occurred infrequently in these series, ranging from zero to 2%. Visceral involvement was not noted in these studies. Zoster complications afflict elderly and immunosuppressed patients with much greater frequency (discussed below).

Based on clinical experience and the absence of zoster-related death in cohort studies, mortality due to zoster appears to be an infrequent event. However, reliable data on zoster mortality rates are not available, not only because it is an unusual event but also because public health officials do not systematically record zoster-related deaths in most countries. Furthermore, death statistics that rely on reports from practitioners are potentially biased by underestimation (zoster-related deaths are not routinely reported), overestimation (death may be from another cause) and lack of a proper denominator to calculate rates. Nonetheless, 1064 zoster-related deaths were reported in 1982–1990 in the US (Weller, 1997). In England and Wales, over 100 zoster-related deaths are estimated to occur each year (Miller et al., 1993).

Geographic and temporal distribution

Geographic

Varicella-zoster virus (VZV) maintains latency and reactivates to cause zoster in populations worldwide but there are no data to indicate that the incidence of zoster differs significantly by geographic region. However, large population based studies of zoster have not been performed in many parts of the world. Given the later onset of varicella in some tropical climates, zoster may occur later in life in persons residing in these areas but this notion requires confirmation from careful epidemiological study of zoster in these populations (Weller, 1983).

Another potential geographic difference is residence in an urban versus rural environment because exposure to factors that influence VZV reactivation may differ in these environments. McGregor reported the occurrence of zoster over a 7 year period in 2400 patients who were two-thirds suburban and one-third rural. Of suburban patients, 3.4% developed zoster compared to 3.3% of rural residents (McGregor, 1957). In the Duke EPESE, urban residence did not increase the lifetime risk of zoster compared to rural residence (odds ratio, 1.01; 95% confidence interval [CI] 0.88–1.07) (Schmader et al., 1995). Thus, available data indicate that site of residence does not affect risk of zoster.

Temporal

Zoster occurs sporadically throughout the year without a seasonal pattern in contrast to the well-known seasonal pattern of varicella in temperate zones (Ragozzino, 1982a; Weller, 1983; Cooper, 1987; Paul & Thiel, 1996). For example, Cooper reported varicella and zoster occurrence by week in 1983 to 1985 in the UK. Within the same population, the incidence of zoster stayed level at 5 cases per 1000 person-years throughout the year 1984–1985 while the incidence of varicella had a well-defined springtime peak as high as 20 cases per 1000 person-years and an autumn trough of roughly 6 per 1000 person-years (Cooper, 1987).

Another temporal issue is whether the incidence of zoster is increasing over time. Prospective population-based data over several decades are not available to answer this question convincingly. The incidence of zoster increased from 1.12 to 1.31 per 1000 person-years in Rochester, Minnesota, US from 1945 to 1959 (Ragozzino et al., 1982a). In addition, the incidence of zoster reported in Boston, US in 1990–1992 was much higher than the incidence reported in Rochester, Minnesota, US, even after excluding zoster cases associated with HIV infection and cancer (Donahue et al., 1995). In a report of zoster cases in 1955–1985 in rural Scotland, the number of cases increased each year between 1969 and 1982 but the number of cases declined after 1982 (Wilson, 1986). However, the incidence of zoster showed no yearly increase in 1947–1962 in England or 1967–1983 in the UK (Hope-Simpson, 1965; Cooper, 1987).

Risk factors and special populations

Age

The cardinal epidemiological feature of herpes zoster is its striking increase in incidence with aging. The increase is so great that the majority of zoster cases occur in the elderly. However, zoster strikes persons of all ages and differs in its characteristics in children.

Elderly

All studies of zoster epidemiology consistently document a sharp increase in the likelihood of zoster with aging as well as a high number of zoster cases in the elderly (Figure 12.1). Hope-Simpson (1965) documented an incidence of 0.74 per 1000 person-years in children under 10 years old, 2.5 per 1000 person-years in adults aged 20–50 years and 7.8 per 1000 person-years in those over 60 years old. Ragozzino et al. (1982a) found a similar dramatic increase with aging in Minnesota, where the incidence of zoster was less than 1 per 1000 person-years under 44 years old but peaked at 4 to 4.5 per 1000 person-years in persons over 75 years old. Donahue et al. (1995) confirmed this relationship when they reported an incidence of 1.9, 2.3, 3.1, 5.7 and 11.8 per 1000 person-years for the age groups 25–34, 35–44, 45–54, 55–64 and 65–75+ years, respectively.

The age at which the sharpest increase in zoster occurs is around 50 to 60 years (Figure 12.1). Furthermore, the slope continues a marked upward course over the age of 60 years. In the Duke EPESE, incidence rates similar to the above studies were reported for elderly persons and the lifetime risk of zoster increased significantly with age even among elderly individuals (odds ratio, 1.20 for every 5 years; 95% CI, 1.10–1.31) (Schmader et al., 1995). These studies estimate the lifetime incidence of zoster to be 10–20% in the general population (Ragozzino at al., 1982a; Straus, 1993; Donahue et al., 1995; Schmader at al., 1995) and as high as 50% of a cohort surviving to age 85 years (Hope-Simpson, 1965). Given these figures and population aging, the total number of zoster cases worldwide will increase significantly in the future.

Another noteworthy age-related epidemiological feature of zoster is an increased prevalence of postherpetic neuralgia (PHN) with aging. For example, the prevalence of pain one or more months after rash onset was zero in the age groups 0–29 years, 3–4% in age groups 30–49 years but 21%, 29%, and 34% in the age groups 60–69, 70–79, and greater than 80 years, respectively, in Cirencester, England (Hope-Simpson, 1965). In Rochester, Minnesota, US, the average age of zoster patients with PHN was 67 years compared to the average age of 46 years in the remainder of the cohort ($P<0.00001$) (Ragozzino et al., 1982a). In Boston, US, patients aged 50 years or older had a 14.7-fold higher prevalence (95% CI, 6.8–32.0) of pain 30 days after rash onset compared to patients younger than 50 years (Choo et al., 1997). In Iceland, 2%, 15%, and 41% of zoster patients aged 0–39, 40–59 and 60 years and older, respectively, had pain more than one month after rash onset (Helgason et al., 1996).

Children

Although much less frequent than in elderly individuals, the incidence of zoster in children and adolescents ranges from 0.42 to 1.06 per 1000 person-years in ages

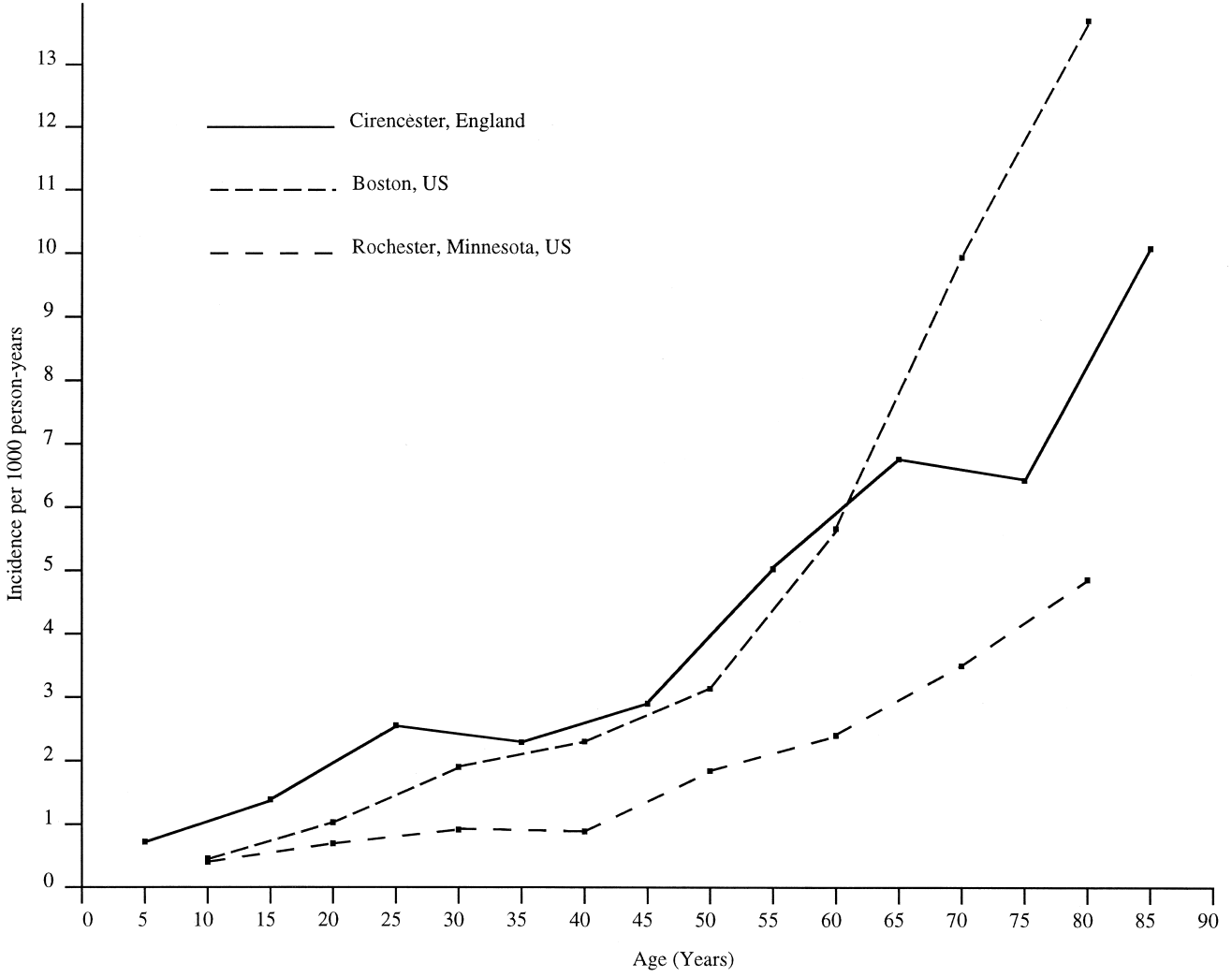

Figure 12.1 Incidence rates of herpes zoster by age in Cirencester, England; Boston, US, and Rochester, Minnesota, US, adapted from the studies of Hope-Simpson, 1965; Donahue et al., 1995; and Ragozzino et al., 1982a, respectively. Zoster incidence rates were reported in 10 year age intervals in these studies. The rates are plotted on the mid-point of each interval in the figure.

0–19 years (Hope-Simpson, 1965; Guess et al., 1985; Donahue, 1995). In children aged 0–9 years, Hope-Simpson (1965) reported an incidence of 0.74 per 1000 person-years and Guess et al. (1985) reported an incidence of 0.25 per 1000 person-years. The incidence rates of zoster in children are affected by the absence of primary VZV infection in some children, diminished immune competence during infancy, and less available time for VZV reactivation compared to adults.

Most studies of zoster in children are case reports and small case series but they are remarkably consistent with respect to two results. First, childhood zoster is a much milder disease in immunocompetent children than in adults, especially with respect to the absence of chronic pain (Brunell et al., 1968; Rogers & Tindall, 1972; Guess et al., 1985; Smith & Glaser, 1996; Kakourou et al., 1998). Second, primary VZV infection of the mother during pregnancy or the child during the first year of life increases susceptibility to zoster during childhood (Timothy & Williams, 1979; Dworsky et al., 1980; Latif & Shope, 1983; Guess et al., 1985; Baba et al., 1986; Terada et al., 1993; Kakourou et al., 1998; Gershon, 1999). In the largest and best designed epidemiological investigation of zoster in childhood, there were no cases of postherpetic neuralgia and 9% of patients had chickenpox in the first year of life compared to an expected 3.3% ($P<0.002$) (Guess et al., 1985). The authors conservatively estimated that children who experienced chickenpox during the first year of life were 2.8 (95% CI, 1.6–4.7) times more likely to develop zoster during childhood than children who experienced chickenpox after the first year of life. Of 173 childhood zoster cases in this study, only 3% occurred in children with malignancy. There was only one case of cancer in 1288 person-years of follow-up, which was no different than that expected from age-specific incidence rates in Rochester, Minnesota, US.

Cellular immunosuppression

An important epidemiological feature of zoster is the increased frequency and complications of zoster in patients with suppressed cell-mediated immunity compared to immunocompetent individuals. Many authors have reported the markedly increased risk for zoster in patients with human immunodeficiency virus infection and/or acquired immunodeficiency syndrome (HIV/AIDS), certain cancers, organ transplants, immune-mediated diseases and immunosuppressive treatments.

HIV/AIDS

HIV infection is an exceedingly potent risk factor for the development and complications of zoster. Table 12.2 lists cohort studies of herpes zoster in HIV-infected individuals. The study populations consisted of HIV seropositive and/or AIDS patients from urban centers in Australia, France, the Netherlands or the US

Table 12.2 Cohort studies of herpes zoster in HIV infected patients

Author & date	Setting & time	No. HIV+	No. zoster cases	Zoster incidence	
				Per 1000 person-years	Cumulative % over time
Buchbinder et al., 1992	San Francisco, US 1987–92	287	58	29.4	30 after 12 years
Rogues et al., 1993	Bordeaux, France 1985–90	1757	101	34.5	6 after 20 months
Glesby et al., 1995	Baltimore, US 1990–93	1344	45	32.0	—
Holmberg et al., 1995	Chicago, Denver & San Francisco, US 1988–92	434	114	34.0	—
Veenstra et al., 1995	Amsterdam, Netherlands 1985–94	331	78	51.5	21 after 5 years (41 after 10 years)
Moore & Chaisson, 1996	Baltimore, US 1989–95	1246	51	38.0	9 after 3 years
McNulty et al., 1997	Sydney & Darlinghurst, Australia 1984–92	146	30	44.4	28 after 2 to 3 years

who either attended HIV clinics or were part of community dwelling cohort studies of homosexual men. Most studies were retrospective and ascertained zoster cases from medical records or patient self-report. The mean age of HIV infected patients in these studies was in the mid-thirties and most studies did not report total person-years of observation.

The incidence of zoster in HIV infected individuals varied from 29 to 51 per 1000 person-years, some 15–25 times greater than the incidence rates of zoster in the general population and 3–7 times greater than the incidence rates of zoster in the elderly (Table 12.2) (Buchbinder et al., 1992; Rogues et al., 1993; Glesby et al., 1995; Holmberg et al., 1995; Veenstra et al., 1995; Moore & Chaisson, 1996; McNulty et al., 1997). Some studies measured zoster incidence rates in non-HIV infected individuals from the same population as HIV infected individuals and found a high relative risk for developing zoster. For example, an age-adjusted relative risk of 16.9 (95% CI, 8.7–32.6) was reported in the San Francisco City Clinic Cohort and a relative risk of 15.3 (95% CI, 8.8–26.6) was reported in the Amsterdam Cohort Study (Buchbinder et al., 1992; Veenstra et al., 1995). The cumulative incidence of zoster in HIV infected persons is estimated to be 30–40% over approximately 10 years of follow-up.

HIV infection increases zoster risk at any age. For example, 27% of HIV infected children with a history of varicella after they were one year old developed zoster an average of 1.9 years after varicella occurred in one study (Gershon et al., 1997). However, increasing age may increase the risk for zoster even among HIV infected adults. In the San Francisco City Clinic Cohort, the incidence of zoster increased with age from an incidence of less than 40 per 1000 person-years in the age groups 20–29, 30–39, and 40–49 years to an incidence of 225 per 1000 person-years in the age group 50–59 years (Buchbinder et al., 1992). No other demographic or clinical risk factors for zoster in HIV infected individuals were reported in the above cohort studies.

Other topics are important in the epidemiology of zoster in HIV infected individuals. One issue is the relationship of zoster to duration of HIV infection. Small, early studies suggested that zoster occurred mostly early in the course of HIV infection but larger, later studies have shown that zoster can occur at any stage of HIV/AIDS (Friedman-Klein et al., 1986; Melby et al., 1987; Buchbinder et al., 1992; Rogues et al., 1993; Holmberg et al., 1996; McNulty et al., 1997). Another issue is whether zoster is a risk factor for progression to AIDS or death. Study results conflict on this point but most studies did not control for key immunological and virological indices such as CD4 count and viral load (Melby et al., 1987; Moss et al., 1988; Van Griensven et al., 1990; Buchbinder et al., 1992; Lindan et al., 1992; Rogues et al., 1993; Glesby et al., 1995; McNulty et al., 1997). One study which did control for CD4 count did not find that zoster was a risk factor for progression to

AIDS or death (Veenstra et al., 1995). However, the frequency of atypical skin lesions (3–11%), severe neurological complications (i.e., meningoencephalitis) (6–8%), ocular complications (6–11%) and a prolonged course of zoster is much higher in HIV infected patients than in the elderly or the general population (Glesby et al., 1995; Veenstra et al., 1996). Finally, multiple authors have documented that patients who presented with zoster in areas of high HIV prevalence often had underlying HIV infection, particularly in Central Africa, some regions of India, and in homosexual groups in US cities, suggesting that zoster may be the first clue to underlying HIV infection when it occurs in groups at high risk for HIV infection (Friedman-Klein et al., 1986; Sandor et al., 1986; Melby et al., 1987; Colebunders et al., 1988; Van de Perre et al., 1988; Dehne et al., 1992; Panda et al., 1994; Tyndall et al., 1995).

Cancer

Patients with malignancy represent another important population at risk for zoster. In community-dwelling cohort studies, cancer preceded zoster in 6% of the population and a past history of cancer significantly increased the lifetime risk of zoster in the elderly (Ragozzino et al., 1982a; Donahue et al., 1995; Schmader et al., 1995). Multiple authors have reported the frequency and clinical characteristics of zoster in cancer, particularly in Hodgkin's diseases, non-Hodgkin's lymphoma, leukemia, and lung cancer in retrospective case series (Goffinet et al., 1972; Schimpff et al., 1972; Wilson et al., 1972; Feldman et al., 1973; Monfardini et al., 1975; Dolin et al., 1978; Mazur & Dolin, 1978; Reboul et al., 1978; Green et al., 1979; Huberman et al., 1980; Feld et al., 1982; Guinee et al., 1985; Novelli et al., 1988; Rusthoven et al., 1988a; Poulsen et al., 1996; Bower et al., 1997). In the largest and strongest study of zoster in a cancer population, Rusthoven et al. collected data on zoster in the medical records of cancer outpatients and inpatients over 15 years old who attended a comprehensive cancer center in Ontario, Canada during 1972–1980 and had a minimum of five years follow-up data from the time of zoster diagnosis (Rusthoven et al., 1988a). From over 7000 new cases of cancer each year, they identified 766 episodes of zoster among 740 adult cancer patients, median age of 57 years (range 16–99 years). The cumulative incidence rate of zoster in this population five years after diagnosis was 62.5 per 1000 person-years. The cumulative incidence was highest in Hodgkin's disease (14%), leukemia (10%) and non-Hodgkin's lymphoma (5%). The risk of second episodes was highest among patients with hematologic malignancies. Among solid tumors, the cumulative incidence was highest in treated patients with breast (2%), lung (2%) and gynecologic malignancies (1%). The median age of zoster patients with hematologic malignancies was significantly younger than patients with solid tumors (51 vs. 59 years, $P<0.005$). Hodgkin's disease ($P<0.001$), non-Hodgkin's lymphoma ($P=0.016$)

and head and neck cancer ($P = 0.043$) were independent risk factors for disseminated zoster infection (Rusthoven et al., 1988b).

The risk for zoster is greater for patients with hematological malignancies than for solid tumors and is greatest for Hodgkin's disease compared to all malignancies (Rusthoven, 1994). In addition to the data reported by Rusthoven et al. above, other investigators have noted a high proportion of patients with Hodgkin's disease who developed zoster. For example, in a record review study of all patients at the clinical center of the NIH, 27 of 107 (25%) zoster cases had Hodgkin's disease, which was highest on a list of underlying diseases (Mazur & Dolin, 1978). At a children's cancer hospital, 22 of 101 (22%) children with zoster had underlying Hodgkin's disease, which was the highest proportion of any underlying cancer (Feldman et al., 1973). In a cancer research center, Hodgkin's disease was the most frequent underlying disease (25%) in the 37 cancer patients who developed zoster over a 2 year period out of 419 cancer patients (Schimpff et al., 1972). Given the importance of Hodgkin's disease in the development of zoster, Guinee et al. (1985) performed a retrospective study of zoster among 717 patients with Hodgkin's disease from six cancer centers over a 3 year period. Via self-report and record review, they identified 116 cases of zoster with a minimum follow-up of 18 months. The cumulative incidence of zoster in these patients was 9.5% after one year, 16.6% after two years and 20.6% after three years.

Organ transplants

Recipients of bone marrow, kidney and heart transplants are often afflicted by zoster. It is difficult to characterize the incidence and risk factors for zoster in these patients because published studies are limited by small samples, retrospective medical record review, variable underlying diseases and treatment regimens, differing time of follow-up, lack of knowledge about whether donors or recipients were VZV seropositive, and the difficulty in determining if the lesions represented zoster or varicella. Despite these limitations, it appears that bone marrow transplant (BMT) recipients are particularly susceptible to developing zoster (Rusthoven, 1994). The frequency of zoster in BMT recipients ranges from 13% to 55% and the majority of cases occur within 12 months of transplantation (Atkinson et al., 1980; Locksley et al., 1985; Schuchter et al., 1989; Wacker et al., 1989; Christiansen et al., 1991; Han et al., 1994; Tzeng et al., 1995; Kawasaki et al., 1996). In a study of 1394 BMT recipients (mostly allogeneic) in Seattle, Washington, US in 1969–1982, 195 (14%) developed zoster (median age, 20 years, range 2–52 years) (Locksley et al., 1985). The median time to onset of zoster was 5 months and 85% of cases occurred within a year. In 153 patients who underwent autologous BMT at the Johns Hopkins Oncology Center in Baltimore, US and survived longer than 100 days after transplant, 33 (22%) developed zoster (median

age, 23 years; range 7–58 years) (Schuchter et al., 1989). The cumulative incidence at a median of one year post-transplant was 19%.

Many of these studies examined issues pertinent to BMT and zoster risk, including type of transplant (allogeneic vs. autologous), graft-versus-host disease (GVHD), underlying disease, and pre-BMT irradiation. For every study that found that these factors increased zoster risk, there is at least one other study that found that they did not. However, all studies agree that cutaneous dissemination (18–23%) and visceral involvement (13–14%) are more common in BMT recipients than in immunocompetent patients.

Data on zoster after renal and cardiac transplantation is limited to a small number of older studies. Of available studies, the frequency of zoster in renal transplant recipients was approximately 7–14% within 2–5 years of transplantation (Rifkind D, 1966; Luby et al., 1977; Naraqi et al., 1977; Lo & Cheng, 1996). Most cases were localized zoster without serious complications. In the largest and most recent study, 49 (14%) cases of zoster developed in 354 renal transplant patients, most of whom received cyclosporine-based immunosuppressive regimens, after a mean of 5 years of follow-up (Lo & Cheng, 1996). The median time to zoster onset was 7.6 months after transplant and 63% occurred within one year of transplantation. Of two studies of cardiac transplant recipients, the frequency of zoster was 13% within 6 months of transplantation and 22% after six years of follow-up (Rand et al., 1977; Preiksaitis et al., 1983).

Immune-mediated diseases

As in organ transplant studies, studies of zoster and immune-mediated diseases are limited by retrospective chart reviews or interviews with patients from referral centers, small samples, and variable treatment regimens and time of follow-up. Nonetheless, investigators have consistently reported a strong association between zoster and systemic lupus erythematosus (SLE). In American and Spanish studies, researchers reported a frequency of zoster of 13–21% in SLE patients and an incidence of 16–22 cases per 1000 person-years (Moutsopoulos, 1978; Kahl, 1994; Manzi et al., 1995; Moga et al., 1995). In Japanese studies, investigators reported a frequency of zoster of 40–47% in SLE patients and an incidence of 55–91 cases per 1000 person-years (Hamaguchi et al., 1970; Nagasawa et al., 1990). In the largest study, researchers used participant interview, an SLE database and matched case-control methods to study zoster in a consecutive series of SLE patients from outpatient and inpatient facilities at the University of Pittsburgh, US between 1979–1989 (Manzi et al., 1995). Of 321 participants (mean age 45 years, range 19–83 years), 48 (15%) developed zoster a median of 6.2 years (range 1 month to 29 years) after the diagnosis of SLE for an estimated incidence of 22 cases per 1000 person-years. Compared to SLE controls, risk factors for zoster included azathio-

prine, cyclophosphamide, nephritis, and malignancy. The sample size was too small for adequate multivariate analyses to determine if these were independent factors. In all SLE studies, zoster was usually localized and had an uncomplicated course even during ongoing immunosuppressive therapy. In addition, the appearance of zoster was not related to SLE activity because cases occurred when SLE disease activity was severe, mild or inactive. The effects of immunological deficits in SLE alone versus those of immunosuppressive therapy on zoster risk were impossible to decipher. However, a high frequency of zoster in SLE was reported before the use of immunosuppressants, which suggests that SLE-induced immune deficits play a role in zoster susceptibility (Hamaguchi et al., 1970).

Zoster occurrence has been reported in rheumatoid arthritis, polymyositis and dermatomyositis but most studies are case reports or small case series (Nagaoka et al., 1990; Antonelli et al., 1991). In a retrospective chart review study of methotrexate treated patients with rheumatoid arthritis in a University of West Virginia, US patient registry, 1979–1989, investigators reported nine zoster patients (mean age = 59) and 187 non-zoster patients (mean age = 56) and calculated an incidence of 14.5 zoster cases per 1000 person-years (Antonelli et al., 1991).

Immunosuppressive treatments

Immunosuppressive treatments are thought to increase the risk of zoster but few data are available on the presence or magnitude of risk and on which immunosuppressive treatments carry greater risk. Furthermore, it is often difficult to disentangle the effects of the underlying disease or treatment on zoster risk. Implicated immunosuppressive treatments include radiation therapy, cancer chemotherapeutic agents, corticosteroids, post-transplant immunosuppressants, and agents for immune-mediated diseases.

Several authors have reported a high frequency of radiation treatments in cancer patients with zoster (Ellis & Stoll, 1949; Rifkind, 1966; Goffinet et al., 1972; Schimpff et al., 1972; Feldman et al., 1973; Mazur & Dolin, 1978; Rusthoven et al., 1988a). For example, Rusthoven et al. reported that 62% of 545 cancer patients who received radiation therapy developed zoster within the radiated area but many patients also received chemotherapy and had tumor at the sites of reactivation and radiation (Rusthoven et al., 1988a). Most investigators did not compare the occurrence of zoster in cancer patients who did or did not receive radiation treatments. In one study of zoster and cancer, 13 of 41 (31%) Hodgkin's disease patients who received radiation therapy developed zoster compared to 4 of 37 (11%) of Hodgkin's disease patients who did not receive radiation therapy, suggesting that radiation therapy alone predisposed to zoster (Schimpff et al., 1972). In an earlier study of zoster in children with cancer, researchers found that none of 38 irradiated patients with acute myelogenous leukemia (AML) developed zoster and one in 100

(1%) of non-irradiated patients with AML developed zoster (Feldman et al., 1973). Of 365 solid tumor patients in this study, 16 of 322 (5%) who were irradiated developed zoster compared to none of 43 who were not irradiated ($P > 0.05$). The VZV serological status of these children was not reported. The effect of radiation therapy on zoster risk in BMT studies shows equally conflicting results. In a Minnesota, US study, radiation therapy in pre-transplant conditioning significantly increased the risk of VZV infection, including zoster, post-BMT (adjusted relative risk, 1.8; 95% CI, 1.09–3.05; $P = 0.023$) (Han et al., 1994). Conversely, radiation therapy was not a significant risk factor for zoster in three other BMT studies (Schuchter et al., 1989; Wacker et al., 1989; Tzeng et al., 1995).

Several authors reported zoster in patients receiving cancer chemotherapy but they were unable to determine the effect of chemotherapy on zoster risk compared to the underlying disease or the risk for zoster related to drugs (Rifkind, 1966; Goffinet et al., 1972; Schimpff et al., 1972; Feldman et al., 1973; Mazur & Dolin, 1978; Feld et al., 1982; Guinee et al., 1985; Rusthoven et al., 1988b; Rusthoven, 1994). However, zoster was significantly more frequent after combination chemotherapy and radiotherapy compared to radiotherapy alone (Reboul et al., 1978; Green et al., 1979; Feld et al., 1982; Guinee et al., 1985). For example, the cumulative 3 year incidence of zoster in Hodgkin's disease was highest in the group receiving induction chemotherapy, radiation therapy and maintenance chemotherapy (51 zoster cases among 185 patients, 27.6%) compared to radiation therapy alone (22 of 193 patients, 11.4%, $P = 0.001$), induction chemotherapy alone (12 of 91 patients, 13.2%, $P = 0.018$) and induction chemotherapy followed by radiation therapy (27 of 134 patients, 20%, $P = 0.011$), a result that remained significant after multivariate analysis (Guinee et al., 1985). Another study reported a higher frequency of zoster in cancer patients who received induction chemotherapy, radiation therapy and maintenance chemotherapy (13 zoster cases among 161 patients, 8.1%) compared to those who received induction chemotherapy followed by radiation therapy alone (2 of 250 patients, 0.8%) and those who received radiation therapy alone (10 of 622 patients, 1.6%) (Feld et al., 1982). The cumulative 3 year probability of zoster was calculated to be 20% for induction chemotherapy plus radiation therapy plus maintenance chemotherapy versus 8% for induction chemotherapy plus radiation therapy ($P = 0.031$) and 2% for radiation therapy alone ($p = 0.0007$).

Clinical experience and case reports suggest that corticosteroids increase zoster risk but there are no well-designed, controlled studies of this relationship. A brief report of a case-control study of prednisone and herpesvirus infections in HIV infection within 30 days of treatment found no difference in VZV infection between treated (0%) and untreated patients (0.3%) (Keiser et al., 1996). In two studies of SLE, univariate analyses revealed that the risk of zoster was higher in

patients who received cyclophosphamide and azathioprine compared to those who did not (Kahl, 1994; Manzi et al., 1995).

Other factors

Sex

Large cohort studies demonstrated no differences in the incidence of zoster by gender. In the studies of Hope-Simpson (1965), Ragozzino et al. (1982a), and Donahue et al. (1995), the incidence of zoster among males versus females was 3.6 vs. 3.2, 1.34 vs. 1.26 and 2.19 vs. 2.11 per 1000 person-years, respectively. Among elderly persons in the Duke EPESE, being female did not significantly increase lifetime zoster risk (odds ratio 1.13; 95% CI, 0.89–1.43) (Schmader et al., 1995).

Race

Most zoster epidemiology studies have focused on all or predominantly white populations in the United States and Europe. In earlier referral center reports of zoster from Philadelphia, US and Durham, NC, US, the authors reported that 9–18% of patients were black but noted that these percentages mirrored the racial make-up of the referral population (Burgoon & Burgoon, 1957; Rogers & Tindall, 1971). In the Duke EPESE, which used a stratified, random sampling technique in community dwelling elderly to achieve an equal mix of black and white participants, blacks had a significantly lower lifetime occurrence and incidence of zoster than whites (Schmader et al., 1995). The results showed that 316 (9.9%) of 3206 subjects had a past occurrence of zoster, affecting fewer blacks (81 zoster cases in 1754 participants [4.6%]) than whites (235 zoster cases among 1452 participants [16.2%], $P<0.0001$). After controlling for age, cancer, and demographic factors, black persons remained four times less likely than whites (adjusted odds ratio, 0.25; 95% CI, 0.18–0.35; $P=0.0001$) to have experienced zoster. In a study of the prospective occurrence of zoster in the Duke EPESE, 4.3% of blacks and 10.9% of whites developed zoster over 6 years ($P<0.001$) (Schmader et al., 1998). After controlling for the above variables, blacks were significantly less likely to develop zoster (adjusted risk ratio, 0.35; 95% CI, 0.24–0.51; $P<0.001$). In these studies, zoster case ascertainment had a false positive rate of 3% and false negative rate of 0%, which was not enough to explain these striking racial differences (Schmader et al., 1994). Hypothesized reasons for the differences include racial differences in VZV immunity, age at onset of varicella, and exposure to varicella over the life course (Dworkin, 1996). However, these findings need confirmation in other studies and indicate the need for epidemiological studies of zoster in nonwhite populations.

In Canada, Ross et al. reported significantly lower rates of zoster among white Hutterites than other white non-Hutterite Canadians (Ross et al., 1995). The study included 5601 Hutterites with life-long residence in Manitoba and 5476 matched

controls from Manitoba of all ages. By age 10 years the Hutterites had 39% less varicella than the controls. A significantly greater number of Hutterites of all ages were seronegative for VZV antibodies (Ross et al., 1997). These results suggest that the age of onset of varicella was later in Hutterites, who may have lowered zoster incidence in later life. In addition, a larger proportion of Hutterites were VZV seronegative and not latently infected and therefore not at risk for zoster.

Other demographic factors

Most epidemiological studies of zoster did not investigate or publish information on marital status, education, socioeconomic status, or occupation. In the Duke EPESE, years of education did not increase zoster risk for lifetime occurrence (adjusted odds ratio, 0.98; 95% CI, 0.88–1.07) or yearly incidence (adjusted relative risk, 1.04; 95% CI, 0.87–1.23) (Schmader et al., 1995). In the same population, being married did not reduce zoster risk (adjusted relative risk, 1.16; 95% CI, 0.78–1.72) (Schmader et al., 1998).

Physical trauma

There are many anecdotal reports of physical trauma and surgery as zoster precipitants but most persons who experience trauma do not develop zoster and there are no studies that publish comparative data and adjust for confounding factors. Hope-Simpson (1965) noted that only two cases of zoster had preceding physical trauma and concluded that these events were probably coincidence. In Rochester, Minnesota, US, 1.9% of his zoster cases had prior trauma but the nature of trauma, its timing to zoster, or the percentage of trauma in non-zoster controls were not reported (Ragozzino et al., 1982a).

Psychological factors

Patients and clinicians alike commonly believe that psychological stress plays a role in the development of zoster. In a retrospective case-control study of 101 zoster patients and 101 age, sex and race-matched controls collected by random digit dialing, zoster patients experienced stressful life events significantly more often than controls in the 2 months before zoster onset (odds ratio, 2.60; 95% CI, 1.13–6.27), 3 months before zoster onset (odds ratio, 2.64; 95% CI, 1.20–6.04), or 6 months before zoster onset (odds ratio, 2.00; 95% CI, 1.04–3.93) and the risk was insignificant by 12 months before zoster onset (Schmader et al., 1990). Recall bias was a potential weakness of this study. In a prospective study of stressful life events and zoster in the Duke EPESE, stressful life events increased the risk of zoster but the result was borderline for statistical significance (adjusted relative risk, 1.38; 95% CI, 0.96–1.97) (Schmader et al., 1998). Being unmarried and lack of social support were not associated with zoster. However, the temporal aspects of the study design

biased against finding a relationship because stressful life events were measured over one year and zoster was measured over the subsequent three years. The available data suggest, but do not prove, that psychological stress is a risk factor.

Zoster as a predictor of disease

In contrast to studies of risk factors for zoster, investigators have studied zoster as a risk factor for cancer, diabetes mellitus, rheumatoid arthritis, multiple sclerosis (MS), SLE, and glioma. The idea that zoster may be a marker for underlying cancer is reasonable given the association of zoster with cancer and immunosuppression but prospective studies of patients with zoster did not demonstrate an increased risk for malignancy (Ragozzino et al., 1982b; Feuyo & Lookingbill, 1984; Wurzel et al., 1986). In Rochester, Minnesota, US, the authors examined 590 zoster cases over 9389 person-years of observation for the development of cancer and compared the data to age and sex specific cancer rates in the entire Rochester population (Ragozzino et al., 1982b). The relative risk for detection of cancer after the diagnosis of zoster was not significantly increased (relative risk, 1.1; 95% CI, 0.9–1.3). The authors used the same database and similar methods to show that zoster was not associated with diabetes mellitus (relative risk, 1.08; 95% CI, 0.74–1.51) or rheumatoid arthritis (relative risk, 1.29; 95% CI, 0.6–2.21) (Ragozzino & Kurland, 1982; Ragozzino et al., 1983). An association between zoster and MS has been suggested by some reports but not by others. In a retrospective study of 50 MS patients and 50 age–sex matched controls with psoriasis, 10 MS cases reported a history of shingles compared to 2 controls ($P<0.05$) (Lenman & Peters, 1969). In a case-control study of Hutterites and non-Hutterites in Canada, investigators noted 40 cases of zoster, 122 cases of varicella, and 5 cases of MS in 5601 Hutterites compared to 76 cases of zoster ($P<0.001$), 172 cases of varicella ($P<0.001$), and 17 cases of MS ($P<0.014$) in 5601 non-Hutterite controls (Ross et al., 1995). These observations raised the question of whether the significantly lower rates of zoster, varicella and MS were related. However, in the above Rochester database, no cases of MS were observed in the 590 zoster patients over 9389 person-years of follow-up as opposed to the expected number of 0.2 cases (Ragozzino et al., 1983). Although the Rochester study is the largest cohort study of this relationship, a larger sample size was required to have adequate power to detect significant differences between groups. Regarding SLE, researchers compared multiple risk factors in 195 cases of SLE and 143 age and sex matched controls in Philadelphia, Pennsylvania, US, between 1985 and 1987 (Strom et al., 1994). A significant association was found between SLE and a history of shingles (adjusted odds ratio, 6.4; 95% CI, 1.4–28.0). Because it was impossible to determine when the immunological deficits of SLE began in the cases, these findings probably confirm

the association of zoster and SLE discussed above. Finally, investigators asked 462 adults with glioma and 443 age, sex, and ethnicity matched controls about their history of shingles in the San Francisco Bay Area Adult Glioma study, 1991–1994 (Wrensch et al., 1997). A history of shingles was significantly less likely in cases than controls (odds ratio, 0.5; 95% CI, 0.3–0.8). This interesting observation will need confirmation in cohort studies.

Transmission

Primary VZV infection is necessary for susceptibility to zoster because zoster is caused by reactivation of latent infection. Therefore, the epidemiology of zoster is dependent on the transmission and spread of VZV in populations. Although varicella is a highly infectious condition that is of prime importance in the transmission of VZV, latent and reactivated VZV infections also play important roles in maintaining VZV infection in populations (Arvin, 1996). Latently infected elderly adults and immunosuppressed patients are particularly important reservoirs of virus because VZV is more likely to reactivate in these groups. When zoster does occur, VZV can be transmitted during the vesicular phase of the rash. VZV transmission results in primary VZV infection when the zoster exposure consists of person to person contact with a seronegative individual. The likelihood of such infection has not been adequately measured but it appears less likely than transmission after exposure to varicella.

What happens if a zoster exposure occurs with a seropositive, latently infected individual? The exposure may result in a subclinical reinfection and boost of humoral and cellular VZV immunity but it is unlikely to cause varicella or herpes zoster in these individuals (Arvin, 1996). In a study involving leukemic children who were immunized against varicella, factors that conferred significant protection from development of zoster were household exposure to varicella and more than one dose of varicella vaccine, both of which were associated with boosting immunity to VZV (Gershon et al., 1996). Investigators have reported clusters of zoster cases over a short period of time in the workplace and have reported zoster after prior exposure to varicella (Berlin & Campbell, 1970; Palmer et al., 1985). It is not clear whether these episodes are coincidence, a clinical manifestation of exogenous reinfection, or stimulation of endogenous VZV reactivation. However, the majority of such exposures with latently infected persons appear to be of little clinical significance. The potentially important epidemiological significance of zoster or varicella exposure in latently infected individuals is boosting of VZV immunity and prevention of zoster.

Epidemiologists can track the spread of infectious agents with molecular biolog-

ical techniques that detect genetic differences in organisms. Molecular epidemiology may have limited application to VZV since the genome is remarkably stable and there is only one serotype. However, investigators raised the possibility of the existence of VZV strains when they examined two polymorphic markers, allele frequency in variable repeat region R5 and the presence or absence of the BglI restriction site in gene 54, in VZV samples from 105 cases of varicella and 144 cases of zoster in inpatients and outpatients from 1971 to 1995 in a multiethnic area of London (Hawrami et al., 1997). R5 allele frequency was no different over time or by age or ethnicity between the zoster and varicella cases. However, a BglI restriction site was present in 63% of zoster cases who had chickenpox in countries with lower adult immunity to varicella (mostly nonwhite individuals) compared to 10% who had chickenpox in countries with higher adult immunity to varicella (mostly white individuals) ($P = 0.0005$). The authors postulated that a geographically distinct, BglI positive viral strain of VZV exists and that the source was immigrants from countries with lower adult immunity to varicella.

Molecular techniques accurately differentiate varicella vaccine virus from wild type VZV (LaRussa et al. 1992). The accurate identification of these viruses will be important for deciphering the potential impact of varicella vaccination on zoster epidemiology because varicella vaccination may change the incidence and morbidity rates of zoster significantly. For example, widespread varicella vaccination of children will change varicella exposure rates in populations, theoretically reducing immune boosting and possibly shifting the age of onset of zoster in latently infected adults. However, vaccination of children may reduce zoster incidence or complications over the long term by preventing primary wild type infection, assuming latent vaccine virus is less likely to reactivate or cause zoster complications when it does reactivate (Hardy et al., 1991). Finally, varicella vaccination of elderly adults may reduce the incidence of zoster or its complications, an hypothesis now being tested in clinical trials. Over the next several decades, the data should become available to determine the true effect of the vaccine on zoster epidemiology.

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13

Clinical manifestations of herpes zoster

Michael N. Oxman

Herpes zoster (shingles) is a localized disease characterized by unilateral radicular pain and a vesicular eruption that is generally limited to the dermatome innervated by a single spinal or cranial sensory ganglion. In contrast to varicella, which follows primary exogenous varicella-zoster virus (VZV) infection, herpes zoster is the result of reactivation of endogenous VZV that had persisted in latent form within sensory ganglia following an earlier attack of varicella.

Herpes zoster (HZ) occurs most often in dermatomes in which the rash of varicella achieves the highest density – those innervated by the first (ophthalmic) division of the trigeminal ganglion and by spinal sensory ganglia from T1 to L2 (Head & Campbell, 1900; Stern, 1937; Denny-Brown et al., 1944; Hope-Simpson, 1965; Ragozzino et al., 1982; Donahue et al., 1995). Presumably, areas of skin with a denser rash during varicella transmit larger amounts of virus to the corresponding sensory ganglia, thereby endowing these ganglia with a higher concentration of latent VZV. If subsequent reactivation occurs at random, HZ would be expected to occur most frequently in dermatomes innervated by ganglia with the highest concentrations of latent VZV. The occurrence of HZ at sites of prior vaccination with live attenuated varicella vaccine is consistent with this model of VZV latency.

Although the latent virus in the ganglia retains its potential for full infectivity, reactivation is sporadic and infrequent, and infectious virus does not appear to be present during latency. The mechanisms involved in the reactivation of VZV are unclear (see Chapter 6), but a number of conditions have been associated with the occurrence and localization of HZ. These include immunosuppression in HIV infection and in Hodgkin's disease and other hematologic malignancies; administration of immunosuppressive drugs, especially corticosteroids; irradiation of the spinal column; spinal surgery; and local trauma, tumor involvement or tuberculosis of the cord, dorsal root ganglion, or adjacent structures (Head & Campbell, 1900; Hope-Simpson, 1965; Oxman, 1986). Most important, though, is the senescence of cellular immune responses to VZV that occurs with increasing age (Hope-Simpson, 1965). Beginning before the fifth decade of life, there is a selective age-related decline in cell-mediated immune responses to VZV, and it is this

decline that appears to explain the increased incidence and severity of HZ and its complications in older persons (Miller, 1980; Berger et al., 1981; Burke et al., 1982; Hayward et al., 1991; Takahashi et al., 1992; Arvin,1992; Oxman, 1995).

In some cases reactivation of latent VZV may occur, but virus is rapidly contained by host immune responses and there are no clinical manifestations. Subclinical reactivation of latent VZV has been documented in immunocompromised patients by the detection of periodic increases in humoral and cell-mediated immunity to VZV and by the detection of VZV DNA by PCR (polymerase chain reaction) in peripheral blood mononuclear cells (Luby et al., 1977; Gershon et al., 1982; Weigle & Grose, 1984; Ljungman et al., 1986; Wilson et al., 1992). Similar increases in immunity to VZV have been observed in normal adults following contact with cases of varicella, reflecting subclinical exogenous reinfection (Arvin et al., 1983; Gershon et al., 1984; Weigle & Grose, 1984). The antigenic stimulation provided by these subclinical reactivations and exogenous reinfections helps to maintain the immunity to VZV that was initiated by childhood varicella and to slow the decline in that immunity that occurs over time. Despite this stimulation, cell-mediated immunity to VZV still declines with increasing age in older persons, and when that immunity falls below a critical level, reactivated virus can no longer be contained. It multiplies and spreads within the ganglion, causing neuronal necrosis and intense inflammation, a process that often results in severe neuralgia. VZV then spreads antidromically down the sensory nerve, causing intense neuritis, and virus is released from sensory nerve endings in the skin, where it produces the characteristic clusters of zoster vesicles. The occurrence of neuralgia several days before the rash appears, and the presence of degenerative changes in cutaneous nerve fibrils on the first day of the eruption, provide additional evidence that VZV replication in the sensory ganglion precedes involvement of the skin (Muller & Winkelmann, 1969).

Spread of the ganglionic infection proximally along the posterior nerve root to the meninges and into adjacent regions of the spinal cord or brainstem results in local leptomeningitis, cerebrospinal fluid pleocytosis, and segmental myelitis. Infection of motor neurons in the anterior horn, as well as inflammation and degeneration of the anterior nerve root, account for the local palsies that may accompany the cutaneous eruption. Extension of infection to the contralateral posterior horn appears to be a frequent event, as evidenced by loss of peripheral neurites in the skin of the contralateral dermatome (Oaklander et al., 1998). There is often involvement of adjacent ganglia on the same side, and extension of infection within the central nervous system may result in transverse myelitis, ascending myelitis or meningoencephalitis, rare complications of HZ (McKendall & Klawans, 1978).

Following reactivation, limited hematogenous dissemination of virus from the

affected ganglion often produces a few scattered vesicles at a distance from the primary dermatome, even in uncomplicated HZ (Oberg & Svedmyr, 1969). Together, the local and disseminated infections stimulate an anamnestic immune response that terminates the infectious process. Sometimes this response is sufficiently rapid to neutralize virus released into the skin and thus prevent the development of recognizable cutaneous lesions; the result is an episode of radicular pain without eruption (zoster sine herpete) (Lewis, 1958; Easton, 1970; Juel-Jensen & MacCallum, 1972; Luby et al., 1977; Gilden et al., 1992). If the anamnestic host response is delayed or deficient, as it is in many immunocompromised patients, the duration and severity of the local infection are increased, and VZV viremia is more prolonged and extensive. The result may be clinically significant cutaneous and visceral dissemination (Shanbrom et al., 1960; Merselis et al., 1964; Sokal & Firat, 1965; Goffinet et al., 1972; Schimpff et al., 1972; Feldman et al., 1973; Ruckdeschel et al., 1977; Dolin et al., l978; Reboul et al., 1978; Gallagher & Merigan, 1979; Patel et al., 1979; Arvin et al., 1980; Locksley et al., 1985; Ljungman et al., 1986; Novelli et al., 1988; Jacobson et al., 1990; Balfour, 1991; Safrin et al., 1991).

Under normal circumstances, an episode of HZ induces a marked and long-lasting increase in cell-mediated immunity to VZV, and this appears to be responsible for the rarity of second episodes of HZ in immunocompetent persons (see Chapter 12). "Recurrent H2" in immunocompetent persons, especialy involving the same dermatome, is likely to be "zosteriform" herpes simplex (Oxman, 1986; Kallman & Laskin, 1986).

Clinical manifestation of herpes zoster in the immunocompetent host

The prodrome

The first manifestation of HZ is usually acute segmental neuralgia with pain and paresthesia in the involved dermatome (Burgoon et al., 1957; de Moragas & Kierland, 1957; Rogers & Tindall, 1971; Hope-Simpson, 1975; Wood et al., 1996; McKendrick et al., 1986; Oxman, 1986; Beutner et al., 1995). This generally precedes the eruption by several days, occasionally by a week or more, and it varies from superficial itching, tingling, or burning to severe deep boring or sharp, stabbing, lancinating pain. The pain may be constant or intermittent and it is often accompanied by tenderness and hyperesthesia in the involved dermatome. The incidence of prodromal pain varies with age. It is uncommon in immunocompetent persons under 30 years of age, but occurs in the majority of persons with HZ who are over the age of 60 years. Constitutional symptoms, including headache, malaise and fever, occur in about 5% of patients, usually children, and may precede the rash by 1 or 2 days (Burgoon et al., 1957; Rogers and Tindall, 1971, 1972). A lymphocytic pleocytosis, with or without an increase in the concentration of

protein in the CSF (cerebrospiral fluid), occurs in about 40% of patients with uncomplicated HZ, and in a greater proportion when cranial nerves are involved. VZV DNA can be detected in the CSF of patients with uncomplicated HZ and infectious VZV can occasionally be isolated (Haanpaa et al., 1998).

The pre-eruptive pain of HZ may simulate the pain of myocardial infarction, pleurisy, duodenal ulcer, cholecystitis, biliary or renal colic, appendicitis, prolapsed intervertebral disc or early glaucoma, and this may lead to serious misdiagnosis. The presence of sensory changes in the skin and tenderness to gentle palpation within the affected dermatome are suggestive of pre-eruptive HZ. Occasionally, patients with HZ report very prolonged periods of prodromal pain, sometimes interspersed with pain-free periods lasting days to weeks (Gilden et al., 1991). This suggests that reactivation of latent VZV may be followed by a subacute ganglionic infection in which host defenses slow virus replication, but are unable to terminate it.

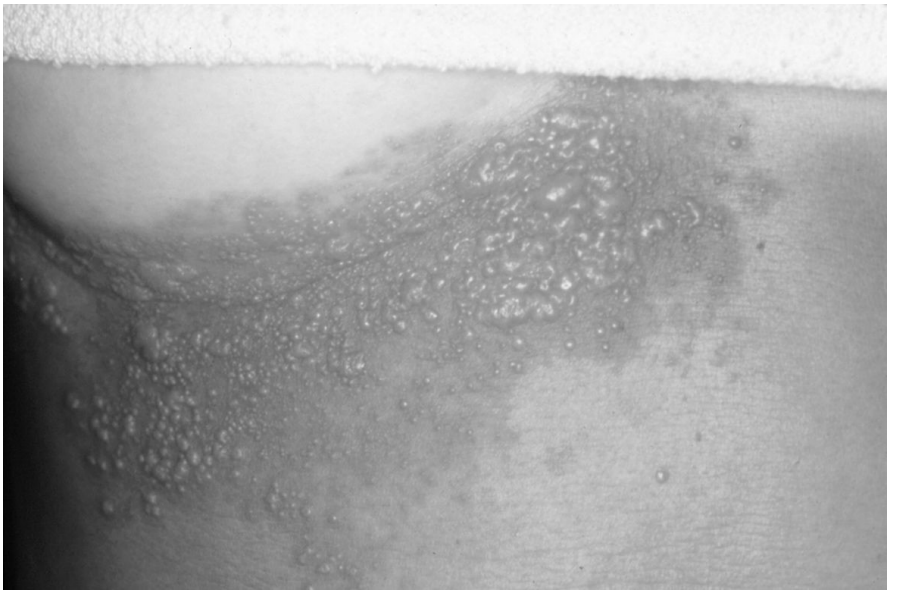
A few patients experience this acute segmental neuralgia without developing a cutaneous eruption, a syndrome called zoster sine herpete. A concurrent rise in the titer of antibodies to VZV provides evidence that these mysterious episodes of neuralgic pain are, indeed, due to reactivation of latent VZV (Lewis, 1958; Easton, 1970; Juel-Jensen & MacCallum, 1972; Luby et al., 1977; Gilden et al., 1992; Barrett et al., 1993).

The rash

The most distinctive feature of HZ is the localization of the rash, which is unilateral, does not cross the midline and is generally limited to the area of skin innervated by a single sensory ganglion (i.e., to a single dermatome) (Stern, 1937; Seiler, 1949; Hope-Simpson, 1965; Oxman, 1986; Helgason et al., 1996) (Figures 13.1, 13.2). Individual sensory ganglia are not attacked at random; HZ occurs with greatest frequency in those dermatomes in which the rash of varicella is most abundant (Stern, 1937; Hope-Simpson, 1965; Brown, 1976; Ragozzino et al., 1982). The area supplied by the ophthalmic division of the trigeminal nerve and by spinal sensory ganglia from T1 to L2 are most frequently affected; ophthalmic zoster accounts for 8% to 15% of reported cases of HZ, and more than 50% involve thoracic dermatomes (Head & Campbell, 1900; Burgoon et al., 1957; Hope-Simpson, 1965; Juel-Jensen & MacCallum, 1972; Brown, 1976; Ragozzino et al., 1982; Donahue et al., 1995; Helgason et al., 1996). Lesions rarely occur below the elbows or knees. Regional lymphadenopathy occurs in the majority of cases of HZ.

The lesions of HZ consist of closely grouped vesicles on an erythematous base, in contrast to the discrete, randomly distributed vesicles of varicella. This difference reflects intraneural spread of virus to the skin in HZ, as opposed to viremic spread in varicella; clusters of vesicles occur where sensory nerve endings introduce VZV

(A)



(B)

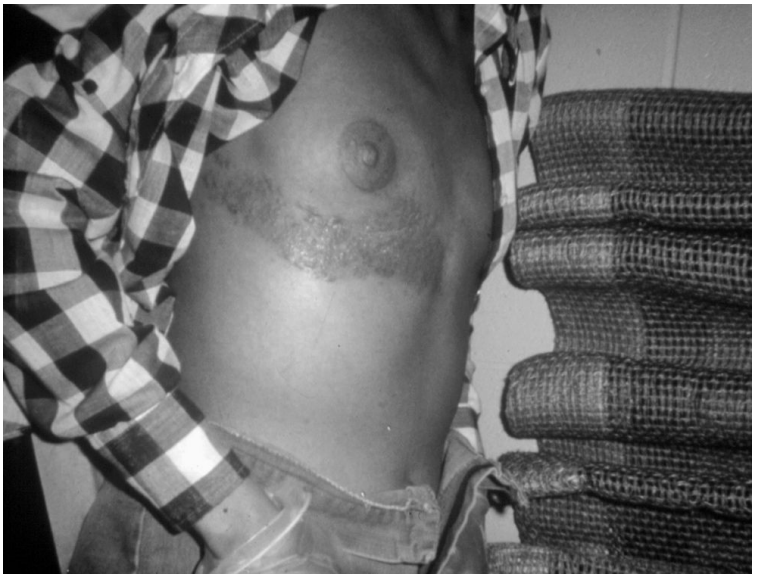


Figure 13.1 (A) Herpes zoster in the right fifth thoracic dermatome. Note the grouped vesicles, some of which are coalescing to form small bullae. (B) Another example of herpes zoster in the right fifth dermatone. Note the involvement of the entire dermatome (Courtesy of Dr. D. A. Lopez).

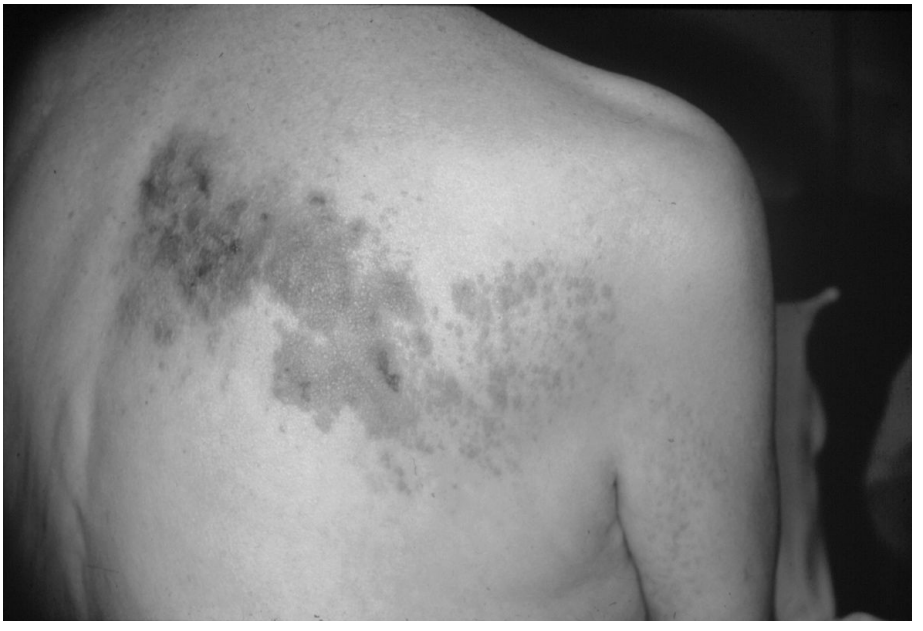


Figure 13.2 Herpes zoster in the right second thoracic dermatome involving primarily skin innervated by the posterior primary division and by the posterior branch of the lateral cutaneous nerve.

into the skin. The lesions begin as erythematous macules and papules, which often first appear where superficial branches of the affected sensory nerve are given off, e.g., the posterior primary division and the lateral and anterior branches of the anterior primary division of spinal nerves (Stern, 1937; Oxman, 1986). In the early papular lesions, the epithelium is slightly elevated due to swelling of infected epithelial cells, and edema and vascular congestion in the underlying dermis. Infected cells in the germinal layer and stratum spinosum of the epithelium show "ballooning degeneration" with loss of intercellular bridges, and they are soon separated by intercellular edema. The papular lesions evolve into intraepidermal vesicles within 12 to 24 hours as a result of the infection and degeneration of increasing numbers of epithelial cells and the continuing influx of edema fluid, which elevates the uninvolved stratum corneum to form a delicate clear vesicle. At this stage, the vesicle fluid contains fibrin, degenerating epithelial cells and large amounts of cell-free infectious VZV. In some cases vesicles may coalesce to form large bullae. Characteristic multinucleated giant cells with eosinophilic intranuclear inclusion bodies, formed by fusion of infected epithelial cells with adjacent infected and uninfected cells, are present at the base and periphery of the vesicle. These multinucleated giant cells are readily identified in Tzanck smears, which may be prepared

at the bedside from material scraped from the base of vesicular lesions. Their presence distinguishes HZ from all other cutaneous diseases except varicella and herpes simplex virus infections (see Chapter 17). By the third day, polymorphonuclear leukocytes and macrophages invade from the underlying dermis and the vesicle fluid becomes cloudy. This transforms the vesicle into a pustule. The fluid is then absorbed and a flat adherent crust is formed by 7 to 10 days. The lesions evolve in the same manner as those of varicella, but more slowly. In immunocompetent individuals, new lesions continue to appear for 1 to 4 days – occasionally for up to 7 days – and virus may be recovered from lesions for as long as a week. Crusts generally persist for 2–3 weeks. They fall off spontaneously, generally within 4 weeks of rash onset, when displaced by the regrowth of subjacent epithelial cells. The rash is most severe and lasts longest in older persons and is least severe and of shortest duration in children. The HZ rash generally resolves completely, but in severe cases there may be changes in skin pigmentation, scarring, and persistent sensory abnormalities.

The histopathology of the skin lesions of HZ and varicella are indistinguishable. However, HZ is accompanied by acute inflammation of the corresponding ganglion and sensory nerve.

Pain

Pain is a major feature of HZ, especially in older persons (de Moragas & Kierland, 1957; Hope-Simpson, 1975). A prodrome of neuralgic pain occurs in most elderly persons with HZ, beginning 3 or more days before the rash in more than half (McKendrick et al., 1986). Pain accompanies the rash in 60–90% of immunocompetent individuals with HZ; it is rare in children and uncommon in younger adults, but it occurs in more than 90% of persons with HZ who are 60 years of age or older (de Moragas & Kierland, 1957; Burgoon et al., 1957; Wood, 1991). It is described as sharp, stabbing, shooting, burning, throbbing, tender, boring, itching or hot, and may be constant or intermittent (Bhala et al., 1988; Wood, 1991; Bowsher, 1993). Although pain during the acute phase of HZ usually resolves as the rash heals, it persists for months or even years beyond rash healing in some patients – an often debilitating complication known as postherpetic neuralgia (PHN).

A number of different but overlapping mechanisms appear to be involved in the pathogenesis of pain in HZ and PHN (Chapter 15). The relative contribution of each of these mechanisms is likely to vary from patient to patient and over the course of the disease in individual patients. However, in every case, the underlying cause of the pain associated with HZ is damage to neural tissues caused by VZV replication.

During the prodrome, the replication and spread of reactivated VZV within the sensory ganglion produces intense lymphocytic inflammation, necrosis of nerve cells and fibers, lymphocytic cuffing of small vessels, focal hemorrhage, and inflammation of the ganglion sheath (Head & Campbell, 1900; Denny-Brown et

al., 1944). Satellite cells and neurons contain characteristic intranuclear inclusion bodies, virus particles, and VZV antigens and nucleic acids. The peripheral nerve shows lymphocytic infiltration and focal hemorrhage with axonal degeneration and demyelination of sensory fibers, and the process extends distally to branches innervating the affected skin. These observations suggest that VZV may spread from the sensory ganglion to the periphery by replicating in Schwann and perineural cells, rather than by axonal transport, which would help explain the long prodrome and intense peripheral neuritis that characterize HZ (Straus & Oxman, 1999).

Infection and inflammation in the ganglion extend proximally to the posterior nerve root and into adjacent regions of the spinal cord or brainstem, producing an ipsilateral segmental myelitis that involves the posterior horn more than the anterior horn. There is degeneration of nerve fibers in the posterior column and inflammatory changes in the gray matter of the posterior and anterior horns (Head & Campbell, 1900; Denny-Brown et al., 1944; Watson et al., 1991). The process may extend for two or more segments from the one corresponding to the HZ rash. There is also inflammation and degeneration of the anterior nerve root within the meninges and in the portion overlying the involved sensory ganglion, producing motor radiculitis. When extensive, the acute inflammatory process is followed by fibrosis of the sensory ganglion and nerve. The acute injury to the peripheral nerve and to neurons in the sensory ganglion triggers afferent signals perceived as pain during the prodrome and following the onset of the HZ rash. The process also induces long-lasting changes in the physiology of second-order neurons in the spinal cord.

With the onset of the HZ rash, VZV replication in the skin itself results in activation and sensitization of peripheral sensory receptors, producing nociceptive signals that further amplify and sustain the pain induced by earlier injury to neural tissues and adding a local cutaneous component to the dermatomal pain. The barrage of afferent signals may also cause excitotoxic injury to interneurons in the spinal cord, adding to the damage caused directly by virus replication. Damaged neurons in the sensory ganglion and spinal cord become spontaneously active and hypersensitive to peripheral stimuli and to sympathetic stimulation. This state persists until the axon is reconnected to the skin. However, this may be prevented by damage to the nerve sheath, and the regenerating axons may form neuromas which also exhibit spontaneous activity and hypersensitivity. Loss of inhibitory interneurons and reorganization of central processing of somatosensory input result in allodynia, a major component of PHN; second-order nociceptive neurons that normally respond to input from nociceptive C-fibers respond instead to input from low-threshold mechanoreceptors carried by myelinated Aβ-fibers. It is the persistence of these changes after the HZ rash has healed that is responsible for PHN.

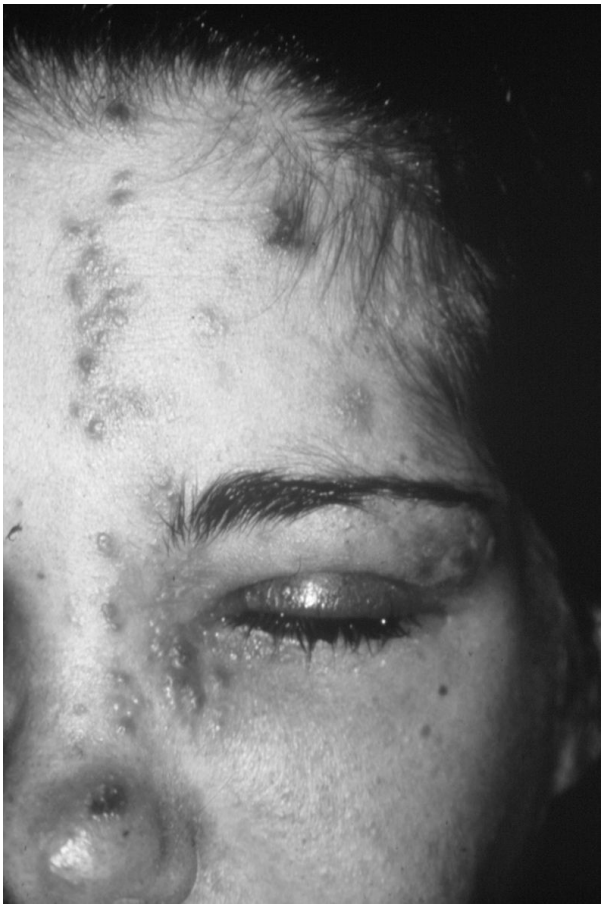


Figure 13.3 Herpes zoster of the ophthalmic division of the fifth cranial nerve. Involvement of the nasociliary branch results in lesions on the tip and side of the nose and unilateral conjunctivitis (courtesy of Dr. D. A. Lopez).

The anatomic and functional changes responsible for PHN appear to be established early in the course of HZ, perhaps even before the rash appears. Consistent with this is the correlation of initial pain severity and the occurrence of prodromal pain with the subsequent development of PHN, and the failure of antiviral therapy initiated promptly after HZ rash onset to prevent this debilitating complication.

As described in Chapter 14, between 8% and 15% of reported cases of HZ involve the ophthalmic division of the trigeminal nerve (Figure 13.3). The rash of ophthalmic HZ may extend from the level of the eye to the vertex of the skull, but it does not cross the midline of the forehead. When only the supratrochlear and supraorbital branches are involved, the eye is usually spared. Involvement of the nasociliary branch, as evidenced by a herpetic rash on the tip and side of the nose,

provides VZV with direct access to intraocular structures. Thus, when ophthalmic zoster involves the tip and the side of the nose, careful attention must be given to the condition of the eye. The eye is involved in 30% to 40% of patients with ophthalmic zoster. Corneal sensation is always impaired and when impairment is severe, it may lead to neurotrophic keratitis and chronic ulceration. VZV is not, however, as directly pathogenic for the cornea as is herpes simplex virus. The advice of an ophthalmologist should be sought in treating patients with ophthalmic zoster.

Complications of herpes zoster (Table 13.1)

The most common complication of HZ is postherpetic neuralgia (PHN), which can result in severe debilitating chronic pain and allodynia. The overall incidence of PHN has been reported to be 9% to 15% in retrospective population-based studies (Burgoon et al., 1957; Hope-Simpson, 1975; Ragozzino et al., 1982; Donahue et al., 1995). Age is the most significant risk factor; PHN is rare in patients under 40 years of age, but occurs in more than 50% of persons with HZ who are over 60 years of age. Other risk factors for PHN include the presence of prodromal pain; severe pain during the acute phase of HZ; HZ involving cranial nerves (as opposed to thoracic or lumber HZ); a more severe and extensive rash in the affected dermatome; and immunosuppression (Whitley et al., 1988, 1999; Wood, 1991; Galil et al., 1997). Patients with PHN describe three types of pain, and almost all experience at least two: (1) spontaneous, constant deep burning, throbbing, aching pain; (2) intermittent sharp, stabbing, shooting, lancinating pain which may also be spontaneous; and (3) dysesthetic pain provoked by light tactile stimulation (allodynia) which usually lasts well beyond the duration of the stimulus (hyperpathia) (Watson et al., 1988; Watson, 1989; Bowsher 1993; Nurmikko,1994). Allodynia, which is present in 90% or more of patients with PHN, is usually the most distressing and debilitating component. Multiple overlapping mechanisms appear to be involved in the pathogenesis of PHN, but the underlying cause is damage to neural tissues caused by VZV replication. When patients with PHN are carefully studied, the involved skin almost always shows pigmentary changes and scarring, and there are major sensory abnormalities in the affected dermatome. These include reduced sensitivity to light touch, pinprick, warm and cold temperature and hot pain, as well as decreased two-point discrimination (Watson et al., 1988, 1991; Rowbotham & Fields, 1989; Nurmikko & Bowsher, 1990; Bowsher, 1993, 1995; Nurmikko, 1994, 1995). Such changes are rarely seen in patients with HZ who recover without PHN. PHN is discussed in Chapter 15.

Anesthesia in the affected dermatome is another common sequela of HZ. It is particularly troublesome when it occurs in the area innervated by the optic nerve

Table 13.1 Complications of herpes zoster

Cutaneous	Visceral	Neurologic
Cutaneous VZV dissemination	Neural Extension of VZV Infection	Postherpetic neuralgia
Bacterial superinfection	Bronchitis	Aseptic meningitis
Scarring	Esophagitis	Meningoencephalitis
Cellulitis	Gastritis	Transverse myelitis
Zoster gangrenosum	Colitis	Ascending myelitis
Septicemia (with metastatic foci of infection)	Cystitis	Peripheral nerve palsies
	Myositis	Motor
	Pericarditis	Autonomic
	Pleuritis	Diaphragmatic paralysis
	Peritonitis	Cranial nerve palsies
	Visceral VZV dissemination	Sensory loss
	Pneumonia	Deafness
	Hepatitis	Vestibular dysfunction
	Myocarditis	Ocular complications
	Pericarditis	Loss of corneal sensation
	Arthritis	Neurotrophic keratitis
		Corneal ulceration
		Secondary bacterial infection
		Panophthalmitis
		Keratitis
		Scleritis
		Uveitis
		Chorioretinitis
		Iridocyclitis
		Optic neuropathy
		Ptosis
		Mydriasis
		Cicatricial lid scarring
		Secondary glaucoma
		Acute retinal necrosis (ARN)
		Progressive outer retinal necrosis
		Granulomatous cerebral
		Angitis (causing delayed contralateral hemiplegia)

and impairs corneal sensation (Womack & Liesegang, 1983; Liesegang, 1991; Juel-Jensen & MacCallum, 1972).

When the HZ rash is particularly severe, as it often is in immunocompromised patients, there may be superficial gangrene with delayed healing and subsequent scarring.

Bacterial superinfection of HZ skin lesions, usually due to *Staphylococcus aureus*, occasionally to group A beta hemolytic streptococcus, may result in delayed healing and scarring. It is less common than bacterial superinfection of the skin lesion of varicella, occurring in about 2% of patients with HZ. Local extension can produce cellulitis and, rarely, necrotizing fasciitis, which requires prompt surgical debridement, fasciotomy, and systemic antibiotics (Jarrett et al., 1998). Septicemia with metastatic foci of infection is rare and seen primarily in immunocompromised patients who are neutropenic.

Most other complications of herpes zoster are a consequence of the spread of VZV from the initially involved sensory ganglion, nerve or skin, either via the bloodstream or by direct neural extension. When immunocompetent patients with HZ are carefully examined, 17% to 35% are found to have at least a few vesicles in areas distant from the involved and immediately overlapping dermatomes; presumably this is due to hematogenous dissemination of virus from the affected ganglion, nerve, or skin. The disseminated lesions usually appear within a week of onset of the dermatonal eruption and, if few in number, are easily overlooked. More extensive dissemination (with 25 to 50 lesions or more), producing a varicella-like eruption (generalized herpes zoster), occurs in 2% to 10% of unselected patients with localized HZ, most of whom are immunocompromised by HIV infection, underlying malignancy (particularly lymphomas); corticosteroids; or immunosuppressive therapy (Shanbrom et al., 1960; Merselis et al., 1964; Oberg & Svedmyr, 1969; Goffinet et al., 1972; Ruckdeschel et al., 1977; Reboul et al 1978; Patterson et al., 1980; Whitley et al., 1982; Locksley et al., 1985; Cohen et al., 1988; Novelli et al., 1988; Gilson et al., 1989). Cutaneous dissemination is rarely associated with clinically significant visceral involvement in immunocompetent patients.

On rare occasions, most often in children or immunocompromised patients, infection may disseminate widely from a small, painless area of HZ (Rogers & Tindall, 1972; Munoz et al., 1998), so that the HZ is unnoticed and the disseminated eruption is mistaken for varicella. This explains some reported second attacks of varicella, as well as some of the cases of "atypical generalized zoster" (a disseminated varicella-like eruption without an accompanying dermatomal rash in a person with a history of varicella) which are reported primarily in immunocompromised patients (Schimpff et al., 1972; Patterson et al., 1980; Locksley et al., 1985). Symptomatic reinfection (i.e., a second episode of varicella) does occur, however, especially in immunocompromised patients and in people whose initial infection

was modified by passively acquired antibody to VZV (Gershon et al., 1984; Weller, 1983; Balfour et al., 1977). These patients have a prolonged incubation period and probably account for many cases of "atypical generalized zoster" (Merselis et al., 1964; Oberg & Svedmyr, 1969; Schimpff et al., 1972; Patterson et al., 1980).

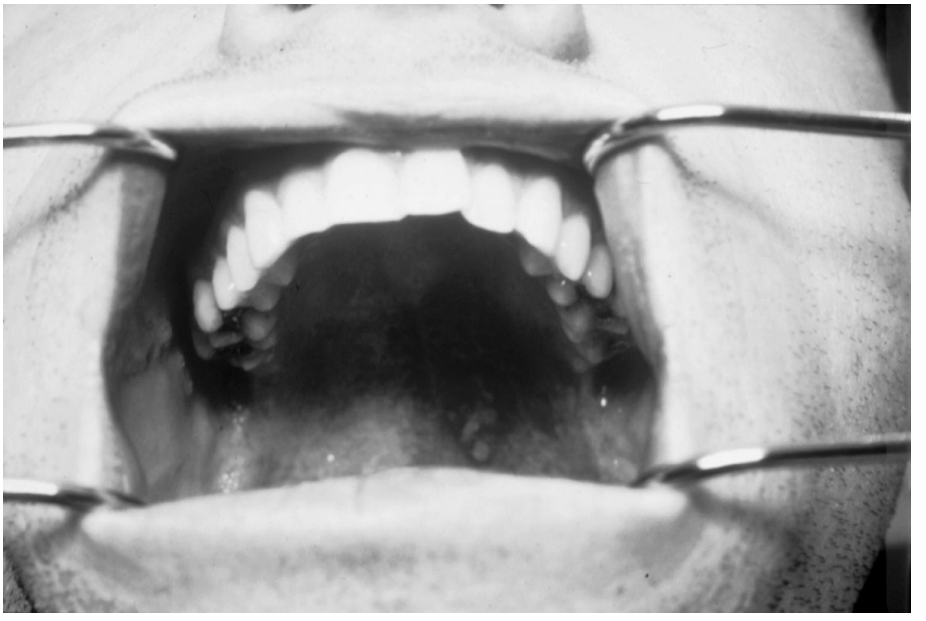
Segmental motor paralysis occurs in 5% or more of patients with HZ. It is caused by the destruction of motor neurons in the anterior spinal horn or by motor radiculitis, and is associated with the direct extension of VZV infection and inflammation from the sensory ganglion to adjacent parts of the nervous system (Head & Campbell, 1900; Denny-Brown et al., 1944; Grant & Rowe, 1961; Juel-Jensen & MacCallum, 1972; Thomas & Howard, 1972). Paralysis usually begins within a week of the onset of the HZ rash, and almost always involves muscles with innervation corresponding to the affected dermatome. Oculomotor and facial palsies are seen with cephalic HZ (Hunt 1908; Devriese, 1968; Devriese & Moesker, 1988; Berrettini et al., 1998; Johnson, 1998), unilateral diaphragmatic paralysis with ipsilateral cervical HZ (Dutt, 1970; Melcher et al., 1990), Horner's syndrome with HZ of the second and third thoracic dermatomes (Wimalaratna et al., 1987), abdominal muscle paralysis with HZ of thoracic dermatomes (Gottschau & Trojaborg, 1991; Glantz & Ristanovic, 1988); paralysis of the trunk and limbs with HZ involving corresponding dermatomes (Kendall, 1957; Grant & Rowe, 1961; Gupta et al., 1969; Thomas & Howard, 1972; Goodman & Kendrick, 1974; Molloy & Goodwill, 1979); and urinary retention or urinary and fecal incontinence with sacral HZ (Jellinek & Tulloch, 1976). When HZ involves the cranial nerves or extremities, the incidence of motor involvement may exceed 10%, and the incidence in thoracolumbar HZ may be higher than reported because mild motor deficits in intercostal and abdominal muscles are often missed (Thomas & Howard, 1972; McKendall & Klawans, 1978; Gottschau & Trojaborg, 1991; Johnson, 1998). Rare cases in which the involved myotome and dermatome are widely separated may reflect more extensive myelitis (Rose et al., 1964; Thomas & Howard, 1972).

The pathogenesis of HZ-associated facial paralysis is unclear and it is probably multifaceted (Devriese, 1968; Johnson, 1998). It can occur with HZ of the face or neck, and there are often additional neurologic findings which reflect involvement of multiple cranial nerves and ganglia (Denny-Brown et al., 1944; Devriese, 1968; Kikuchi et al., 1995). During varicella, vesicles in the oropharynx lead to latent infection of the trigeminal ganglia, as well as the petrosal ganglia via the glossopharyngeal nerve. Later reactivation may result in HZ of the oropharyngeal mucosa. Similarly, somatic sensory fibers of the facial nerve innervating the auditory meatus may carry virus to the geniculate ganglia during varicella (Furuta et al., 1992); later reactivation may lead to HZ of the auditory meatus, loss of taste in the anterior two-thirds of the tongue, and ipsilateral facial palsy (the Ramsey Hunt syndrome). The ear and external auditory canal are innervated by the fifth, seventh,

ninth and tenth cranial nerves and by the upper cervical nerves, and the facial nerve anastomoses with all of them. Thus when HZ involves the ganglia of any of these nerves it may cause facial paralysis and cutaneous lesions on or around the ear (Denny-Brown et al., 1944; Devriese, 1968; Kikuchi et al., 1995). Facial paralysis may also occur in association with HZ of the cervical dermatomes. Eighth nerve dysfunction, with sensorineural hearing loss or vertigo, may accompany facial paralysis or occur independently. HZ may also cause facial paralysis in association with oropharyngeal lesions, which are often not recognized (Figure 13.4). On rare occasions, HZ can cause facial paralysis without any skin or mucosal lesions (i.e., with zoster sine herpete). However, most cases of idiopathic facial palsy (Bell's palsy) appear to be caused by herpes simplex virus infection (Murakami et al., 1996). Paralysis usually occurs during the first week of the HZ rash, but it precedes the rash in about 30% of cases. Recovery of function is related to the extent of the paralysis. The majority of patients with incomplete facial paralysis recover most or all motor function in 3 to 15 months; when paralysis is complete only a minority recover without a significant deficit (Devriese & Moesker, 1988). Ophthalmic HZ is frequently complicated by oculomotor palsies, ptosis and paralytic mydriasis. In otic HZ with facial palsy, there is often also hearing loss and vertigo (Hunt, 1908; Devriese, 1968; Johnson, 1998).

Dorsal root ganglia contain the cell bodies of visceral as well as cutaneous afferents, and this explains the occurrence of visceral as well as cutaneous lesions in patients with HZ. The affected viscera usually have afferent innervation corresponding to the infected dermatome. Thus, HZ lesions in the gastric mucosa have been observed in patients with thoracic HZ (Wisloff et al., 1979); aseptic arthritis of the knee has been observed with ipsilateral L1–L2 HZ (Aarons & Beeching, 1993); and hemicystitis has frequently been observed in patients with sacral HZ (Gibbon, 1956; Richmond, 1974). Other mucosal surfaces may also be affected, resulting in esophagitis, pleuritis or peritonitis. Visceral afferents also originate in mechanoreceptors involved with gastrointestinal and bladder motility and sphincter function. Thus cutaneous HZ has been associated with gastroparesis; ileus; colitis; constipation; hypomotility; symptoms of gastrointestinal obstruction with evidence of VZV infection of the myenteric plexus; and with dysfunction of the bladder and anus (Figiel & Figiel, 1957; Wayburn-Mason, 1957; Chang et al., 1978, Kesner & Bar-Maor, 1979; Kebede et al., 1987; Okimura et al., 1996). Myositis in association with HZ appears to represent direct infection of muscle by VZV transmitted from the affected ganglion via proprioceptive afferents (Rubin & Fusfeld, 1965; Norris et al., 1969; Schmidbauer et al., 1992). VZV is the principal cause of acute retinal necrosis (ARN), a fulminant sight-threatening disease that occurs primarily in otherwise healthy individuals (Culbertson et al., 1986; Soushi et al., 1988; Engstrom et al., 1994; Holland, 1994; Garweg & Bohnke, 1997). Although it is

(A)



(B)

Figure 13.4 Herpes zoster involving the palatal branch of the right facial nerve (A) complicated by right facial palsy (B) in an immunocompetent adult. The facial palsy gradually resolved over a period of 3 months.

caused by reactivation of latent VZV, ARN is rarely accompanied by cutaneous manifestations of HZ. A high index of suspicion is essential, because prompt initiation of effective antiviral therapy offers the best chance of preserving sight.

Although lymphocytic pleocytosis, with or without an increase in the concentration of protein in the cerebrospinal fluid, is a regular feature of uncomplicated HZ, the incidence of acute symptomatic meningoencephalitis and myelitis is low (0.2% to 0.5%). When these complications do occur, their onset usually follows the onset of rash by 7 to 10 days, but they may precede the rash by a week or more or follow it by up to 2 months (Gold & Robbins, 1958; Applebaum et al., 1962; McCormick et al., 1969; Norris et al., 1970; Gershon et al., 1980; Jemsek et al., 1983; Peterslund, 1988). The pathogenesis is not understood, but it may involve both virus-induced and immunopathologically mediated injury. Most cases appear to involve VZV infection, with virus reaching the CNS by the hematogenous route in the course of disseminated HZ or by direct extension from the involved sensory ganglion. Cerebral vasculitis, with VZV infection of vessel walls, may also play a role, especially in severely immunocompromised patients (Amlie-Lefond et al., 1995). Clinical manifestations include fever, altered sensorium (frequently with delirium and hallucinations), headache, meningismus, and cranial or extracranial nerve palsies, often at a cord or brainstem level corresponding to the rash. There is a lymphocytic cerebrospinal fluid pleocytosis, with the cell count usually ranging from 10 to 500/mm^3, a moderate elevation in protein concentration, and a normal glucose concentration. However, the cell count may occasionally exceed 1000/mm^3, there may sometimes be 30–40% neutrophils, and the glucose concentration may be low. The incidence of meningoencephalitis is increased in cranial HZ and in immunocompromised patients, in whom most cases occur in association with VZV dissemination. Immunocompetent patients generally recover and return to their pre-encephalitis cognitive status, but many are left with postherpetic neuralgia, chronic ophthalmic infections, and motor palsies (Jemsek et al., 1983; Peterslund, 1988; Johnson, 1998). HZ myelitis reflects direct involvement of the spinal cord by VZV which, in immunocompetent patients, appears to reach the CNS by direct extension from the involved sensory ganglion. Infection and inflammation are most severe at the level corresponding to the HZ rash, but the process may extend to higher and lower levels. Clinical manifestations include motor weakness and bladder dysfunction, as well as reflex abnormalities and sensory loss below the level of the rash. Severe cases result in transverse myelitis with clinical manifestations of spinal cord transection (Hogan & Krigman, 1973; Heller et al., 1990). HZ myelitis has been observed in the absence of a HZ rash. It is more frequent and severe in immunocompromised patients. Aseptic meningitis, usually asymptomatic, occurs in about 40% of HZ patients and in a higher proportion of those with cephalic HZ. It appears that some cases of aseptic

meningitis caused by reactivation of latent VZV occur in the absence of HZ rash (Echevarria et al., 1987; Gilden et al., 1992).

VZV-induced segmented granulomatous angiitis of cerebral arteries is responsible for a syndrome of ophthalmic zoster and delayed contralateral hemiplegia (Rosenblum & Hadfield, 1972; Bourdette et al., 1983; Hilt et al., 1983, Reshef et al., 1985; Eidelberg et al., 1986; Herkes et al., 1987; Gilden et al., 1996). It usually occurs weeks to months after the episode of ophthalmic zoster (average interval 7 to 8 weeks) and may present as an isolated cerebral infarction, multiple cerebral infarctions, stroke-in-evolution, transient ischemic attacks, or sudden death. Because the clinical manifestations are similar to those of hypertensive strokes and the delayed onset may obscure the relationship to herpes zoster, the syndrome is probably underdiagnosed. Cerebral arteriograms usually reveal segmental narrowing or occlusion of cerebral arteries ipsilateral to the ophthalmic zoster. Although most cases have followed ophthalmic HZ and involve the middle and anterior cerebral arteries, some have followed HZ of other cranial nerves, as well as cervical HZ (Filloux & Townsend, 1985; Fukomoto et al., 1986; Snow & Simcock, 1988; Willeit & Schmutzhard, 1991) and have resulted in central retinal artery occlusion; thalamic, brainstem and cerebellar infarctions; and a ruptured basilar artery aneurism. Pathologic studies have documented VZV infection of the walls of the involved arteries (Ross et al., 1990; Gilden et al., 1996; Kleinschmidt-DeMasters et al., 1996; Johnson, 1998). It seems likely that virus reaches these arteries via nerves that originate in the involved ganglia and innervate the arterial walls. Although multiple strokes may occur for several weeks, later recurrences are rare and the disease appears to be self-limited. The mortality in reported cases is about 20%. The long interval between the episode of HZ and the clinical manifestations of arteritis indicate that VZV is capable of producing a subacute smoldering infection in immunocompetent individuals, as well as those who are immunocompromised.

Clinical manifestation of herpes zoster in the immunocompromised host

Infection with the HIV, certain types of malignancy, especially Hodgkin's disease and lymphocytic leukemia, and the administration of immunosuppressive therapy (e.g., radiation, antimetabolites, antilymphocyte serum, and corticosteroids) to patients with malignant and nonmalignant diseases markedly increase the incidence and severity of HZ (Shanbrom et al., 1960; Merselis et al., 1964; Sokal & Firat, 1965; Goffinet et al., 1972; Schimpff et al., 1972; Feldman et al., 1973; Stevens, 1975; Goodman et al., 1976; Luby et al., 1977; Dolin et al., 1978; Reboul et al., 1978; Meyers et al., 1980; Pollard et al., 1982; Cohen et al., 1988; Novelli et al., 1988; Rusthoven et al., 1988; Leboit et al 1992; Wilson et al., 1992; Whitley, 1992; Veenstra et al., 1996). In fact, except for PHN and complications of ophthalmic zoster,

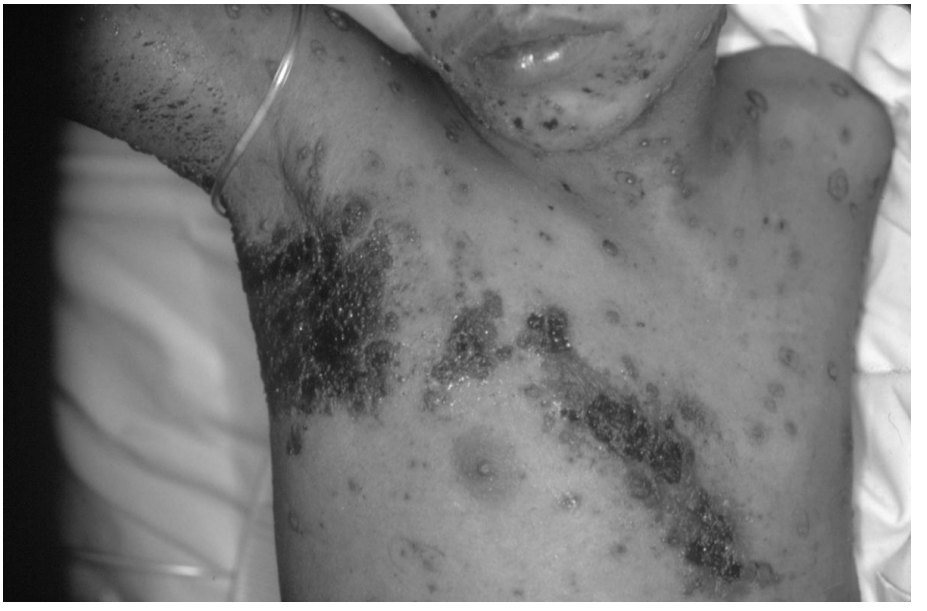


Figure 13.5 Herpes zoster in the right third thoracic dermatome in a patient with Hodgkin's Disease. Note the cutaneous dissemination and the necrosis of the involved skin in the primary dermatome.

serious complications of HZ occur predominantly in immunocompromised patients.

From 20% to 50% of patients with Hodgkin's disease develop HZ within 18 months following diagnosis, with the highest incidence in patients with far-advanced disease and those receiving radiation and combination chemotherapy. When HZ occurs in these patients, it is usually within 1 month of chemotherapy or within 7 months of X-ray therapy.

The severity of HZ and the risk of complications are also increased in immunocompromised patients – necrosis of skin and scarring are fairly common (Figure 13.5), and the incidence of cutaneous dissemination may be as high as 25% to 50%. Approximately 10% of patients with cutaneous dissemination manifest widespread, often fatal visceral dissemination, particularly to the lungs, liver, and brain (Shanbrom et al., 1960; Merselis et al., 1964; Pek & Gikas, 1965; Dolin et al., 1978; Whitley, 1992; Veenstra et al., 1996). The incidence and severity of HZ are also markedly increased in immunosuppressed recipients of solid organ and bone marrow transplants. Between 30% to 40% of allogeneic bone marrow transplant recipients develop HZ within 1 year of transplantation, usually between 3 to 9 months (Locksley et al., 1985); 10% to 15% have significant visceral dissemination, often without a recognizable cutaneous focus of localized HZ. PHN, scarring, and

bacterial superinfection are also frequent complications of HZ in these patients. Allogeneic bone marrow transplant recipients may present with a syndrome of acute abdominal pain, nausea and vomiting that is associated with visceral dissemination and extensive involvement by VZV of intraabdominal organs, including liver, spleen, pancreas, bowel and terminal esophagus (Locksley et al., 1985; Schiller et al., 1991; Verdonk et al., 1993; Munoz et al., 1998). Some develop, in addition, VZV interstitial pneumonia. The visceral dissemination frequently precedes the appearance of any cutaneous lesions by several days, and in some cases cutaneous lesions never appear. The mortality rate is 20% to 40%.

The incidence of HZ is greatly increased in persons infected with HIV; it tends to occur early in the course of HIV infection and is often the first sign of immune deficiency (Friedman-Kien et al., 1986; Colebunders et al., 1988). The incidence and severity of HZ in HIV infected patients increases with decreasing CD4+ lymphocyte counts, but HZ is not an independent predictor of HIV disease progression or death (Veenstra et al., 1995; McNulty et al., 1997; Chaisson et al., 1998). HIV infected patients are fairly unique in their tendency to suffer multiple recurrences of HZ as their disease progresses; HZ may recur in the same or in a different dermatome, or in several contiguous or noncontiguous dermatomes. Patients with AIDS may develop severe HZ with cutaneous and visceral dissemination (Cohen et al., 1988; Pahwa et al., 1988; Jura et al., 1989; Cohen & Grossman, 1989; Petrozza et al., 1993; Fraisse et al., 1998). They can experience chronic, often acyclovir-resistant cutaneous lesions with unusual verrucous, hyperkeratotic, or ecthymatous features (Figure 13.6) (Alessi et al., 1988; Hoppenjams et al., 1990; Leboit et al., 1992; Tappero et al., 1995; Asensi et al., 1996; Breton et al., 1998). AIDS patients also experience progressive outer retinal necrosis (PORN), a blinding infection that can be distinguished from cytomegalovirus retinitis and acute retinal necrosis (ARN) caused by VZV or herpes simplex virus (Engstrom et al., 1994; van den Horn et al., 1996).

A syndrome resembling progressive multifocal leukoencephalopathy (PML) has been reported following HZ in several immunocompromised patients, mostly patients with advanced AIDS (Horton et al., 1981; Gray et al., 1992, 1994). These patients exhibit progressive, asymmetric, multifocal neurologic deficits, impaired mental function and focal seizures, and die within months to a year or so after onset. Autopsy reveals multifocal lesions, primarily at the gray–white cortical junction, with demyelination, necrosis, and eosinophilic Cowdry type A intranuclear inclusion bodies in oligodendrocytes, neurons, and astrocytes. These lesions contain abundant viral antigens, particles, and nucleic acids. The pathogenesis of these lesions seems to involve a vasculopathy affecting primarily smaller vessels which are infected by VZV, producing small areas of infarction, and transmitting the virus to glial cells, which leads to local demyelination (Schmidbauer et al., 1992;

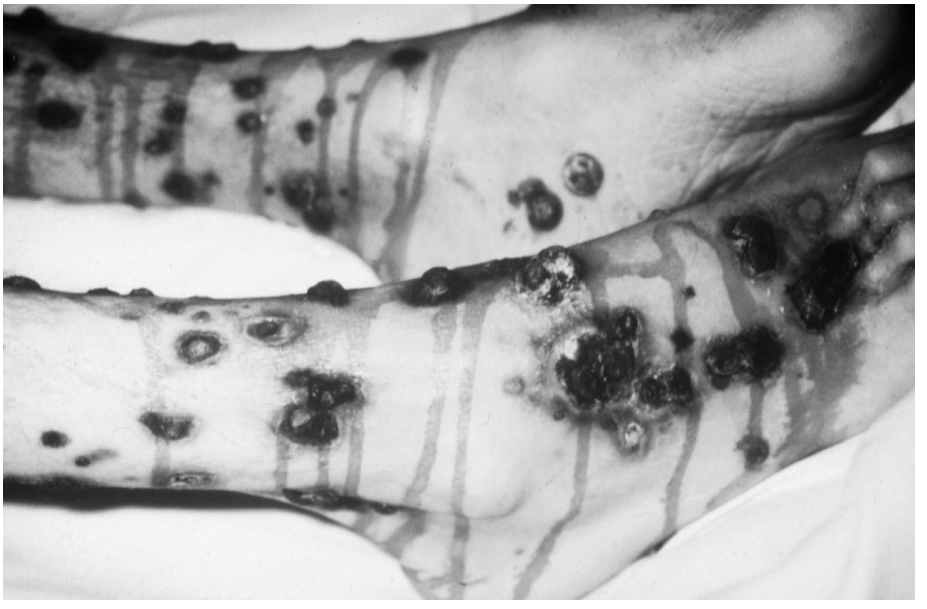


Figure 13.6 Chronic recurrent disseminated HZ caused by acyclovir-resistant VZV in a male hemophiliac with AIDS and a CD4 cell count of 5/mm^3. The large verrucous hyperkeratotic lesions evolved from small vesiculopustular lesions during therapy with acyclovir (courtesy of Dr. V. Asensi).

Amlie-Lefond et al., 1995; Kleinschmidt-DeMasters et al., 1996). A remarkable feature of some of these cases has been the long interval (up to 20 months) between the episode of cutaneous HZ and the onset of neurologic symptoms. This observation, as well as the long interval between ophthalmic zoster and the onset of symptoms in patients with segmental granulomatous cerebral angiitis, and the occurrence of chronic cutaneous HZ in patients with AIDS suggest that in addition to established latent infections, VZV can produce prolonged, chronic, "smoldering" subclinical and clinical infections.

A variety of visceral and central nervous system VZV infections have been recognized in HIV infected and other profoundly immunocompromised patients. They have often presented long after dermatomal HZ or in the absence of any recognized cutaneous lesions. They include myocarditis (Chauhan et al., 1996), pericarditis (Winfield & Joseph, 1980), nodular pneumonia (Pek & Gikas, 1965; Wingard et al., 1985; Fraisse et al., 1998; Kim et al., 1999), recurrent HZ encephalitis (O'Donnell et al., 1981), chronic progressive VZV encephalitis (Ryder et al., 1986; Gilden et al., 1988), brainstem encephalaitis (Moulignier et al., 1995) and necrotizing encephalitis (Manian et al., 1995; Lionnet et al., 1996), as well as a variety of manifestations of ocular VZV infections (see Chapter 14).

In reviewing the wide variety of complications of HZ, it is remarkable how often the afflicted individuals have received systemic corticosteroids. The central role of vasculitis in many of these complications is also noteworthy. Finally, since most complications of HZ are ultimately dependent upon VZV replication, a high index of suspicion and the early initiation of effective antiviral therapy will do much to reduce the morbidity and mortality of HZ until preventive measures have been developed.

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14

Ophthalmic zoster

Deborah Pavan-Langston

Involvement of the trigeminal nerve in recurrent varicella-zoster virus (VZV) infection (herpes zoster ophthalmicus, HZO) is second in frequency only to thoracic zoster but can exact a much greater toll in terms of long term complications and marked disruption of the quality of life.

Epidemiology

Of the one million doctor visits for zoster in the United States annually, up to 250 000 involve the eye and, of those, 50–72% suffer chronic, recurring ocular disease and visual loss. Disturbance of cell-mediated immunity (CMI) is a critical precipitating factor, with the incidence of zoster now increasing due to the HIV epidemic, an aging population, and other causes of immunosuppression (Hope-Simpson, 1965; Ragozzino et al., 1982; Harding et al., 1987; Donahue et al., 1995; Weller, 1995; Pepose, 1997).

In a 1975 to 1980 study of 86 cases of HZO, Womack & Liesegang (1983) reported a predominance in female patients, involvement of the left eye, and a peak incidence in the seventh and eighth decades of life. This differed from other series showing a male predominance or equal distribution between the sexes and a peak incidence in the fourth or fifth to the seventh decade of life (Edgerton, 1945; Scott, 1957; Scheie, 1970; Donahue et al., 1995; Pavan-Langston & Dunkel, 1996). In all these studies ocular involvement ranged from 50 to 72%.

Pathogenesis and histopathology of HZO

Mahalingam et al. (1990, 1993), reported that after primary infection with varicella, VZV becomes latent far more often in the trigeminal than in any thoracic ganglion, the most common area involved clinically. They estimated that 10–25% of all zoster infections involve the eye.

Inflammation and destruction of the ganglion ensue with viral proliferation (Esiri & Tomlinson, 1972; Bastian et al., 1973; Hedges & Albert, 1982). VZV may not be

restricted to the trigeminal sensory nerves, but may also travel to the CNS to cause necrosis in the corresponding sensory nuclei (Linnemann, 1980). Studies of the histopathology of acute HZO revealed normal corneal stroma, nongranulomatous inflammation of the iris and ciliary body with extension into the choroid, macrophage and other inflammatory cell infiltration of the trabecular meshwork; all of these abnormalities appeared to be reversible (Hedges & Albert, 1982).

Acute retinal necrosis (ARN) is characterized by an extensive plasma/lymphocytic infiltrate in the ciliary body with inflammatory retinal necrosis, choroid thickened by focal lymphocytic/mononuclear infiltration with intranuclear viral inclusions alternating with areas of granulomatous infiltration and vascular thrombosis. This process can also involve the optic and posterior ciliary nerves (Culbertson et al., 1986; Culbertson & Dix, 1996; Pepose, 1997).

Histopathologic studies of chronic HZO describe a keratitis with various combinations of epidermalization of epithelium, lipid keratopathy, stromal vascular scarring, and granulomatous reaction to Descemet's membrane (Hedges & Albert, 1982; Wenkel et al., 1993). Naumann et al. also reported a lymphocytic infiltration of the posterior ciliary nerves and vessels, chronic inflammation and vasculitis of the iris and ciliary body with patchy necrosis of the iris and pars plicata, perivascular cuffing by chronic inflammatory cells in the retina, and granulomatous choroiditis. HZO extraocular muscle palsies are the result of perineuritis and perivasculitis (Naumann et al., 1968). VZV DNA was located in corneal keratocytes, epithelial cells, and mononuclear cells but not in the endothelium despite the proximity of the granulomatous reaction (Wenkel et al., 1993).

Neuronal relationships

The ophthalmic (V-1) division of the trigeminal nerve is affected about 20 times more often than are other divisions. The frontal nerve is the most frequently affected main branch (Juel-Jensen & MacCallum, 1972). Involvement of the tip of the nose by the acute rash is called *Hutchinson's sign*, a variably reliable indication that the eye may be seriously affected by VZV because of the involvement of the nasal branch of the nasociliary nerve of V-1.

By direct neural connections, VZV can cause various complications and varying degrees of disease severity. In brief these manifestations may include cicatricial lid retraction or loss, paralytic ptosis, conjunctivitis, episcleritis, scleritis, keratitis (infectious or immune), iridocyclitis, retinitis, choroiditis, optic neuritis, optic atrophy, retrobulbar neuritis, Argyll Robertson pupil, exophthalmos, extraocular muscle palsies, and glaucoma (Edgerton, 1945; Womack & Liesegang, 1983; Liesegang, 1985; Harding et al., 1987; Harding, 1993; Pavan-Langston et al., 1995; Pavan-Langston & Dunkel, 1996; Wilson, 1996; Pepose, 1997).

Clinical disease findings

The trigeminal illness may begin with unilateral headache, fever, malaise, and chills, preceded or followed by lancinating and/or constant boring and often severe neuralgia over the distribution of V-1. It may become impossible to bear the pain of combing the hair or wearing a hat. Acute pain occurs in 93% of patients with an overall persistence in 31% of cases at 6 months and the highest incidence being greater than 70% in those over 80 years of age (Harding, 1993).

Dermatitis

Within 1 to 3 days of neuralgia affecting the ocular and forehead area, hot, flushed hyperesthesia and edema of the dermatome develop, followed by eruption of multiple crops of clear vesicles from which virus may be cultured for approximately 3 days. The vesicles then become turbid and yellow, forming deep eschars that frequently leave behind permanent pitted scars. The acute inflammatory period lasts 10 to 20 days. (Figure 14.1A,B). The skin ulceration often takes many weeks to heal and results in either little scarring or the equivalent of third-degree burns, with significant loss and scarring of ocular and periocular tissues. On occasion, dermatitis may never develop (zoster sine herpete). VZV DNA has been isolated from the aqueous in an "idiopathic" keratouveitis without skin lesions (Yamamoto et al., 1995).

Conjunctivitis, episcleritis, and scleritis

Inflammation of the conjunctiva is extremely common and characterized by watery hyperemia, follicular hypertrophy with or without regional adenopathy, and rarely, severe necrotizing membranous inflammation. Sectoral episcleritis involves the fibrous tissues between the superficial membranous conjuctiva and the tough scleral coat of the eye. It may be flat or nodular. Scleritis tends to be focal and may involve several areas particularly in the perilimbal region, either as a flat or a nodular process. Episcleritis or scleritis may occur during the acute disease or several months after the cutaneous eruption has cleared. As the scleritis resolves, scleral thinning is frequently noted as focal dark areas on the "white" of the eye (Figure 14.2A)

Keratitis

Epithelial

Some form of keratitis is seen in nearly two-thirds of all HZO. Keratitis is almost invariably associated with marked decrease in corneal sensation due to significant corneal damage and/or to the necrotic ganglionitis. The keratitis may precede or follow the neuralgia or skin lesions by several days and may assume a variety of

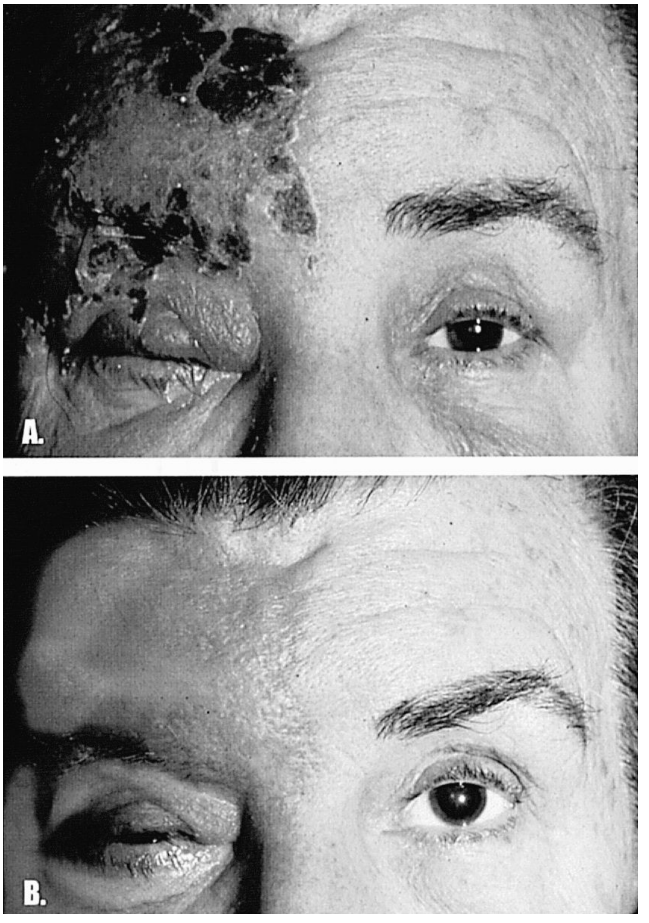

Figure 14.1 (A) Acute herpes zoster ophthalmicus O.D. (i.e. the right eye) with lid closure, hemorrhagic crusting, and sympathetic contralateral lid edema. (B) Two months post-acute disease with residual scarred hyperemia over V-1 dermatome, ptosis, and lid edema OD.

forms (Table 14.1). Most common are the infectious forms of epithelial keratitis. This form may be dendritic (branching) in pattern, which may be mistaken for herpes simplex virus keratitis (Piebenga & Laibson, 1973, Forrest & Kaufman, 1976; Marsh, 1976; Marsh & Cooper, 1987; Yamamoto, 1994). VZV has been cultured from the acute dendritic lesions by Pavan-Langston and McCulley and demonstrated by immunofluorescence (Pavan-Langston & McCulley, 1973; Liesegang, 1985). The lesions clear spontaneously within a few days, leaving behind mild anterior stromal infiltrates in 52% of patients.

"Delayed corneal mucous plaques" (delayed pseudodendrites) may appear up to 2 years after the acute disease (Liesegang, 1985). They cause a foreign body

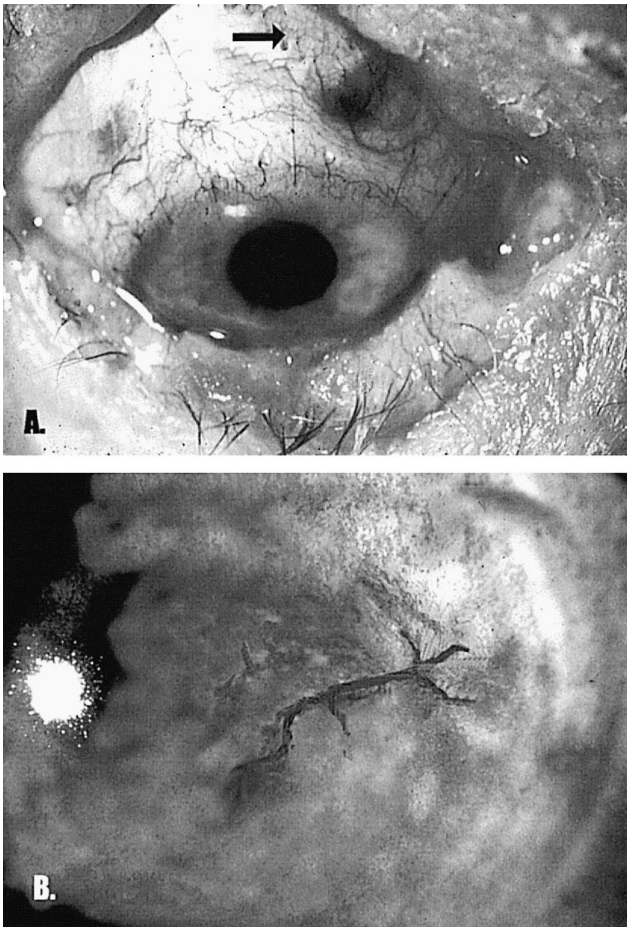

Figure 14.2 (A) HZO scleritis with moderate focal hyperemia (arrow) extending from midline nasally, and surrounding focal dark area of scleral thinning. (B) Delayed dendritiform zoster corneal plaque forming one year post acute disease and PCR positive for varicella-zoster DNA.

sensation and are grey-white, epithelial cells piled in plaques or a dendritiform shape on the surface of the cornea (Figure 14.2B). They are both transient and migratory and are often associated with a neurotrophic keratitis or previous corneal inflammation. Pavan-Langston et al. (1995) have reported PCR (polymerase chain reaction) detection of VZV DNA in several of these late-onset pseudodendrites in both immunocompetent and immunocompromised patients.

Stromal/endothelial keratitis

Thinning of the peripheral cornea (peripheral ulcerative keratitis, PUK) is commonly associated with collagen vascular disease but it is also noted in 7% of HZO

Table 14.1 Herpes zoster ophthalmicus: complications*

Complication	Incidence (%)
1. Lids/tear canaliculi	14
2. Cornea	54
3. Sclera	4
4. Iritis	43
5. Glaucoma	12
6. Cataract	8
7. Neuro-ophthalmic	8
8. Postherpetic neuralgia	17

Note: *86 patients

Sources: Adapted from Womack & Liesegang (1983).

patients (Mondino et al., 1978; Liesegang, 1985). Topical steroid therapy is effective but some corneas may require tissue adhesive and a therapeutic soft lens to enhance healing.

Steroid-sensitive keratouveitis, with or without endotheliitis, is seen in about one-third of patients and may have its onset immediately during acute disease or several weeks to months later. Frequently focal or diffuse keratic precipitates, folds in Descemet's membrane, and diffuse or focal stromal edema appear. Acute glaucoma develops in 33% of these patients, presumably due to an associated trabeculitis with inflammatory cells blocking the trabecular meshwork. A severe vasculitis with hypopyon or hyphema and pars plicata ischemia may develop, leading to anterior segment ischemia (Crock, 1967). The endotheliitis may result in significant loss of endothelial cells with permanent corneal swelling (Marsh, 1976; Reijo et al., 1983). Further, a necrotic interstitial keratitis or Wessley ring consisting of antigen–antibody–complement mediated reaction with PMN infiltration may be seen. The end-stage of this form of stromal disease is characterized by white patches of lipid deposition and fibrovascular scarring, often with deep neovascularization despite adequate topical steroid treatment.

Non-necrotic translucent immune disciform keratitis may occur any time within the first year after the acute event, most commonly at 3 to 4 months. There may be full-thickness stromal edema and associated necrotic interstitial keratitis (IK), Wessley rings, or vasculitis (Figure 14.3A). The response of this immune reaction to topical steroids is moderate to rapid initially, but tapering of steroids must often be slow, with many patients requiring minimal daily doses to prevent rebound immune disease (Green & Zimmerman, 1967; Naumann et al., 1968; Hedges & Albert, 1982; Liesegang, 1991; Pavan-Langston, 1994; Pavan-Langston & Dunkel,

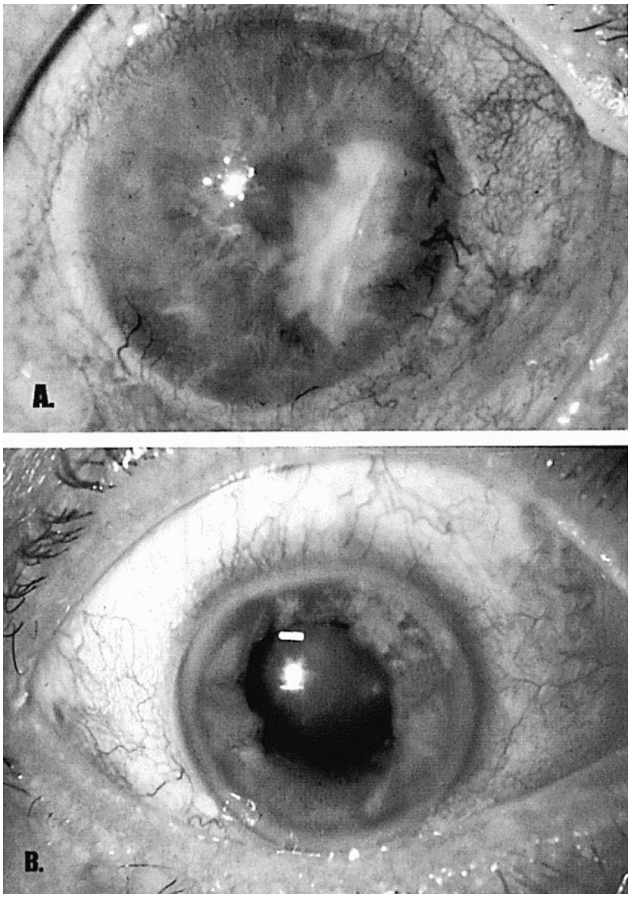

Figure 14.3 (A) Chronic stromal immune zoster keratitis with peripheral neovascularization, focal, white necrotic interstitial keratitis, and diffuse grey disciform edematous haze. (B) Chronic HZO iritis with limbal hyperemia nasally, iris stromal and sphincter atrophy with irregular, poorly reactive pupil.

1996). Assays for VZV DNA have been positive in some reported cases (Wenkel et al., 1993; Yu et al., 1993; Pavan-Langston et al., 1995). As with iritis (see below), disciform keratitis may occur as a manifestation of zoster sine herpete. VZV DNA had been detected in the aqueous of such a patient (Silverstein et al., 1997).

Mechanical

Nearly half of all patients develop neurotrophic keratitis, the result of corneal anesthesia secondary to the ravages of VZV ganglionitis and to aqueous tear deficiency due to loss of the nasolacrimal reflex (Womack & Liesegang, 1983; Liesegang, 1985; Heigle & Pflugfelder, 1996). About 25% of all HZO patients will, however, develop

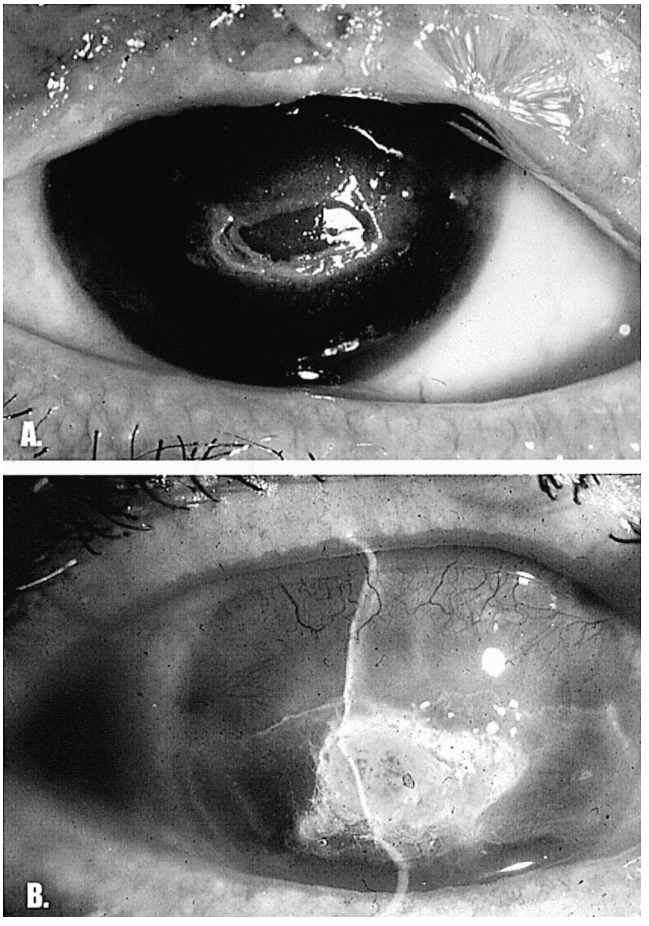

Figure 14.4 (A) Sterile, melting (thinning) chronic stromal ulcer in anaesthetic cornea of HIV-infected patient. Lid scarring prevents adequate closure. (B) Tissue adhesive (cyanoacrylate glue) used to fill a melting trophic ulcer in an anesthetic, severely scarred post-zoster cornea. Over several months healing occurred under the adhesive.

clinical signs of neurotrophic keratitis due to permanent and marked corneal anesthesia (0–1/6 on Cochet–Bonnet testing).

Clinical findings include a dull or irregular corneal surface with mild coarse punctate epithelial keratitis. The tear film is highly unstable, blink frequency is reduced in these anesthetic eyes, and exposure may further aggravate the condition. As the corneal epithelium becomes progressively more unhealthy, ovoid epithelial defects frequently develop in the palpebral fissure or lower corneal area with subsequent melting and corneal thinning; perforation is possible if no scarring neovascular pannus grows in (Liesegang, 1985) (Figure 14.4).

Iridocyclitis

Inflammation of the iris is second in frequency to corneal involvement in HZO. In Womack and Liesegang's studies, anterior uveitis, white blood cells and protein flare in the aqueous fluid was seen in 43% of patients. This figure is in general agreement with other studies, as were the characteristic findings of circumcorneal hyperemia, lymphocytic precipitates on the corneal endothelium (keratic precipitates, KPs), iris scarring to the lens, sectoral iris pigment atrophy, and sphincter damage (Womack & Liesegang, 1983) (Figure 14.3B). Fluorescein angiography revealed occlusion of iris vessels from vasculitis at the sites of atrophy (Marsh et al., 1974; Womack & Liesegang, 1983; Liesegang, 1984). Involvement may occur early or many months or years after the onset of acute disease and independent of corneal activity. It is felt to be an immune reaction probably triggered by direct invasion of the uveal structure by infectious VZV. Late-onset uveitis may be due to immune reaction to the antigenic residua of the virus. VZV DNA has been detected in the aqueous (Liesegang, 1984; Yamamoto et al., 1995; Wilson, 1996).

Glaucoma/intraocular pressure (IOP)

Marked elevation of IOP may be seen in about 10% of patients, especially those with peripheral corneal endotheliitis with an associated open angle trabeculitis, with isolated trabeculitis and no corneal involvement, or secondary to cicatricial closure of the angle. Occasionally, a marked decrease in IOP due to necrosis of the pars plicata of the iris ciliary body, with subsequent decreased aqueous production, is more than counterbalanced by impairment of outflow facility by clogging of the trabecular meshwork with inflammatory cellular debris. Depending on the balance between decreased aqueous production and decreased outflow, the IOP may be low, normal, or elevated (Naumann et al., 1968; Womack & Liesegang, 1983; Liesegang, 1984).

Muscle palsies and posterior segment disease

Palsies of nerves III, IV, and VI are not uncommon and may cause distressing double vision (Chang-Godinich et al., 1997). With the exception of an almost invariable residual partial lid ptosis (droop), these palsies clear completely over several weeks to months regardless of initial severity (Figure 14.5A).

Retinitis

Posterior segment involvement is uncommon but may include optic neuritis, central retinal vein occlusion, central retinal artery occlusion, necrotizing retinitis (acute retinal necrosis [ARN] or progressive outer retinal necrosis [PORN]), delayed thrombophlebitis, optic neuropathy, and localized arteritis with or without exudates (Culbertson et al., 1986; Browning et al., 1987; Engstrom et al., 1994;

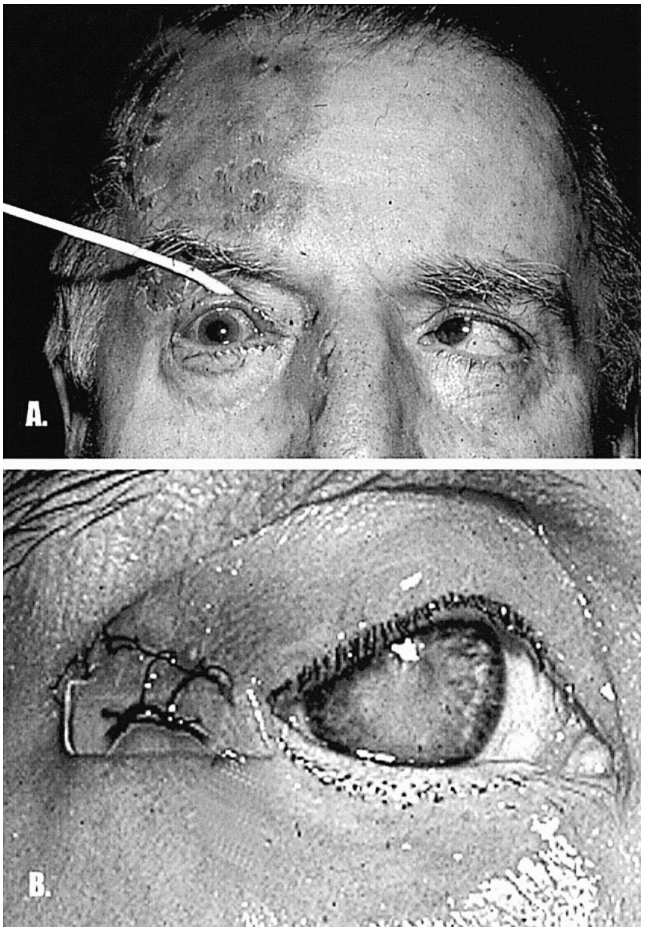

Figure 14.5 (A) Third, fourth and sixth nerve paralysis such that eye is "frozen" in orbit in all fields of gaze at one month post acute HZO. Full eye motion was recovered within 18 months. (B) Lateral 4 mm of lids sutured shut to provide greater protection for anaesthetic cornea at risk of ulceration and thinning and scarred by immune stromal disease.

Culbertson & Dix, 1996). Of these, the most prevalent are ARN and PORN, either of which may develop during primary infection or from reactivation of latent VZV. The differential diagnosis of VZV retinitis includes herpes simplex virus, which is another cause of ARN, cytomegalovirus, toxoplasmosis, syphilis, large cell lymphoma and Behçet's disease.

ARN is seen in either immunocompetent or immunocompromised patients while PORN is an affliction of those with advanced AIDS. While any age group may be affected, incidence peaks in the fifth to seventh decades of life. Temporally, ARN may develop just days to months after cutaneous zoster, which need not involve the

trigeminal dermatome or may be contralateral to the eye with HZO. Unilateral cases may become bilateral in 5 to 20 days. In contrast, PORN is temporally much more closely associated with recent cutaneous zoster, although, again, not necessarily HZO.

Clinically, the initial symptoms of ARN are discomfort on eye movement, photophobia, and floaters. The eye(s) become(s) inflamed and a significant iritis with corneal and vitreous white cell precipitates (KPs) may make fundus view difficult and mandate dilation for exam. The earliest retinal lesions are oval, deep, yellow-white lesions in the retina involving the postequatorial fundus (peripheral two-thirds) and sparing the macular region. Optic nerve edema is common. The necrotizing retinal lesions spread rapidly over 5 to 10 days, involving just one quadrant or the entire peripheral retina with infiltrate, blotchy hemorrhages, arteriolitis and phlebitis. Vision deteriorates rapidly as these lesions progress.

Both eyes become involved in 24–80% of untreated patients (Engstrom et al., 1994; Culbertson & Dix, 1996). Regression begins within 5 days in acyclovir treated patients and in 3 weeks in untreated patients. The atrophic retina has a salt and pepper appearance, the optic nerve is pale, vision may be good to poor, and retinal detachment occurs within 8 weeks in 75% of the most severely involved eyes.

PORN symptoms are minimal with painless, quiet constriction of peripheral vision and ultimately loss of central vision. There is minimal anterior inflammation and no vitritis. Multiple areas of necrotic retinitis develop throughout the back of the eye, including the macular area and spreading progressively to the far periphery. Optic neuritis and vasculitis are less common than with ARN, but bilaterality is much more common (70%). Prognosis for vision is guarded because the therapeutic response in HIV patients is often poor and the incidence of retinal detachment is high.

Diagnosis is based largely on clinical impression but the etiology may be proved by viral isolation or PCR assay of vitreous or retinal biopsies, antibody titers of intraocular fluids (aqueous or vitreous) and intrathecal antibody production. Serum antibody titers are of little value.

Acute and postherpetic neuralgia (PHN)

HZO has a much higher incidence of postherpetic neuralgia (PHN) than other forms of zoster, especially in patients over the age of 50 years (Ragozzino et al., 1982; Cobo et al., 1987; Donahue et al., 1995). In a study of 916 patients with ocular zoster seen between the years 1935 and 1949, deMoragas and Kierland reported an incidence of 12.5% acute neuralgia in those patients less than 20 years old, approximately 40% in those in the third and fourth decades of life, and 20% in those in the sixth and seventh decades (deMoragas & Kierland, 1957). In contrast, within this same population the incidence of chronic postherpetic neuralgic (PHN) pain

lasting more than 1 year fell to less than 4% in the age group of under 20 years, and to about 10% in those patients in the third and fourth decades of life, but rose to nearly 50% in those patients in the sixth and seventh decades of life. This concurs with another study noting PHN persisting in 17% of patients, with the most severe cases being in those patients in the sixth to eighth decades of life. Four patients had been treated with prednisone in the course of disease. Ragozzino and coworkers found a 9.3% incidence also in the older patient population (Ragozzino et al., 1982; Womack & Liesegang, 1983).

The efficacy of systemic acyclovir for preventing or minimizing the PHN of HZO varies, with some studies reporting no efficacy, and others a significant decrease in severity and incidence (Cobo et al., 1986; Harding & Porter, 1991; Hoang-Xuan et al., 1992; Aylward et al., 1994; McGill & White, 1994; Pepose, 1997). Famciclovir and valaciclovir are more effective against PHN than acyclovir in nontrigeminal zoster but no studies of ocular zoster have been published (Beutner et al., 1995; Tyring et al., 1995; Pepose, 1997; Dworkin et al., 1998; Wood et al., 1998). Two HZO studies have reported that famciclovir and valaciclovir are equivalent to acyclovir in acute disease. The effect on trigeminal PHN is, as yet, undetermined (Tyring et al., 1998; Colin et al., 2000).

HZO in immunocompromised patients

Jabs' review of 1163 HIV patients indicates that the overall incidence of HZO is 3%, regardless of the stage of immunosuppression (Jabs, 1995). With the advent of this epidemic, physicians are seeing more HZO patients in younger age groups. In 1984, Cole et al. reported four cases of HZO in HIV-infected men ranging in age from 26 to 41 years. Only one patient was treated with systemic antiviral therapy (vidarabine). All received topical steroids for ocular immune reactions, and one received systemic prednisone therapy, yet all recovered without immediate adverse consequences of the HZO.

HZO in the HIV-infected patient is characterized by marked and prolonged dermatitis, keratitis, and iritis variably responsive to intravenous and oral antivirals (Cole et al., 1984; Engstrom & Holland, 1988; Litoff & Catalano, 1990). Margolis et al. (1998) noted that, in 48 HIV-infected patients with trigeminal zoster, 31% had no ocular involvement, 35% has stromal keratitis (mostly mild), 4% had infectious psedudodendritic keratitis, 50% had iritis, and 4% had postherpetic neuralgia. More recently, Chern et al. (1998) have reported 16 HIV-infected patients with HZO who had chronic dendritiform epithelial keratitis ranging in onset from day 1 of acute disease to 6 years post rash. All were either culture or PCR positive for VZV. Two patients never had a dermatitis and five had previous nontrigeminal zoster. The lesions were similar to some of those described under "Keratitis" above

in being thick and discontinuous with no terminal bulbs but far more long-lasting (weeks to months) and tending to cross the limbus. In 75% of patients, pain was particularly severe and was relieved by topical anaesthetics but not by TCAs (tricyclic antidepressants), indicating that it was local and not PHN. Response to antivirals was highly variable. The recommended initial drugs are topical trifluridine, vidarabine ointment, or oral acyclovir. Oral famciclovir, foscarnet or cidofovir have been given in treatment failures. The efficacy of foscarnet and cidofovir against VZV has not been proved and many treatment failures are attributable to poor host responses rather than VZV resistance to antiviral drugs.

Therapy of HZO

Prevention: varicella vaccine

One case of HZO has occurred in a child 3.5 years after vaccination but it could not be determined whether this was due to the vaccine strain or a natural strain of VZV (Matsubara et al., 1995). The marked drop in cell-mediated immunity to zoster by age 50 and the excellent immune response to vaccine in the older population suggests that vaccinating even those patients who have had chickenpox in childhood may reduce the incidence of zoster, including HZO in older adults (Levin et al., 1998).

Antiviral drugs

Zaal et al. (1991) reported that at 3 months post onset of HZO, patients who received 3% topical acyclovir (ACV) had longer durations of periocular lesions and significantly more visual loss compared to the group receiving oral ACV, and that all patients given combined topical ACV and dexamethasone drops developed chronic disease. This experience indicates that systemic antiviral therapy is the route of choice and that topical steroids should be withheld, if possible, during the periacute stage of HZO.

Controlled clinical trials in immunocompetent patients with acute HZO revealed a significant superiority of ACV therapy in comparison to placebo (Cobo et al., 1985, 1986). ACV patients benefiting most were those in whom treatment began within 72 h of onset of disease (but benefit was *not* confined to this early treatment group) and received doses of 800 mg p.o. 5i.d. for 7 days. There was a prompt resolution of skin rash, cessation of pain, more rapid healing, reduced duration of viral shedding, and reduced duration of new lesion formation. There was also a significant reduction in the incidence and severity of acute dendritiform keratopathy, scleritis, episcleritis, iritis, the incidence (but not the severity if it occurred) of corneal stromal immune keratitis, and the incidence of late-onset ocular inflammatory disease.

Other studies in more than 400 immunocompetent patients with HZO demonstrated similar ocular benefits but also showed a reduced incidence of pain, particularly PHN (29% versus 50–70% untreated patients in other studies) (Borruat et al., 1991; Harding & Porter, 1991; Hoang-Xuan et al., 1992). One contrasting report is a 5 year retrospective case-controlled study of 419 patients reporting that ACV had no effect on the rate of ocular complications in HZO (Aylward et al., 1994).

The salient effect on PHN noted in all but the last HZO study is consistent with results of large ACV (800 mg q.d. × 7d) versus placebo trials in patients with non-ocular zoster (Huff et al., 1993; Malin, 1993; Wood et al., 1996). Use of ACV in HIV or other immunocompromised patients is much more complicated, particularly in posterior segment disease. Nonetheless, for anterior segment HZO, the usual dose of ACV 800 mg p.o. 5i.d. usually suffices but should probably be continued for 2 to 3 weeks. For more severely ill patients or for retinitis, hospitalization and intravenous ACV, 10 mg/kg q. 8 h, and often combined with intravenous foscarnet 60–80 mg/kg q. 12 h, is indicated for 14 days followed by 6 to 14 weeks of oral ACV (Palay et al., 1991; Johnston et al., 1993). ACV resistant strains of VZV have been isolated from HIV patients, which seems to be related to longer term therapy. Foscarnet is the alternative for treatments of these cases (Safrin et al., 1991). Other management of posterior segment disease is discussed below.

Famciclovir (FCV), 500 mg p.o. t.i.d. for one week, was significantly better than placebo or acyclovir in significantly decreasing the duration and incidence of acute pain, viral shedding, and lesion duration, and in reducing the duration of PHN in non-ocular zoster (Tyring et al., 1995; Tyring, 1996). deGreef et al. reported equivalence of FCV to ACV (deGreef, 1995) and FCV has been shown to be safe and effective at this dose in HIV-infected patients (Schacker et al., 1998). Unfortunately, there are no published reports of the efficacy of FCV for HZO. As noted under the heading postherpetic neuralgia above, a recently completed but as yet unpublished multicenter, masked study comparing ACV 800 mg p.o. 5i.d. to FCV 500 mg p.o. t.i.d. for 7 days in acute HZO, showed that both drugs were comparable in every parameter evaluated (Tyring et al., 1998).

In Beutner et al.'s study comparing valaciclovir (VCV), 1 g p.o. t.i.d. × 7 d, with ACV 800 mg p.o. 5i.d. × 7 d in 1141 immunocompetent patients with zoster, VCV was comparable to ACV in effects on the duration of skin lesions but significantly better than ACV in acute pain resolution and reduced duration of PHN up to 1 year. The study included 35 patients with HZO but the HZO group was too small to permit a separate analysis of efficacy (Beutner et al., 1995). In a double-blind, randomized trial comparing ACV with VCV in 121 immunocompetent patients with acute HZO, Colin et al. (2000) reported that one week of treatment with regimens like those used by Beutner et al., resulted in a similar incidence of keratitis, uveitis, and episcleritis in each group. Neither group had any incidence of

neurotrophic keratitis or scleritis, and acute pain was noted in about two-thirds of patients in each group. VCV is considered a valid alternative to ACV in treatment of HZO. VCV is not approved for use in immunocompromised patients because of reports of fatal thrombotic/ thrombocytopenic purpura and hemolytic uremic syndrome (Pepose, 1997). Studies of the incidence or severity of PHN in HZO treated with VCV have not been reported.

Corticosteroids

The only apparent role for steroids in ophthalmic zoster is in combination with high-dose systemic antivirals in patients with such severe acute orbital edema that the ocular circulation was compromised, or in those with HZO complicated by CNS vasculitis (Terborg & Busse, 1995).

Analgesics

The current management of pain in acute HZO and trigeminal PHN does not differ from methods used for non-ocular zoster. The use of such agents as tricyclic antidepressants, anticonvulsants, slow release opioids, and topical capsaicin in the treatment of acute and PHN of HZO is adopted from therapies developed in studies on non-ocular zoster (Galen, 1995; Bowsher, 1997; Dworkin et al., 1998).

Surgical procedures in HZO

Surgical procedures for complications of HZO include lid tarsorrhaphy for neurotrophic keratitis; cryotherapy for trichiasis, entropion, or ectropion repair; dacryocystorhinostomy for canaliculus obstruction; conjunctival transplant or conjunctival flap or tissue adhesive for corneal melting and penetrating keratoplasty (Thoft, 1977; Pavan-Langston & Dunkel, 1996; Wilson, 1996).

Exposure or neurotrophic keratopathy of the anesthetic cornea is the most common indication for surgical intervention for HZO. If lid structures are basically intact but closure is incomplete due to scarring, or the epithelium is rough and fragile, suturing the lateral one-third of the lids together (lateral tarsorrhaphy) will frequently suffice to protect the anterior segment (Baum, 1987) (Figure 14.5B). If lid tissue has been lost, plastic reconstruction may be required (Waring & Ekins, 1981).

Lateral tarsorrhaphy with copious artificial tear lubricants and ointments is also indicated for the anesthetic eye that may not be exposed but which, because of a state of poor innervation, manifests gray unhealthy epithelium with threatening breakdown. If an epithelial defect develops with or without thinning, however, a therapeutic soft contact lens and/or tissue adhesive is useful. Alternatives in this situation include pulling down a conjunctival flap or a conjunctival transplant (Thoft, 1977; Lugo & Arentsen, 1987).

Penetrating keratoplasty (corneal transplant, PK) has a limited role in HZO. As the majority of these eyes are anesthetic, transplanted eyes are prone to melting, superinfection, and wound dehiscence (Makensen et al., 1984). A scarred cornea that has retained a reasonable amount of sensation may be considered a candidate for such a surgical procedure in an effort to restore vision. If this is done, however, a lateral tarsorrhaphy should be placed at the same time to protect the new graft (Reed et al., 1989). Keratoprosthesis, a more recent procedure, holds great promise for success in these cases (C. H. Dohlman, personal communication).

Summary of therapeutic approaches to HZO

The therapeutic guidelines currently recommended for management of HZO are as follows. (Note: i.v., intravenously; p.o., by mouth; b.i.d., twice a day; t.i.d., three times a day; q.i.d., four times a day; 5i.d., five times a day; q.d., every day; q.8 h, every 8 hours; q.h.s., at bedtime; p.r.n., as required.

1. Acute HZO

Antivirals: Preferably started within 72 h of onset of rash.

(a) Famciclovir (Famvir™) 500 mg p.o. t.i.d. for 7 days (immunocompetent or compromised) *or*

(b) Valaciclovir (Valtrex™) 1 g p.o. t.i.d. (immunocompetent), *or*

(c) Acyclovir (Zovirax™) 800 mg p.o. 5i.d. for 7 days. In immunocompromised patients, i.v. ACV 10 mg/kg q.8 h for 10 days in adults and 500 mg/sq m q.8 h for children under 12 years.

Pain prevention/management

(a) Nortriptylene, desipramine, or other TCA 10–100 mg p.o. q. h.s. × 3 m (or longer p.r.n.) preferably starting lowest dose as early as possible after acute disease onset and increasing p.r.n. over weeks to highest dose.

(b) Non-narcotic or short term narcotic analgesics, e.g., oxycodone, codeine, propoxyphene.

Topical therapy

(a) Cool wet compresses (if tolerable) to keep involved skin clean.

Anterior segment care

(a) Corneal exposure: topical antibiotic ointment t.i.d.

(b) Corneal dendritiform keratopathy: topical (3% vidarabine or 1% trifluridine 5i.d. or p.o. antivirals ×2–3 wks (variable efficacy).

(c) Corneal immune disease (moderate to severe), episcleritis, scleritis or iritis: topical steroids (0.125% prednisolone b.i.d. to q.i.d. up to 0.1% dexamethasone in a frequency warranted by disease) followed by slow taper over weeks to months. Try to avoid topical steroids in acute disease. Oral NSAIDS, e.g.

ibuprofen 400 mg p.o. t.i.d. Antivirals unnecessary. Mydriatic/cycloplegia for iritis (scopolamine q.d.).

(d) Glaucoma: topical beta-blockers such as timolol or carteolol b.i.d. May add other agents such as latanaprost q.d., brinzolamide or dorzolamide b.i.d. *No* miotics, e.g. pilocarpine. Add topical steroids if glaucoma due to inflammatory trabeculitis.

2. Long-term or chronic problems
   (a) Corneal immune disease, episcleritis, scleritis, iritis, or dendritiform keratopathy: as under Acute HZO above.
   (b) Unhealthy epithelium in anaesthetic cornea: early lateral tarsorrhaphy and copious lubrication with artificial tears and ointments. Allow vascularization to progress to aid in healing any defect. Topical steroids with caution and only at low doses to keep the eye quiet.
   (c) Exposure keratopathy or corneal ulceration or thinning: lateral tarsorrhaphy, therapeutic soft contact lens, e.g. Permalens™ or Kontur™ lenses with Dermabond™ tissue adhesive if thinning, conjunctival flap, or transplant.

   See Surgical procedures in HZO.

   (d) Glaucoma: As under Acute HZO above.
3. Postherpetic neuralgia
   (a) TCA antidepressant (nortriptyline, desipramine, or other TCA antidepressants) 10 mg titrated up to 100 mg q.h.s. p.r.n.
   (b) Gabapentin 300 mg p.o. q.d. starting dose. Efficacy may not be reached until 600 mg t.i.d.–q.i.d. Some patients will not respond at all.
   (c) Long-acting opioids added if TCAs +/or gabapentin insufficiently effective, e.g. Oxycontin-SR™ 10–40 mg p.o. q. 12 h.
   (d) Capsaicin cream one to four times daily to skin as tolerated.
   (e) Sympathetic blockade or invasive pain management techniques, e.g., trigeminal ganglion ablation, rarely warranted as rarely successful.

Retinitis

Lack of controlled studies limits our comparison of various antiviral agents. Acyclovir has been used both i.v. and intravitreally in ARN (acute retinal necrosis) (Pepose, 1997). While resolution of disease begins within 4 days of treatment, the long-term prognosis for the involved eye does not seem to differ from untreated eyes particularly with reference to ultimate retinal detachment or vision loss. There is, however, a significant reduction in the incidence of involvement of the fellow eye in treated versus untreated patients (13% vs. 70%) (Blumenkranz et al., 1986). Oral famciclovir or valaciclovir have been successful as adjuctive therapy with intravenous ACV or foscarnet (Figueroa et al., 1997; Miller et al., 1997). Oral or intravenous steroids have been used along with anticoagulants in efforts to reduce

intraocular inflammation, vasculopathy and neuropathy with no apparent adverse effects but only a clinical impression without objective proof that this treatment is effective. PORN (progressive outer retinal necrosis) is very resistant to treatment, perhaps due to poor patient immune status. Combinations of acyclovir and ganciclovir do not appear to alter the relentless clinical course but intravitreal ganciclovir and intravenous foscarnet coupled with laser photo-coagulation have been successful (Engstrom et al., 1994; Perez-Blazquez et al., 1997).

Conclusion

HZO remains one of the most common and most devastating infectious diseases of the eye. In recent years, however, significant progress has been made in developing effective antiviral drugs, and better agents for management of acute and late inflammatory and neurological complications. Over time, prevention of varicella by immunization may decrease the risk of HZO. With such continued progress, this often incapacitating illness will be relegated to the past.

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15

Postherpetic neuralgia and other neurologic complications

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Zoster (shingles) epidemiology

Herpes zoster is a common disorder. More than 300 000 cases are estimated to occur annually in the United States. With the exception of immunocompromised individuals (particularly AIDS patients), zoster is a disease of the elderly. The incidence among people over age 50 is double that of people under 50 (Harnisch, 1984), which translates into an 8–10 fold increased frequency in people over age 60 compared with those under 60. As the aging population increases (currently 13% of Americans are older than 65), the incidence of zoster-associated morbidity and mortality is also expected to increase. Varicella in infancy may predispose to zoster earlier in life (Guess et al., 1985). The incidence of recurrent zoster is less than 5% (Hope-Simpson, 1965). Although varicella outbreaks occur most often in the spring, zoster may develop at any time of the year. The risk of zoster in vaccinated individuals compared with those who developed naturally occurring chickenpox will not be known for decades. Meanwhile, some investigators have predicted an increased incidence of zoster with widespread use of the live attenuated varicella vaccine (Garnett & Grenfell, 1992; Wharton, 1996).

Pathology and pathogenesis

Despite the ubiquitous nature and frequency of VZV infection, the pathogenesis of zoster remains largely unknown. Our present understanding of virus spread, localization and replication is based on in vitro studies of infected human or primate cells in tissue culture, correlation of the presence of VZV in human tissues with pathologic changes in different clinical situations, and attempts to produce disease experimentally.

The histologic features caused by zoster focus on the dorsal root ganglia and adjacent nerves. Pathologic changes in ganglia corresponding to the segmental distribution of rash were first noted by von Barensprung (1863), and more extensively detailed by Head & Campbell (1900) and Denny-Brown et al. (1944). The older

literature accurately reflects the pathology of zoster, since the lesions described were those of localized zoster in immunocompetent individuals, except perhaps for occasional zoster that developed in syphilis patients treated with arsenic. The cardinal pathologic features were inflammation and hemorrhagic necrosis of dorsal root ganglia, often associated with neuritis, localized leptomeningitis in adjacent spinal cord, unilateral segmental poliomyelitis, and degeneration of related motor and sensory roots. Demyelination was also seen in areas of mononuclear cell infiltration and microglial proliferation.

Detection of intranuclear inclusions (Cheatham et al., 1956; Ghatak & Zimmerman, 1973), viral antigen and herpesvirus particles (Esiri & Tomlinson, 1972; Nagashima et al., 1975) in ganglia did not occur until much later. VZV was first isolated from ganglia by Bastian et al. (1974). However, those studies were performed on ganglia from patients with underlying malignancies or other disorders of immune function who developed disseminated zoster just before death. There is a single report in which VZV antigen was detected and virus isolated from ganglia of a fatal case of bacterial pneumonitis on which acute thoracic zoster was superimposed (Shibuta et al., 1974).

Zoster is presumed to reflect reactivation and retrograde transport of virus from ganglia to skin in a host partially immune to VZV. Viremia has also been demonstrated in otherwise immunocompetent individuals with acute zoster (Gilden et al., 1987). Although the significance of viremia in zoster patients remains to be determined, VZV DNA has been detected by in situ hybridization (ISH) in blood mononuclear cells (MNCs) of four uncomplicated zoster patients for 3–7 weeks after rash (Gilden et al., 1988), coinciding with the period during which these patients experienced pain.

In immunocompromised patients with localized and disseminated zoster, VZV can also be isolated from blood (Gold, 1966; Feldman & Epp, 1976; Feldman et al., 1977; Gershon et al., 1978; Myers, 1979), suggesting a role for hematogenous spread in the pathogenesis of zoster in such individuals. VZV in blood is cell-associated (Myers, 1979) and has been detected by electron microscopy in monocytes (Twomey et al., 1974). A loss of cell-mediated immunity to VZV may be responsible for an increased risk of zoster in immunocompromised patients (Wharton, 1996). Such a notion is supported by an increased detection of VZV by PCR (polymerase chain reaction) in bone marrow transplant recipients with or without zoster (Wilson et al., 1992).

The detection of VZV in macrophages (Arbeit et al., 1982), in B cells (Leventon-Kriss et al., 1979; Cauda et al., 1986), and in T cells (Gilden et al., 1987), particularly in activated T lymphocytes (Koropchak et al., 1989), provides indirect evidence that various types of blood MNCs represent a site for VZV persistence. Nucleic acid hybridization studies revealed that VZV DNA did not replicate in

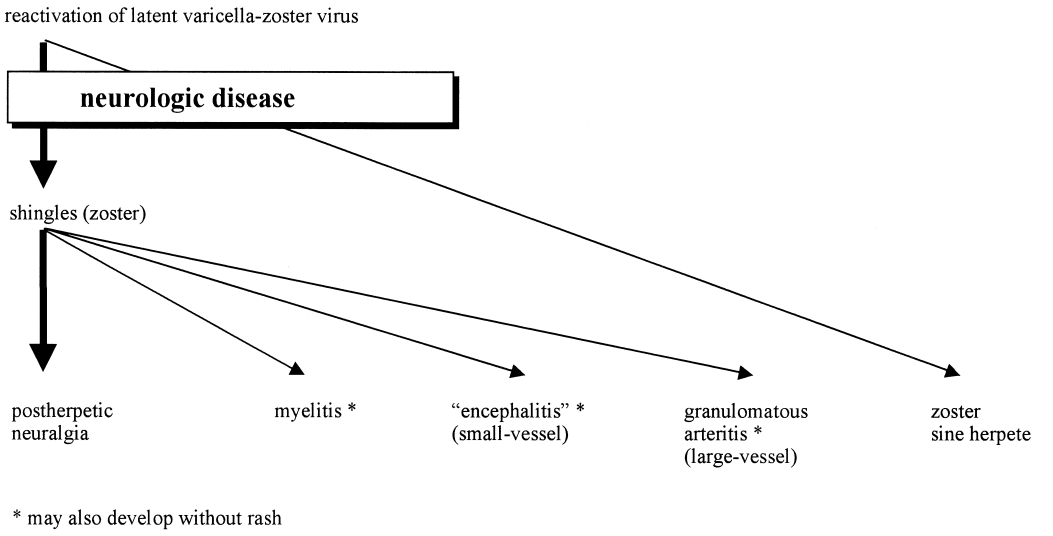

Figure 15.1 Neurologic disease produced by reactivation of varicella-zoster virus.

human MNCs (Gilden et al., 1987), a finding confirmed by Koropchak et al. (1989), suggesting that VZV persists in MNCs, but that active viral replication does not occur. Nevertheless, because VZV infection is more common in immunocompromised individuals, it should be noted that in experimentally infected SCID mice, VZV does replicate in T cells and infectious virus is released (Moffat et al., 1995).

Neurologic complications of VZV reactivation

Ganglionitis is the main neurologic complication of VZV reactivation, which manifests clinically as zoster (shingles). Zoster and postherpetic neuralgia (persistent pain months after rash disappears) are the most common clinical features of the ganglionitis. Other neurologic complications are far less common. Occasionally, after VZV reactivates, virus invades the spinal cord, leading to myelitis, or the cerebral arteries, producing granulomatous arteritis or small-vessel VZV encephalitis. Although rash usually precedes all the neurologic complications of VZV reactivation depicted in Figure 15.1, each neurologic disorder may develop in the absence of rash.

Zoster (shingles, ganglionitis)

Herpes zoster is characterized by pain and a vesicular eruption on an erythematous base in one to three dermatomes. All levels of the neuraxis may be involved.

Thoracic zoster is most common, followed by lesions on the face, most often in the ophthalmic division of the trigeminal nerve. The latter is frequently accompanied by zoster keratitis, a potential cause of blindness if not recognized and treated promptly. All patients with ophthalmic distribution zoster should have an immediate slitlamp examination by an ophthalmologist. Maxillary and mandibular trigeminal distribution zoster with osteonecrosis and spontaneous tooth exfoliation has also been described in adults (Manz et al., 1986) and children (Garty et al., 1985). The seventh cranial nerve is also commonly involved. Weakness of all facial muscles of one side develops in conjunction with rash in the ear (zoster oticus) or on the ipsilateral anterior two-thirds of the tongue or hard palate. Unless searched for in patients with facial weakness, vesicles in either site are easily overlooked. The combination of zoster oticus and peripheral facial weakness constitutes the Ramsay Hunt syndrome, and the prognosis for recovery from facial weakness or paralysis is not as favorable as in idiopathic Bell's palsy. Zoster may be accompanied by ophthalmoplegia, most commonly affecting the third cranial nerve (Thomas & Howard, 1972), optic neuritis (Miller et al., 1986), or both (Carroll & Mastaglia, 1979), and less often lower cranial nerve palsies (Crabtree, 1968; Steffen & Selby, 1972). Zoster-associated cranial neuropathy often occurs weeks after acute VZV infection. One explanation for this late-onset phenomenon is that VZV spreads slowly along trigeminal and other ganglionic afferent fibers to small vessels supplying cranial nerves. This appears to happen in granulomatous arteritis (described below) preceded weeks earlier by trigeminal distribution zoster. All cranial nerves receive their blood supply from the carotid circulation (Lapresle & Lasjaunias, 1986). It is likely, but not yet proven, that the vasa vasorum of cranial nerves may also receive trigeminal afferents, as has been shown for larger extracranial and intracranial blood vessels (Mayberg et al., 1984). Possibly, virus may travel from small neural afferents to the vasa vasorum and result in arteritis and cranial nerve infarcts.

Postherpetic neuralgia

Most neurologic complications of zoster manifest as postherpetic neuralgia (PHN), operationally defined as pain persisting more than 4–6 weeks after rash. Age is the most important factor in predicting the development of PHN (Brown, 1976; Ragozzino et al., 1982). The risk of PHN in zoster patients over age 50 is in the range of 43–47.5%. The incidence of PHN also appears to be slightly greater in women (Hope-Simpson, 1975) and after trigeminal distribution zoster (DeMoragas & Kierland, 1957; Rogers & Tindall, 1971; Hope-Simpson, 1975).

The mechanism of PHN is unknown. The detection of VZV-specific proteins in MNCs of patients with PHN (Vafai et al., 1988b) suggested that persistence of VZV may result in PHN. Later, VZV DNA was shown to persist in blood MNCs of PHN

patients (Mahalingam et al., 1995) compared with zoster patients without PHN, thus providing further presumptive (albeit indirect) evidence that the abundance of VZV in ganglia of PHN patients may be greater than during latency. It is possible that MNCs trafficking through such ganglia encounter and engulf virus whose DNA can then be amplified by PCR. To prove this hypothesis, ganglia need to be analyzed at autopsy from individuals who suffered from PHN at the time of death. If a greater virus burden could be demonstrated by quantitative PCR in these ganglia than has been found during latency (Mahalingam et al., 1993), this would provide a rationale for aggressive treatment of PHN patients with antivirals. Meanwhile, the existence of ganglionitis without rash is further supported by the presence of radicular pain up to 100 days preceding zoster (Gilden et al., 1991), so-called preherpetic neuralgia. Further, a recent report described four patients with acute trigeminal distribution zoster who, after years free from pain, developed severe trigeminal "PHN" (Schott, 1998).

Zoster paresis

Zoster in cervical, thoracic and lumbosacral dermatome distributions may be associated with muscle weakness developing 1–5 weeks after rash. Cervical distribution zoster has been associated with arm weakness, and rarely, diaphragmatic paralysis (Brostoff, 1966; Stowasser et al., 1990). The small incidence of thoracic zoster paresis probably reflects difficulty in diagnosing intercostal muscle weakness at the bedside. Lumbosacral distribution zoster is more easily appreciated clinically and may be associated with leg weakness, as well as impairment of bladder and bowel function. Urinary retention, hemorrhagic cystitis and massive bladder hemorrhage have all been described with sacral distribution zoster (Izumi & Edwards, 1973; Jellinek & Tulloch, 1976). The incidence of zoster paresis has been estimated from as low as 0.5% to as high as 31%. Approximately 11% of patients with segmental zoster paresis have malignant disease (Thomas & Howard, 1972).

VZV encephalitis–arteritis

What has previously been called VZV encephalitis is for the most part a VZV vasculopathy which affects large or small vessels of the CNS, and often, both. Large-vessel artery disease (granulomatous arteritis) and small-vessel artery encephalitis are discussed below.

Zoster large-vessel encephalitis (granulomatous arteritis)

Zoster large-vessel encephalitis (granulomatous arteritis) is characterized by acute focal deficit that develops weeks to months after contralateral trigeminal

distribution zoster. Stroke results from a necrotizing arteritis, primarily of large cerebral arteries. In a comprehensive review from the pre-AIDS era by Hilt et al. (1983), most patients with zoster large-vessel encephalitis were over age 60, without sex predilection. The mean onset of neurologic disease was 7 weeks, and the longest interval was 6 months after zoster. Transient ischemic attacks and mental symptoms were common. Twenty-five percent of patients died. Laboratory and radiographic studies aid in the diagnosis. The majority of patients had CSF pleocytosis, usually fewer than 100 cells (predominantly mononuclear), oligoclonal bands, and increased CSF IgG. Besides contralateral hemiplegia, ipsilateral central retinal artery occlusion (Hall et al., 1983) and posterior circulation involvement have been described. Angiographic examination reveals focal constriction and segmental narrowing primarily in middle cerebral, internal carotid and anterior cerebral arteries. Microscopic examination readily explained the clinical features. Findings usually included necrotizing arteritis, primarily involving the intima and adventitia, inflammation with multinucleated giant cells, VZV antigen, Cowdry A inclusions and herpes virus particles. Most zoster-associated granulomatous angiitis infarcts are pale (Kuroiwa & Furukawa, 1981), but hemorrhagic infarction also occurs (Eible, 1983). Afferent trigeminal ganglionic fibers to both intracranial and extracranial blood vessels (Mayberg et al., 1984) provide an anatomic pathway for the spread of virus. How much blood vessel inflammation is due to the lytic effects of virus versus a viral-induced immunopathology, or both, is unknown. Based on the collective pathologic changes, granulomatous arteritis patients should receive intravenous acyclovir (10–15 mg/kg three times daily for 7–10 days) to kill persistent virus, and a short course of steroids (prednisone 60–80 mg daily for 3–5 days) can be added for their anti-inflammatory effect.

Zoster small-vessel encephalitis

Zoster small-vessel encephalitis is the most common form of CNS involvement. Disease usually develops on a background of cancer, immunosuppression (Horton et al., 1981) or AIDS. Neurologic disease is subacute and death is common. Zoster encephalitis presents with headache, fever, vomiting, mental changes, seizures and focal deficit. Brain MRI (magnetic resonance imaging) reveals large and small ischemic or hemorrhagic infarcts, often both, of cortex and subcortical gray and white matter (Figure 15.2). Deep-seated white matter lesions often predominate, and are ischemic or demyelinative, depending on the size of blood vessels involved and the amount of additional demyelination. The demyelinative lesions are smaller and less coalescent than those seen in progressive multifocal leukoencephalopathy. The CSF (cerebrospinal fluid) shows a mild pleocytosis (predominantly mononuclear), normal or mild elevation of protein, and a normal glucose, findings that do not differ significantly from zoster without encephalitis. Two reports describe

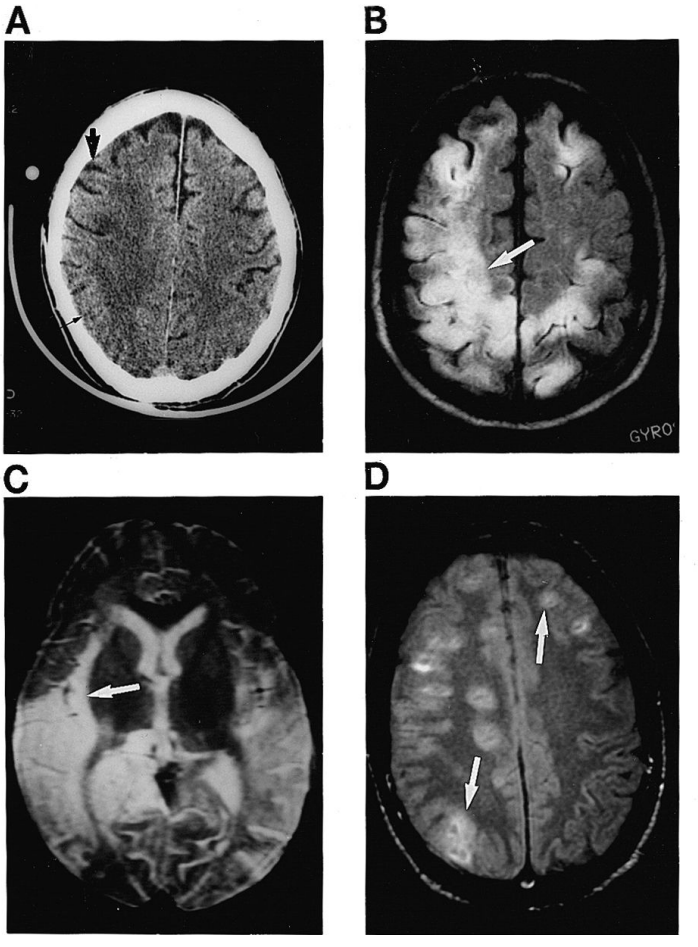

Figure 15.2 (A) Brain computed tomography (CT) changes that occur in most viral encephalitides. The scan demonstrates relative effacement of sulci posteriorly in both hemispheres (thin arrow), compared with normal sulcal spaces anteriorly (thick arrow). (B) MRI changes that occur in most viral encephalitides. T_2-weighted inversion recovery [fluid-attenuated inversion recovery (FLAIR)] MRI brain scan of the same patient demonstrates areas of heightened signal in both hemispheres, greater on the right and even more so posteriorly (arrow), reflecting increased water content in mildly swollen brain. (C) Herpes simplex virus encephalitis. T_2-weighted MRI brain scan demonstrates bilateral involvement of temporal lobes. The exaggerated signal does not extend beyond the insular cortex (arrow). (D) Varicella-zoster virus encephalitis. Proton-density brain MRI scan shows multiple areas of infarction in both hemispheres (arrows).

hypoglycorrhachia in zoster meningoencephalitis (Wolf, 1974; Reimer & Reller, 1981). In suspected cases of zoster small-vessel encephalitis, the CSF may be studied for both VZV DNA and antibody to VZV. PCR for VZV DNA is the most useful diagnostic test on CSF. If possible, the CSF should also be tested for antibody to VZV. Its presence in CSF, with or without the presence of VZV DNA, may be helpful to diagnose infection of the central or peripheral nervous system by VZV. Since VZV antibody is not usually present in the CSF of normal humans, and since nearly all humans have serum antibody to VZV, serum/CSF ratios are not a requirement. Overall, the presence of either, or both, in CSF, in the typical clinical setting described above, is strong presumptive evidence of VZV small-vessel encephalitis (Gilden et al., 1998), and should be treated with acyclovir, 15–30 mg/kg/day for 10 days. Severely immunocompromised patients may require longer treatment.

VZV ventriculitis and meningitis

Finally, two other documented unusual presentations of VZV encephalitis have occurred in immunocompromised patients. The first was ventriculitis characterized by profound infection of ependymal cells by VZV in several cases. Although ventriculitis and ependymitis were usually mixed with ovoid ischemic and demyelinative lesions, pure forms of VZV ventriculitis have also been described (Kleinschmidt-DeMasters et al., 1996). The second unusual presentation was that of a necrotizing vasculitis primarily affecting meninges. Patients developed a profound CSF pleocytosis and protein elevation. Either the brain (Kleinschmidt-DeMasters et al., 1998) or spinal cord (Meyers et al., 1972) may bear the brunt of disease.

VZV myelitis

Myelitis, a rare complication of acute varicella or zoster, usually develops 1–2 weeks after rash. Clinical features are those of paraparesis with a sensory level and sphincter impairment. The CSF is either normal or may show a mild pleocytosis with a normal or mild elevation of protein. Although recovery is variable, most patients improve significantly, but some experience persistent lower extremity stiffness and weakness. This form of VZV-associated transverse myelitis has been presumed to be postinfectious, but requires pathological, virological and immunological verification.

In immunocompromised individuals, the development of myelopathy is often more insidious, progressive, and sometimes fatal. Spinal cord MRI scanning shows longitudinal serpiginous enhancing lesions (Gilden et al., 1994a). Autopsy studies have demonstrated frank invasion of the spinal cord by VZV. A fatal case of ascending VZV myelitis was described after steroid treatment for thoracic zoster (Hogan

& Krigman, 1973). The severity of the myelitis could have been influenced by steroid therapy, a notion supported by the development of zoster meningoencephalitis that occurred in a diabetic who had been treated for months with low-dose steroids (Tako & Rado, 1965). As in VZV encephalitis, an early diagnosis is important, particularly since aggressive treatment with acyclovir, even in AIDS patients, may produce a gratifying response (de Silva et al., 1996).

Finally, although VZV myelopathy is usually diagnosed by the close temporal relationship between rash and the onset of myelopathy, VZV myelopathy spans a wide spectrum. Disease may be acute, chronic, or remitting–exacerbating; and when myelopathy recurs, rash usually does not (Gilden et al., 1994a).

Neuropathy

Postinfectious polyneuritis or the Guillain-Barré syndrome (GBS) is an uncommon, but well-documented neurologic complication of both varicella and zoster. No neurologic features distinguish polyneuritis after varicella or zoster from that seen in other clinical settings. The average interval between rash and neurologic disease was 12 days (Underwood, 1935). Bilateral facial paralysis occurred less often in VZV-associated GBS than in GBS unassociated with varicella (Underwood, 1935; Miller et al., 1956). GBS after chickenpox is rare, as evidenced by its occurrence in only eight of 2534 cases of chickenpox (Bullowa & Wishik, 1935), and by the total absence of associated chickenpox in 50 extensively studied cases of GBS (Haymaker & Kernohan, 1949).

GBS after zoster is equally rare. Only 16 cases have been described since the initial report by Wohlwill (1924) and the first compilation of cases by Dayan et al. (1972). Polyneuritis usually occurs many days to a few weeks after rash, and in some instances 2 months later. The clinical course is usually acute, but occasionally subacute or indolent, especially in the cases that developed 1–2 months after rash. Neuropathologic examination of teased nerve fibers revealed acute demyelination and remyelination. In addition, fibrinoid necrosis of blood vessels and infarction were seen in severely affected spinal ganglia, suggestive of an Arthus-type immunopathology (Dayan et al., 1972).

VZV associated with Reye's syndrome

Reye's syndrome is a rapidly progressive, often fatal, noninflammatory disorder of children and adolescents. The two most affected organs are the brain, which swells, and the liver, which becomes infiltrated by fat. Disease is associated with infection by the influenza viruses or VZV. Reye's typically follows childhood chickenpox, but has not been documented after zoster. An initial, apparently mild upper respiratory

infection, or an episode of classic chickenpox, is followed by an asymptomatic few days, after which patients develop intractable vomiting, seizures, lethargy, coma, and often death (Hurwitz et al., 1982). Therapeutic amounts of salicylates increase the risk of developing Reye's syndrome (Remington et al., 1986). Characteristic laboratory abnormalities include elevated serum transaminase and ammonia levels, as well as hypoglycemia in ~40% of patients. A brain MRI may reveal sulcal effacement, loss of gray–white matter junctions, and small ventricles, all consistent with brain swelling. The exact metabolic abnormality in Reye's syndrome has not been determined. Patients require intensive care treatment by hyperventilation and intravenous mannitol to reverse rapidly developing brain edema. Steroids have not been shown to be effective, probably because brain edema is thought to be cytotoxic.

Zoster sine herpete

The concept of zoster sine herpete, defined as shingles without rash, is nearly 100 years old. The first proposed case was that of a 38-year-old man who developed acute thoracic-distribution pain and hyperesthesia, a dilated pupil, a CSF pleocytosis (predominantly mononuclear), and a negative serologic test for syphilis (Widal, 1907). He was presumed to have zoster sine herpete although serologic tests for VZV were unavailable at the time. Similarly, two patients who experienced segmental pain, hyperesthesias and focal weakness were presumed to have zoster sine herpete despite lack of serologic confirmation (Weber, 1916). The notion of zoster sine herpete received further credence when Lewis (1958) described numerous zoster patients who, days later, also developed pain without rash in a different dermatome distribution, often on the opposite side.

The first serologic evidence of zoster sine herpete occurred in a physician who developed acute trigeminal distribution pain associated with a 4-fold rise in complement-fixing antibody to VZV, but not to HSV (Easton, 1970). Virologic confirmation of zoster sine herpete did not come until analysis of two men with thoracic-distribution radicular pain that had lasted for months to years revealed amplifiable VZV DNA by PCR, but not HSV DNA, in their CSF and blood MNCs (Gilden et al., 1994b). After diagnosis, both men were treated successfully with intravenous acyclovir. A third virologically confirmed case of thoracic-distribution zoster sine herpete persisting for years included the demonstration of frequent fibrillation potentials restricted to chronically painful thoracic root segments (Amlie-Lefond et al., 1996). Unlike the first two patients treated with antivirals who had a favorable response, the third patient did not improve after treatment with intravenous acyclovir and oral famciclovir.

Although the nosologic entity of zoster sine herpete as a clinical variant has now

been established, its prevalence will not be known until more patients with prolonged radicular pain have been studied virologically. Analysis needs to include PCR to amplify VZV DNA in CSF and in blood MNCs, as well as a search for antibody to VZV in CSF. Antibody to VZV in CSF, even in the absence of amplifiable VZV DNA, has been useful to support the diagnosis of encephalitis and myelitis produced by VZV without rash (Gilden et al., 1998). Analysis of serum anti-VZV antibody is of no value in the workup of patients with prolonged pain, because anti-VZV antibodies persist in nearly all adults throughout life, and the presence of antibodies in serum to different VZV glycoproteins and nonglycosylated proteins is variable (Vafai et al., 1988a).

Other non-zosteriform VZV infection of the nervous system without rash

Zoster sine herpete (reviewed above) is essentially a disorder of the peripheral nervous system (ganglioradiculopathy) produced by VZV without rash. VZV also produces disease of the CNS without rash. While cases are rare, at the University of Colorado Health Sciences Center we have encountered more cases of VZV infection of the CNS (encephalitis and myelitis) without rash than we have of VZV infection of the peripheral nervous system (ganglioneuropathy) without rash.

In pathologically verified cases of VZV encephalitis without rash, the typical clinical picture has been an immunocompromised individual (usually AIDS) who develops CNS disease at the time of acute zoster, or may have a history of zoster weeks to months earlier, or even recurrent zoster. CNS disease in such patients develops more often in the absence of acute zoster than at the time of acute zoster (Amlie-Lefond et al., 1995). Encephalitis is usually of the "small-vessel" type described above (see VZV encephalitis), and disease is usually protracted. Diagnosis may be difficult unless the clinician is alert to the history of recurrent zoster followed by the typical clinical features and multifocal lesions seen by brain MRI in "small-vessel" encephalitis. Many patients die of chronic progressive VZV encephalitis without ever having developed rash (Amlie-Lefond et al., 1995; Gilden et al., 1996). The most extreme example of VZV infection of the nervous system we have encountered was a 77-year-old man with T-cell lymphoma who developed meningoradiculitis and died three weeks after the onset of neurologic disease (Dueland et al., 1991). He did not develop zoster before or during neurologic disease. At autopsy, hemorrhagic, inflammatory lesions with Cowdry A inclusions were found in meninges and nerve roots extending from cranial nerve roots to the cauda equina. The same lesions were present, although to a lesser extent, in the brain as well. We saw VZV antigen and nucleic acid, but not HSV (herpes simplex virus) or CMV (cytomegalovirus) antigen or nucleic acid, in infected tissue at all levels of the neuraxis. Thus, VZV may be included in the differential diagnosis of

acute encephalomyeloradiculopathy, particularly since antiviral treatment is available.

Acute VZV myelopathy may also occur in the absence of rash. Heller et al. (1990) initially described a 31-year-old immunocompetent man who developed transverse myelitis with partial recovery. Disease was attributed to VZV based on the development of antibody in CSF. Later, we also encountered two patients with VZV myelopathy in the absence of rash (Gilden et al., 1994b). The first patient developed zoster followed by myelopathy five months later, at which time amplifiable VZV DNA was detected in CSF. The second equally fascinating patient developed myelopathy at the time of acute zoster. The myelopathy resolved, but recurred 6 months later. Five months after the recurrence of myelopathy, the patient's CSF contained amplifiable VZV DNA as well as antibody to VZV. Overall, the spectrum of VZV myelopathy can be broad, ranging from acute to chronic, and rarely, recurrent myelopathy.

Further VZV involvement of the CNS without rash was verified by the intrathecal synthesis of antibodies to VZV in two patients with aseptic meningitis (Martinez-Martin et al., 1985), later in four additional patients with aseptic meningitis (Echevarria et al., 1987), and in one patient with acute meningoencephalitis (Vartdal et al., 1982). We recently encountered an adult man who was taking low-dose methotrexate and developed acute encephalitis (fever, aphasia and a profound CSF mononuclear pleocytosis). His CSF contained amplifiable VZV and Epstein-Barr virus DNA. Zosteriform rash never developed, and he recovered completely after treatment with intravenous acyclovir.

There have been two instances of polyneuritis cranialis produced by VZV. The first occurred in a 70-year-old man who seroconverted to VZV during acute disease (Mayo & Booss, 1989). Another report described a 43-year-old man with acute polyneuritis cranialis who developed antibody in CSF to VZV, but not to other human herpesviruses, or to multiple ubiquitous paramyxoviruses or togaviruses (Osaki et al., 1995). Both men were apparently immunocompetent. Finally, cases of acute unilateral facial (Bell's) palsy that developed in the absence of zosteriform rash have been attributed to VZV infection (so-called geniculate zoster sine herpete) based on "a positive serum complement fixation test" (Aitken & Brain, 1933). Unfortunately, the serum was not tested for seroconversion to other human herpesviruses.

VZV diagnosis: Amplifiable VZV DNA by PCR and antibody to VZV

The polymerase chain reaction (PCR) technique for viral DNA amplification has detected VZV DNA in blood MNCs of elderly patients with postherpetic neuralgia (Mahalingam et al., 1995), in all of seven (100%) zoster patients with various

neurological features (Puchhammer-Stockl et al., 1991), in CSF from six of 84 (7%) of HIV-infected patients presenting with neurological symptoms, and in CSF of three of five (60%) children with post-varicella cerebellitis. The latter finding is particularly important, since it suggests that cerebellar ataxia days to weeks after chickenpox, thought to be immune-mediated, is more likely due to active virus infection.

Recently, PCR has been used to detect VZV DNA in vitreous biopsy specimens from patients with viral retinitis (Knox et al., 1998). VZV DNA has also been detected in cornea of seven of 14 (50%) patients after herpes zoster ophthalmicus (Mietz et al., 1997). Also, using PCR, VZV has been shown to be the most likely pathogen of atypical necrotizing herpetic retinopathies (Garweg & Bohnke, 1997).

As discussed above, the presence of either VZV DNA or antibody to VZV, or both, in CSF, in the right clinical setting (i.e., acute or chronic spinal cord disease; acute or chronic progressive encephalitis; or chronic pain, even without rash) can provide presumptive evidence of VZV myelitis (Gilden et al., 1994a), large- or small-vessel encephalitis (Gilden et al., 1998) or zoster sine herpete (Gilden et al., 1994b).

Overall, the combination of PCR and the detection of antibody to VZV in CSF are extremely useful not only to evaluate disorders caused by VZV, but also to diagnose subclinical reactivation of VZV.

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16

Varicella and herpes zoster in pregnancy and the newborn

Gisela Enders and Elizabeth Miller

Introduction

When primary or recurrent infection with VZV occurs during pregnancy, the consequences for both the mother and the fetus must be considered. Chickenpox in pregnancy may be associated with severe maternal disease, fetal death and rarely, during the first two trimesters, with congenital varicella syndrome (CVS). Maternal varicella around term carries the risk of serious neonatal disease. Various congenital defects have been reported after maternal zoster in pregnancy, but evidence for a causal association with maternal infection is lacking. This chapter reviews the outcome and management of maternal chickenpox and zoster, and the associated risks to the fetus.

Varicella

Epidemiology of infection in pregnancy

The incidence of varicella in pregnancy is determined by the proportion of women of childbearing age who are susceptible to infection and their opportunity for exposure to VZV during pregnancy. Serological surveys in temperate climates have shown that fewer than 10% of the adult population is susceptible (Ndumbe et al., 1985). In Germany, the seroprevalence to varicella was studied annually from 1984 to 1997 in more than 26 000 pregnant women aged 20–40 years; the seronegative rate was about 5.6%, with no significant change over the period (Enders, 1985; Enders et al., 1994) (Enders, 1998, unpublished). In the US, a recent survey found that the proportion of susceptible adults aged 20–29 years was 5%, dropping to 1.1% in those aged 30–39 years (Chapter 10). However, the proportion susceptible is greater in some populations, for example, black Americans born in the US and individuals born in tropical countries (Gershon et al., 1976; Ooi et al., 1992; Garnett et al., 1993; Dworkin, 1996).

Reliable data on the incidence of varicella in pregnancy are not available but extrapolation from the consultation rates for chickenpox in adults aged 15–44 years

in the United Kingdom suggest an infection risk of approximately 2–3 per 1000 pregnancies (Fairley & Miller, 1996). Current age specific incidence data from the US generated through the National Health Interview Survey show annual rates of 4.6 per 1000 in 15–19 year olds and 1.5 in those aged 20 years and over in 1990–94 (Chapter 10). These rates are consistent with the UK incidence rates in adults. If, as shown for rubella (Miller et al., 1982) and parvovirus B19 (Public Health Laboratory Service Working Party on Fifth Disease, 1990), the incidence of infection in pregnancy is higher for parous women due to exposure to their own children, then the risk of varicella infection for women in a second or subsequent pregnancy may be substantially higher than the overall incidence rates reported in adults.

The mean weekly rate for chickenpox in the 15–44 year age group reported in England and Wales from sentinel surveillance in primary care during the period 1995–7 was 4.2 per 100 000, which gives an indication of the likely risk of maternal varicella in the critical period around delivery (usually considered to be 5 days before to 2 days after), when severe neonatal infection can occur. Based on this estimate, it would be expected that around 160 neonates each year in the US would be at risk (34 in Germany and 30 in the UK). The risk for congenital varicella syndrome (CVS) is more difficult to calculate because the period during early gestation when this risk occurs is less certain; however, most cases occur before 20 weeks.

Current rates of infection in pregnancy appear to be substantially higher than those reported by Sever & White (1968) who, in a US survey conducted between 1959 and 1964, found 20 cases of chickenpox in 30 000 pregnancies (0.7 per 1000). This suggests that the incidence in pregnancy has increased during the last two decades, a trend which is consistent with that seen in adults in general in England (Gray et al., 1990; Miller et al., 1993b; Fairley & Miller, 1996) (and Jane Seward, personal communication).

Adverse effects of varicella in pregnancy

Anecdotal evidence suggests that varicella is more severe in pregnant women than in other adults, particularly the risk of varicella pneumonia. However, there are no reliable population-based prospective studies to confirm this impression (Nathwani et al., 1998). A review of severe varicella pneumonia in a Swedish population of 1.3 million people (Nilsson & Örtqvist 1996) found only one hospital admission and no deaths among pregnant women due to varicella pneumonia over a 10 year period. Bovill and Bannister reviewed admissions for varicella to an infectious disease unit in London over a 26-year period. Only three of the 683 admissions were pregnant women; none died and only one had pneumonia (Bovill & Bannister, 1998). Among 1383 pregnant women with varicella followed up prospectively by the Stuttgart laboratory between 1980 and 1999, no cases of

pneumonia and no deaths were recorded (Enders et al., 1994; G. Enders, unpublished).

These studies indicate that varicella pneumonia associated with pregnancy is rare, although mortality data from the UK suggest that the risk of a fatal infection may be higher in pregnant women than in other adults. In England and Wales between 1985–98, there were nine deaths reported to be due to varicella in pregnant women (Department of Health, 1996b), an average mortality rate of approximately 1 per million pregnancies. Assuming an incidence of varicella of 2 per 1000 pregnancies, the estimated case fatality rate in pregnancy is 1 per 2000. The total deaths reported to the Office of National Statistics attributed to varicella were 41 in non-pregnant women aged 15–44 years in England and Wales in 1985–98, giving an average annual mortality rate of approximately 0.3 per million. Since a third of the deaths in the nonpregnant group will be in high risk immunocompromised patients (Miller et al., 1993c), the risk of fatal varicella appears to be about five-fold higher in pregnant than nonpregnant immunocompetent adults.

VZV pneumonia is the most common serious maternal complication in pregnancy (Haake et al., 1990; Katz et al., 1995) and usually develops within one week of the rash. The predominant signs and symptoms are fever, cough, dyspnea and tachypnea. The outcome is unpredictable and there may be rapid progress to hypoxia and respiratory failure. Pneumonia is regarded as a medical emergency requiring prompt diagnosis and treatment. Studies reporting the outcome of varicella pneumonia in pregnancy show an excess of cases in the third trimester, suggesting that the risk to the mother may be greatest towards term. For example, 28 of 34 cases reported by Smego & Asperilla (1991) and Esmonde et al. (1989) occurred on the third trimester (Esmonde & Asperilla 1989; Smego & Asperilla 1991). Of the nine deaths reported as due to varicella in pregnancy in England and Wales between 1985 and 1998, seven occurred between 27 and 32 weeks' gestation.

In pregnancies complicated by varicella, spontaneous abortion, stillbirth and prematurity do not seem to be significantly increased (Paryani & Arvin, 1986; Balducci et al., 1992; Pastuszak et al., 1994). In a prospective study of 1373 pregnancies in which varicella occurred between conception and 36 weeks gestation, the incidence of fetal loss due to spontaneous abortion during the first 20 weeks was 2.6% and of intrauterine death after the 20th week was 0.7% (Enders et al., 1994). The results found in a further 342 pregnancies followed up prospectively by the Stuttgart laboratory are very similar.

Effects on the fetus

Congenital varicella syndrome

In 1947, Laforet & Lynch (1947) first described an association between maternal varicella infection in early pregnancy and congenital anomalies in the newborn.

Since this description and its re-discovery in 1974, well over 50 cases of CVS have been reported in the English language medical literature (Srabstein et al., 1974; Alkalay et al., 1987; Wutzler et al., 1990; Birthistle & Carrington, 1998, Sauerbrei, 1998). Prior to 1991, a causal association with maternal infection had been virologically confirmed in only a few cases; these include isolation of VZV from skin lesions at birth (Da Silva et al., 1990), detection of genomic VZV DNA in the fetal tissues of a live-born infant (Scharf et al., 1990), detection of VZV DNA in amniotic fluid and blood of fetus from a mother with acute varicella complicated by encephalitis (Gottardi et al., 1991), in amniotic fluid of a fetus with multiorgan involvement and intrauterine death in the 22nd week of gestation (Mehraein et al., 1991) and in amniotic fluid of a similar case with therapeutic abortion in the 22nd week (Puchhammer-Stöckl et al., 1994).

More recently, clinical, virological and serological findings were described in five cases of CVS (in one of whom VZV DNA was detected in postmortem tissues) which had occurred between 1985 and 1997 in the eastern part of Germany (Sauerbrei et al., 1996, Sauerbrei, 1998). Detection of VZV DNA in fluids and tissues of a fetus showing characteristic signs of CVS by ultrasonography (e.g. limb hypoplasia) with confirmation by gross examination following therapeutic abortion or death postpartum (Pons et al., 1995; Hartung et al., 1999) has been reported. The association of characteristic congenital stigmata with virological and serological evidence of intrauterine VZV infection has proved that maternal varicella causes CVS.

Clinical manifestations

The clinical manifestations of CVS range from severe multisystem involvement resulting in death in the neonatal period to dermatomal skin scarring and/or limb hypoplasia as the only defects at birth. Sequelae involving the eye, the CNS and the gastrointestinal tract (e.g. chorioretinitis, paresis, gastro-esophagal refluxes) may only be recognized later (Smith & Sinha, 1993). The full spectrum of this disorder has been reviewed recently (Birthistle & Carrington, 1998). Table 16.1 compares the main clinical features of CVS involving skin, skeleton, eye, CNS and other organs and their relative frequencies in a prospective and a retrospective case series. The proportion of female infants was not as high as previously reported in a smaller series (Alkalay et al., 1987). Low birthweight is a consistent finding in infants with CVS (Birthistle & Carrington, 1998).

Figure 16.1 shows the most characteristic features of the congenital varicella syndrome. Table 16.2 presents the type and combination of clinical features of CVS, the outcome and the immunological responses in the infant according to week of gestation (WG) in our prospective series. Eight of the 13 (62%) affected infants were female and the latest gestational age at which CVS occurred was WG 19. Cases

Table 16.1 Congenital varicella syndrome: Clinical features and their relative frequency in prospective and retrospective cases ascertained by the authors

Main anomalies	Number of infants		
	Prospective ($n=13$*)	Retrospective ($n=12$**)	total ($n=25$)
Skin: Dermatomal cicatricial skin lesions, contractures	8 (62%)	10 (83%)	18 (72%)
Skeleton: Limb hypoplasia associated with reduction deformaties	11 (85%)	7 (58%)	18 (72%)
Eye: Microphthalmia, chorioretinitis, cataract, Horner syndrome	3 (23%)	8 (67%)	11 (44%)
CNS: Microcephaly, brain atrophy, paralysis, convulsions, encephalitis	4 (31%)	8 (67%)	12 (48%)
Mental retardation	1 (8%)	1 (9%)	2 (8%)
Other organ defects (e.g. gastrointestinal, genitourinary)	3 (23%)	2 (17%)	5 (20%)
Multiorgan involvement, e.g. haemorrhagic rash and dystrophy	3 (23%)	3 (25%)	6 (24%)
Death postpartum and later	6 (46%)	3 (25%)	9 (36%)
Female	8 (62%)	5 (42%)	13 (52%)
Male	5 (38%)	7 (58%)	12 (48%)

Notes:

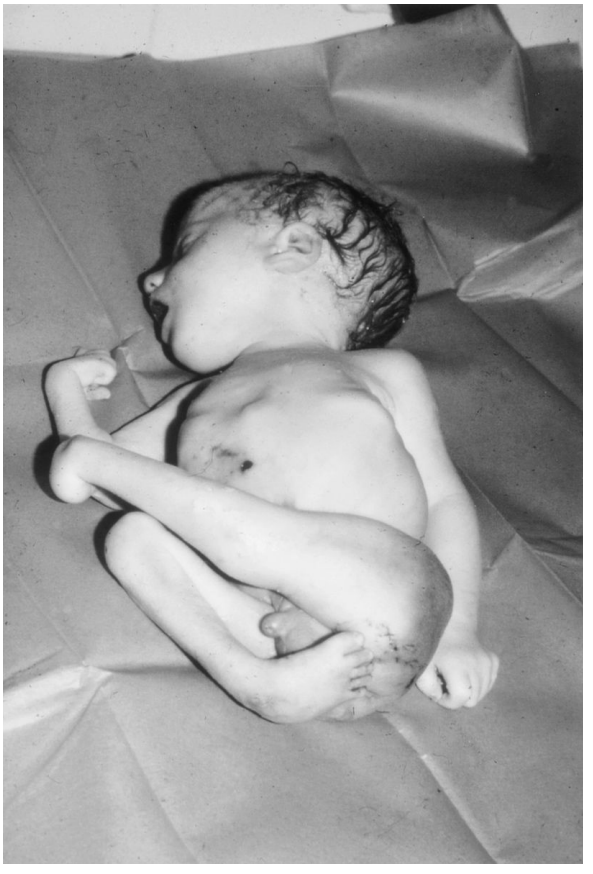
* Lancet study (Enders et al., 1994) and update 1994–99, G. Enders, unpublished

** Stuttgart cases 1980–1994, and update 1994–1999, Enders G., unpublished

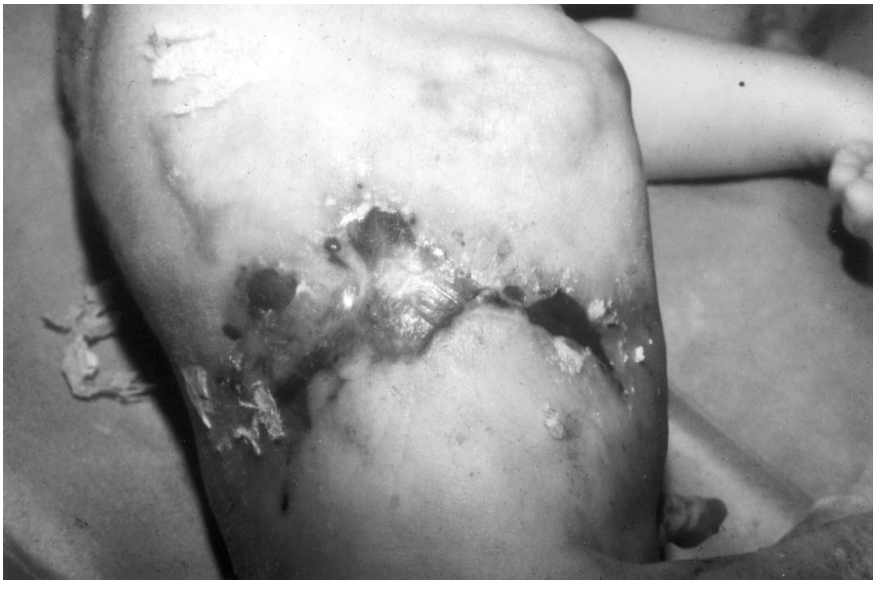
1–8 had multiple features and were severe whereas cases 9–13 were mild with one or two defects only. No relationship was identified between number of clinical features, gestational age of maternal varicella and immune response in the infant (either IgM antibody or cellular immunity response after birth, or persisting IgG antibodies at or over 7 months of age). Furthermore, there was no relationship between IgM at birth and persisting IgG antibodies. Severe cases of CVS (cases 2 and 4) occurred without skin scarring but with various other defects (Smith & Sinha, 1993). Case 12 had a missing elbow joint and club-hand but no other signs of CVS and no persisting IgG antibodies, suggesting a possible unrelated orthopedic deformity, since this defect is not uncommon. Defects in infants born to mothers with varicella in pregnancy should not be considered to have been caused by VZV in all instances.

Pathogenesis of CVS

The precise mechanism of infection with VZV in utero is unknown. Transplacental transmission of VZV probably takes place during the viremic phase, resulting in congenital infection (Trlifojova et al., 1986). The pattern of severe defects of CVS, with the peculiar segmental manifestations of anomalies, suggests the effect of VZV reactivation on the developing neurological system of the fetus (Gershon, 1990). In adults, VZV reactivation results in necrosis of nerve cells, axon demyelination and



(A)



(B)

Figure 16.1 (A) Infant with severe stigmata of congenital varicella syndrome. Severe limb hypoplasia and cutaneous scarring (Enders, 1991). (B) A close-up view of the cicatricial (zig-zag) scar over the left flank. Note the similarity to typical herpes zoster. The child died shortly after birth (Enders, 1991).

Table 16.2 Congenital varicella syndrome: Combination of main symptoms in relation to gestational stage of maternal varicella and immune response in the infant in 13 cases of the prospective study*

Infant no.	Sex	Low birth weight	Limb	Skin scarring	Eye	CNS	Others	Outcome	Maternal VZV WG	IgM at birth (RIA[1]/EIA[2])	CMI at age	Persistent IgG** (age)***
1 (1983)	f	+	+	+	−	+	−	⇒	12	neg.[1]	—	pos. (19 mon)
2 (1992)	f	+	+	−	+	+	+	*zoster* at 1¾ yr. death age 4½ yr.	13	neg.[1]	neg. (4 yr)	pos. (22 mon)
3 (1997)	f	−	+	+	+	+	+	TOP 23rd WG	14	(pos.)	—	—
4 (1997)	m	+	+	−	+	+	+	⇒	14	neg.[2]	—	pos. (7 mon)
5 (1997)	f	+	+	+	+	+	+	death early pp., IUG	15	neg.[2]	—	—
6 (1984)	f	+	+	+	+	+	+	death early pp.	18	pos.[1]	—	—
7 (1990)	m	hemorrhagic rash, multiorgan involvement						IUD 22nd WG	19	—	—	—
8 (1983)	m	n.i.	+	+	+	−	−	⇒	14	neg.[1]	—	pos. (17 mon)
9 (1991)	f	−	+	+	−	−	−	*zoster* at 7 mon.	14	pos.[1]	—	pos. (9 mon)
10 (1997)	f	−	−	+	+	−	−	⇒	14	neg.[2]	pos. (1 mon)	pos. (12 mon)
11 (1989)	f	−	+	−	−	−	−	TOP WG 23	3	neg.[1]	—	
12 (1991)	m	−	+	−	−	−	−	⇒	15	neg.[2]	neg. (11, 22, 26 mon)	neg. (11+22 mon)
13 (1981)	m	−	−	+	−	−	−	⇒	19	neg.[1]	—	neg. (11 mon)
Female	8 (62%)								pos./tested:	3/12 (25%)		6/8 (75%)
Male	5 (38%)											

Notes:

CMI, cell-mediated immunity by lymphocyte proliferation assay; IUG, intrauterine growth retardation; TOP, termination of pregnancy; IUD, intrauterine death; pp., postpartum; ⇒, alive; +, yes; −, no; n.i., no information; ***, ≥7 months of age; WG, week of gestation (completed weeks between LMP and onset of rash); RIA, radioimmunoassay; EIA, enzyme immunoessay.

* Lancet study (Enders et al., 1994) and update 1994–99 (Enders, unpublished).

** IgG titer ranging 1:512–1:2048, neg. < 1:16.

Table 16.3 Clinical manifestations of congenital varicella infection following chickenpox in pregnancy

Stage of maternal infection	Sequelae
First and second trimester	Congenital varicella syndrome
Second and third trimester	Herpes zoster in infancy or childhood
Perinatal	Disseminated neonatal varicella

Note: From Miller, Marshall & Vurdien (1993a).

inflammatory exudate around dorsal root ganglia. Whereas latency established postnatally is maintained for years or decades by effective T-cell immunity, the manifestations of CVS suggest an extremely short period between fetal infection and reactivation of fetal virus in neural tissue, leading to fetal zoster at multiple sites. These effects may be the consequence of an inadequate cell mediated immune response before 20 weeks gestation (Brunell, 1966; Higa et al., 1987).

The clinical consequences of congenital VZV infection depend on the stage of pregnancy at which maternal infection occurs (Table 16.3). However, intrauterine varicella infection can occur without clinical sequelae at any stage of pregnancy, with an increasing rate of asymptomatic infection occurring towards the 36th week of gestation. In some cases, fetal infection early in pregnancy may result in clinical manifestions with healing of lesions before birth, as suggested by an infant born with a pock scar whose mother had varicella at 15 weeks and who had persisting IgG at age 16 months (Enders et al., 1994). The proportion of infants with probable asymptomatic intrauterine infection, as measured by IgM antibodies at birth, persisting IgG antibodies over age 7 months or herpes zoster in infancy, rose from 5–10% in the first and second trimester to 25% near WG 36, and reached approximately 50% when maternal varicella occurred 1–4 weeks before delivery. A third of the babies infected in this later period developed clinical varicella despite high titers of passively acquired maternal antibody (Miller et al., 1989). Similar observations have been made by Enders (unpublished). All of these markers must be used because the limitation of IgM production in fetal varicella and the differing sensitivity of various IgM tests make the detection of VZV IgM in asymptomatic newborns an unreliable test for assessing the rate of intrauterine VZV transmission.

The risk of zoster in early infancy or early childhood following maternal varicella in the second and third trimester as shown in the prospective study (Enders et al., 1994) is estimated to be overall 1.2%. The observed risk after maternal varicella at 13–24 weeks was 4/477 (0.8%) and 6/345 (1.7%) for infections at 25–36 weeks (Enders et al., 1994). This risk is similar to the overall risk for CVS of 1.1% after maternal varicella in the first half of gestation.

Risk of congenital varicella syndrome

Several prospective studies have been undertaken to estimate the risk of CVS following maternal varicella infection at various stages of pregnancy (Enders, 1984; Paryani & Arvin, 1986; Balducci et al., 1992; Jones et al., 1994; Pastuszak et al., 1994). The largest reported prospective study includes 1373 women who had varicella and 366 who had zoster (Enders et al., 1994). The estimated risk of CVS according to gestational age was 0.4% for infection at 0–12 weeks and 2.0% for infections between 13–20 weeks. The overall risk during the first 20 weeks of gestation was approximately 1.0%. The latest gestational age at which CVS was identified was WG 19, which is consistent with the findings in one retrospective report (Alkalay et al., 1987). Four CVS cases were observed in an additional 342 pregnancies complicated by VZV and followed prospectively by the Stuttgart laboratory between 1994 and April, 1999; CVS occurred in three of 321 live-born babies and one therapeutically aborted fetus (WG 23) (Table 16.4). In two of the four cases, maternal varicella occurred in WG 13/14 and in WG 15 in the other two cases. Based on these estimates of risk, together with the incidence of chickenpox in pregnancy and the annual number of births, the number of CVS cases expected to occur each year in the USA is 44, in England/Wales (UK) 8, and in Germany 9 (Table 16.5).

Neonatal varicella

Severe neonatal disease is attributed to intrauterine VZV infection of infants who lack maternal antibody. If the mother's rash appears more than 7 days before delivery, the infant invariably has maternal antibodies. When onset is less than 7 days before delivery, only some infants have significant antibody titers and all of those born less than 3 days after onset of maternal rash, or infants whose mothers develop varicella after delivery, are IgG negative (Miller et al., 1989). Infants at greatest risk of severe or fatal illness are those whose mother's rash appears 5 days before to 2 days after delivery (Gershon, 1975; Miller et al., 1989). In one retrospective series, the clinical attack rate was estimated to be 17% (Meyers, 1974) and the fatality rate 30% (Gershon, 1975). However, a clinical attack rate of more than 50% was found in a large prospective study of infants whose mothers developed varicella during this high risk period, with a further 8% infected asymptomatically; 14% of the infants had severe varicella but there were no fatalities (Miller et al., 1989). All of the infants in this study were given varicella-zoster immunoglobulin (VZIG) at birth, which may have reduced severity, but the commonly cited case fatality rate of 30%, for infants whose mothers develop varicella in the period 4 days before to 2 days after delivery, may be an overestimate due to selective reporting of fatal outcomes in the retrospective study (Miller et al., 1989).

The interval between the onset of rash in the mother and infant is usually 12–13

Table 16.4 Fetal outcome after varicella infection in pregnancy according to stage of gestation of maternal infection: prospective study

Outcome	Lancet study[1] 1980–93 ($n = 1373$)	Only Stgt. 1994–May 99 new cases ($n = 342$)	Lancet + Stgt. study[2] 1980–May 99 ($n = 1721$)
Fetal loss			
Therapeutic abortion	43 (3.1%) (CI: 2.3–4.2%)	6 (1.8%) (CI: 0.7–3.8 %)	49 (2.9%) (CI: 2.1–3.8 %)
Spontaneous abortion < 20th WG	36 (2.6%) (CI: 1.8–3.6%)	13 (3.8%) (CI: 2.0–6.4%)	49 (2.9%) (CI: 2.1–38%)
Intrauterine death > 20th WG	9 (0.7%) (CI: 0.3–1.2%)	2 (0.6%) (CI: 0.07–2.1%)	11 (0.6%) (CI: 0.3–1.1%)
Total (risk)	**88 (6.4%) (CI: 5.2–7.8%)**	**21 (6.1%) (CI: 3.8–9.2%)**	**109 (6.3%) (CI: 5.2–7.6%)**
Live-born infants	1291 (6 sets of twins)	321	1612 (6 sets of twins)
CVS	9	4	13
Overall risk during first 20 weeks of gestation	**1.1%** (CI: 0.5–2.07%) (823 patients, 9 CVS cases)	**1.8%** (CI: 0.49–4.53%) (223 patients, 4 CVS cases)	**1.2%** (CI: 0.66–2.12%) (1046 patients, 13 CVS cases)
1st–12th WG	**0.4%** (CI: 0.05–1.52%) (472 patients, 2 CVS cases)	**0%** (CI: 0.0–2.58%) (141 patients, 0 CVS cases)	**0.3%** (CI: 0.04–1.17%) (613 patients, 2 CVS cases)
Highest risk 13th–20th WG	**2%** (CI: 0.81–4.07%) (351 patients, 7 CVS cases)	**4.9%** (CI: 1.34–12.02%) (82 patients, 4 CVS cases)	**2.5%** (CI: 1.27–4.50%) (433 patients, 11 CVS cases)
Latest gestational age at which CVS occurred	WG 19	WG 15	WG 19

Notes:

[1] Enders et al. (1994).

[2] Enders et al. (1994); Enders, unpublished data 1999.

WG = week of gestation (completed weeks between LMP and onset of rash)

CVS = congenital varicella syndrome

CI = 95% Confidence interval

Table 16.5 Estimated cases of CVS per year in USA, England and Germany

	USA	England and Wales	Germany
Birthrate (1997)	4 000 000	700 000	800 000
Chickenpox in pregnancy per year[1]	8000	1400	1600
In WG 1–20	4000	700	800
CVS cases per year[2]	44	8	9

Notes:

[1] Estimated on the suggested infection risk in pregnancy of 2/1000 in the UK in all adults aged 15–44 years

[2] Based on a risk of 1.1 % for CVS

days, but may be as brief as 2 days, suggesting transplacental infection. A severe outcome of neonatal varicella is not limited to transplacentally acquired infection, but occasionally occurs in infants who have clinical signs of varicella in the early postnatal period (minimum incubation period 8 days after birth) despite the use of VZIG at high dosage. VZV reactivation as herpes zoster is not uncommon after neonatal varicella, giving rise to zoster within a few months or years after the primary infection. Further zoster episodes have not been reported in these children, suggesting that effective cell-mediated immunity develops after reactivation.

Most of the concern surrounding neonatal varicella relates to infants whose mothers develop varicella. However, there have been occasional reports of varicella in neonates of mothers with a past history of chickenpox (Bendig et al., 1998). Miller et al. (1989) followed up 36 infants with documented antibody to VZV who had a home exposure to chickenpox. Six infants (17%) were infected, three with symptoms (two mild, one severe) and three without clinical features. Clearly, passively acquired VZV antibody provides incomplete protection, particularly under conditions of close exposure.

Diagnosis

Diagnosis of maternal varicella

Ideally, a clinical diagnosis of varicella in pregnancy should be verified serologically using paired sera obtained during the acute illness and after its resolution (Chapter 17). For assessing the varicella immune status in adults it should be remembered that the strong cross-reaction between varicella and HSV-1 detectable in various ELISA tests with complete or gP antigen may lead to false positive VZV antibody values, particularly in the case of low titers (Enders, unpublished). In women with complications, such as pneumonia or encephalitis, rapid tests for virus detection are employed to guide antiviral therapy. The possibility of re-infection with adverse

consequences for the fetus, has been raised (Martin et al., 1994). However, the fact that close exposure to varicella may result in a boost in IgG antibodies and also in production of IgM antibodies among immune individuals complicates the use of serologic assays to evaluate pregnant women for VZV infection. Among 2814 pregnant women with borderline or low IgG levels (65–300 IU/l) and negative IgM antibodies (< 1:40) detected within 1–3 days of exposure, a significant rise in IgG titers (500–4000 IU/l) was found in 255 women (9%) who had a follow-up serum obtained 3–4 weeks later. In 99 women, specific IgM antibody (38.8%) was also detectable at medium to high levels. IgA antibodies were not initially tested in the serum but were found in 6.7% (17/255) at medium to high levels (150–4000 IU/l). Nevertheless, in contrast to the report of Martin et al. (1994), none of the women with serological evidence of possible reinfection had symptoms of VZV infection and no congenital defects consistent with CVS were reported in their offspring (Enders, unpublished).

Diagnosis of CVS in the fetus

Recent technologic advances have expanded the role of prenatal ultrasound to identify anomalies and to guide invasive diagnostic procedures. Prenatal diagnosis to detect fetal VZV infection is feasible using PCR (polymerase chain reaction) for VZV DNA but it is not generally recommended because of the low risk of CVS. Even if VZV DNA is detected in the amniotic fluid, many infants have asymptomatic intrauterine VZV infection.

The number of publications that describe prenatal diagnosis of fetal VZV infection by detection of ultrasound abnormalities only (Pretorius et al., 1992; Hofmeyr et al., 1996; Crino, 1999), by ultrasound followed by detection of VZV DNA in fetal fluids and tissues (Liesnard et al., 1994; Pons et al., 1995; Kustermann et al., 1996; Mouly et al., 1997) or attempts to measure VZV IgM antibody in fetal blood (Liesnard et al., 1994; Mouly et al., 1997) is increasing. The majority of the reports confirm that VZV-specific fetal anomalies are detected in fetuses of mothers with onset of varicella mainly in the first 20 WG and exceptionally, up to WG 23. The abnormalities which can be seen by ultrasonography in congenitally infected fetuses include the more nonspecific signs, such as fetal hydrops, abnormal amniotic fluid volumes, hyperechogenic areas in the liver and parenchymal liver calcifications. The more specific signs are, especially, limb deformities, cerebral ventriculomegaly, microphthalmia, and fetal growth restriction. Often several of these abnormalities can be demonstrated (Pretorius et al., 1992; Hofmeyr et al., 1996; Crino, 1999). In some reports, the suspicious findings by ultrasound have been confirmed as VZV-specific by virus detection in amniotic fluid (Pons et al., 1995). In another such study (Liesnard et al., 1994), prenatal diagnosis was performed in 17 mothers with varicella during the first 20 weeks of gestation; fetal

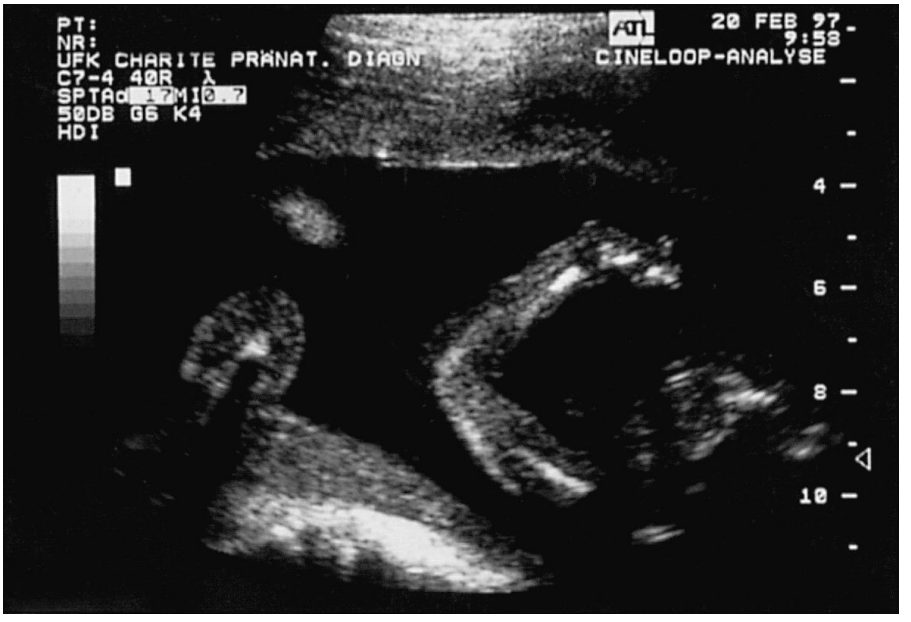
blood samples were investigated for IgM antibodies, and were tested retrospectively for VZV DNA by PCR along with nine amniotic fluid samples. Virus-specific IgM was found by IFA in four of 17 blood samples (23%), whereas VZV DNA was not detectable in eight of the IgM negative fetal blood samples or in the amniotic fluid samples. No ultrasound abnormalities were detectable throughout the pregnancies.

In another study (Kustermann et al., 1996), 14 women underwent prenatal diagnosis between WG 10 and 24 using a combination of chorionic villi sampling, amniotic fluid and fetal blood. The rate of detection of placental fetal infection was much higher (36%) than reported in other studies and was not consistent with prospective studies of fetal risk (Mouly et al., 1997; Enders, 1999, unpublished; Puchhammer-Stöckl et al., 1994; Hartung et al., 1999). VZV DNA was found in two aborted fetuses with hydrocephaly in most of the tissues examined, including brain. The nine women who tested negative at prenatal investigation delivered healthy neonates whose VZV-specific IgM antibodies were negative and none of them developed herpes zoster infection later.

One recent case report (Hartung et al., 1999) used all the available technical advances for diagnosing CVS, including a high-level ultrasound technique and detection of VZV DNA using PCR in fetal fluid samples and in fetal tissues following therapeutic abortion. This report gives information on the sonographic findings, showing the contractures of the upper extremity (Figure 16.2a) and contractures of the upper and lower extremities were found by gross examination of the fetus following therapeutic abortion (Figure 16.2b). The cicatrical skin lesions were not seen. Furthermore, VZV DNA was found in high copy numbers by one-round PCR with verification by the nested PCR in amniotic fluid and fetal blood, and in several fetal tissues following therapeutic abortion; attempts to isolate the virus were unsuccessful. The low-positive IgM value in the fetal blood is questionable and may be due to contamination with maternal blood, which was still IgM-antibody positive at medium level 7 to 8 weeks after onset of maternal infection.

The largest published prospective study of prenatal VZV diagnosis evaluated 107 women with acute varicella up to the 24th week of pregnancy (Mouly et al., 1997). Time of prenatal diagnosis was between WG 21 and 23. VZV DNA was detected by PCR in 9 of 107 (8.4%) amniotic fluid samples. The PCR protocol was similar to those used in other studies (Kido et al., 1991; Puchhammer-Stöckl et al., 1994). Eighty-two fetal blood samples were tested for IgM antibodies (by Enzygnost IgM EIA) but none was positive for VZV IgM antibodies, including five sera which corresponded to the nine amniotic fluid samples that were PCR-positive. Virus detection in fetal blood by PCR was not attempted. The outcome of pregnancies was known in 99 cases, which included 94 live-born infants who were followed for 26 months (range 7–60 months of age). The estimated risk for VZV transmission in

(A)



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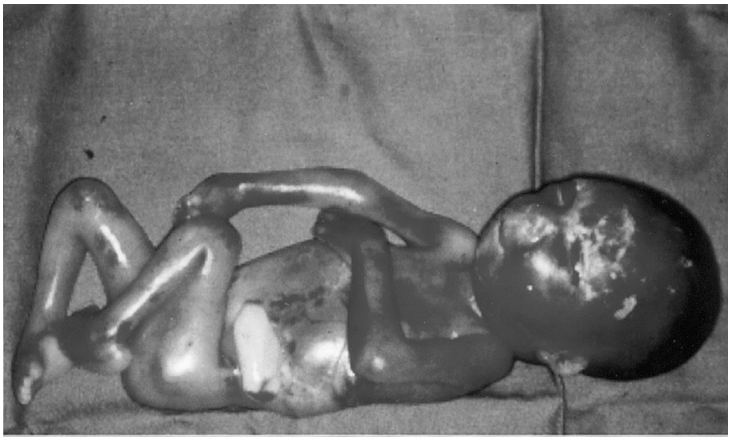

Figure 16.2 (A) Sonographical demonstration of contraction of the upper extremity (Hartung et al., 1999). (B) Gross examination of the fetus with congenital varicella syndrome, illustrating the upper and lower limb contractions (Hartung et al., 1999).

maternal varicella occurring before the 24th week of pregnancy was 8.4% by laboratory tests, 2.8% based upon occurrence of CVS and 3.8% using zoster in asymptomatic newborns in early infancy as the marker. Of the 34 women who received VZIG, only one within 72 hours after exposure, and two women who received acyclovir, prenatal diagnosis was negative and the infants of these women were delivered at term and were normal. Thus the effect of these interventions on fetal risk cannot be assessed because only three cases were evaluated.

In a prospective study of prenatal diagnosis done in 991 women who had acute varicella up to WG 24, who were evaluated from 1991 to April, 1999 (Enders, unpublished), 140 (14%) underwent prenatal diagnosis between WG 14 to 27. VZV DNA was detected by PCR in 10 of 126 amniotic fluid samples and in five of 28 fetal blood samples. In five fetuses with specific signs of fetal varicella infection by ultrascan, fetal blood and amniotic fluid were DNA positive by PCR. These cases are included in the large prospective study of the risk of CVS (Enders et al., 1994). None of the 28 fetal blood samples obtained between WG 21 and 24, of which five were VZV DNA positive, had detectable VZV IgM antibodies using the Enzygnost ELISA. In this study, the transplacental VZV transmission as estimated by the DNA PCR positive rate in amniotic fluid and fetal blood, was 7.9%.

The available experience with prenatal diagnosis demonstrates that amniotic fluid taken at WG 18–23 and over 4–6 weeks after the onset of maternal varicella is technically easy to obtain and yields the best results for detecting fetal VZV infection by PCR if the assay is done in a laboratory with extensive experience with the method. Nevertheless, it is essential to understand that a positive PCR result in amniotic fluid is **not** predictive for fetal abnormalities in the absence of consistent abnormal findings by ultrasound. Therefore, high level ultrasound screening at WG 19–23/24 is recommended for all women with varicella in the first 20–21 weeks of pregnancy. If the findings are abnormal, fetal blood and amniotic fluid should be tested for VZV DNA. Tests for VZV IgM in fetal blood are not helpful.

Ultrasonographic scans to detect major abnormalities should focus on the limbs and cerebral and ocular structures. Skeletal defects may be evident earlier but cerebral and ocular abnormalities are seldom recognized before WG 22 to 23. They may not become apparent until much later in pregnancy, when therapeutic abortion is not an option. However, in the hands of very experienced sonosists, magnetic resonance imaging of the fetus may enable earlier detection of more discrete defects, such as microphthalmia, micropolygyria and signs of microcephaly (Mouly et al., 1997).

Although the literature includes anecdotal reports of the use of various methods for the prenatal diagnosis of CVS, it is necessary to define the positive and negative predictive value of these methods when applied in prospective studies with known outcomes of pregnancy. The necessary information includes detection of the agent

in the fetal material, in the case of spontaneous or therapeutic abortion, and clinical and laboratory investigation of the newborn, with follow-up in the first and second years of life. The prognostic value of prenatal diagnosis, based on the data obtained in the Mouly study and in the Stuttgart laboratory, is as follows. In the absence of abnormal findings by ultrasound and positive VZV PCR in amniotic fluid at WG 17–21, the risk of CVS is questionable. If no abnormalities are found in a repeated sonographic scanning at WG 23–24, the risk of CVS is remote but cannot totally be excluded (Mouly et al., 1997). The risk of CVS is very high if VZV-specific signs are present by ultrasound, in combination with detection of VZV DNA in amniotic fluid or fetal blood and, particularly, if found in both samples in WG 22–24 and later. A negative result for VZV DNA detection by one round PCR or nested PCR (nPCR) in amniotic fluid of WG 18–22 and a normal high level ultrasound at or after WG 23 suggests a low risk of intrauterine infection with serious sequelae.

Diagnosis in infancy

The criteria for the diagnosis of fetal varicella syndrome include maternal symptomatic varicella infection during pregnancy, presence of cicatrial skin lesions that correspond to a dermatomal distribution or immunological evidence of in utero VZV infection. The latter should include either the demonstration of specific IgM antibody after birth, the detection of a positive cellular immune response over the age of 1 month, and/or persisting IgG antibodies after 7 months of age, in the absence of postnatal varicella. In addition to the immunological criteria, virus detection by VZV PCR should also be attempted in body fluids, blood or tissue biopsy of newborn and infants. In cases of fetal death, postpartum the detection of VZV DNA in tissues of various organs of the infant and the demonstration of calcified necrosis by histopathological techniques is also possible (Hartung et al., 1999). Laboratory confirmation of intrauterine VZV infection is of greater importance in cases with less typical features. The confirmation of fetal VZV infection at an early stage appears only possible by detection of VZV DNA in body fluids, blood or tissue because immunological evidence is unreliable. IgM antibody may not be detectable even in infants with typical features of CVS. In the few cases with IgM antibody detection, it was of low concentration and only positive in the RIA (Enders et al., 1994) or the indirect immunofluorescence test (Liesnard et al., 1994). The IgM response in CVS differs from that in congenital rubella syndrome, which is associated with chronic multisystem infection, in which specific IgM is nearly always detectable in infected fetuses and symptomatic or asymptomatic newborns for some weeks after birth (Enders, 1982; Enders, 1985). A positive cellular immune response in newborns at or over 1 month of age detected by an in vitro lymphocyte proliferation assay (Paryani & Arvin, 1986) is also indicative of intrauterine infec-

Table 16.6 Frequency of immunological and clinical criteria for intrauterine infection in newborns and infants with symptoms of congenital varicella syndrome

	Number of infants*		
Markers	Prospective 1981–99 ($n = 13$)	Retrospective 1979–99 ($n = 12$)	Total
Time of maternal infection (WG)	3–19	8–23	
IgM at birth	3/12 (25%) (2 RIA, 1 ELISA)	4/10 (40%) (RIA)	7/22 (32%)
CMI: lymphocyte proliferation assay >30 days to 4 years	1[a]/3 (33%)	1[b]/4 (25%)	2/7 (29%)
Persisting IgG (age ≥10 mon)	6/8 (75%)	8/9 (89%) titer range (65–4000 IU/l)	14/17 (82%)
Zoster in early infancy	2/8 (25%) (7 mon, 21 mon)	2/9 (22%) (4 mon, 7 mon)	4/17 (24%)

Notes:

[a] at 1 mon positive

[b] 3, 9, 12 mon positive, but negative at age of 14–21 mon

* Lancet study (Enders et al., 1994) and update 1994–99, Enders, unpublished.

tion. However, evaluation of cellular immunity is carried out only infrequently in CVS cases because of the special requirements for performance of this assay.

Recently, the diagnostic value of VZV DNA detection in blood of newborns and infants with CVS was investigated as a marker of intrauterine infection. In four of nine CVS cases, VZV DNA was detected by nPCR from shortly after birth up to 3.5 months of age and, in one case, up to 7 months of age but not longer (Enders, unpublished). The detection of VZV DNA appears not to be a reliable marker for confirming intrauterine VZV infection (Ozaki et al., 1994) but it may be useful when IgM or persistent IgG markers are negative. In addition to persisting IgG, an even later criterion of intrauterine VZV infection is the occurrence of zoster in early infancy.

The immunological and clinical findings in 13 prospectively and 12 retrospectively ascertained CVS cases are summarized in Table 16.6. The rate of detection of VZV IgM at birth is low and positive IgM antibodies were found mainly by RIA. For the measurement of the CMI (cell mediated immunity) response suitable blood samples were available from only seven infants. One of the infants of the prospective series had a positive lymphocyte transformation to VZV antigen at 4

weeks of life. No further blood sample for CMI testing was available. The other infant in the retrospective series had a positive CMI response at ages 3, 9, and 12 months but not at age 14 and 21 months, while IgG antibodies were measurable at high titers. Persisting IgG antibodies were found in 82% and zoster in early infancy occurred in 4 of 17 cases (24%). The lymphocyte transformation assay was performed in only one of those infants with zoster and was negative at an age of 4 years. The two infants with positive CMI response had persisting antibodies at age of over 1–4 years in relatively high titers and no zoster episode had been reported at the age of 2 and 4 years. These results support the observation that infants with a good CMI response are at low risk of zoster in early childhood. There are only three reports of CVS cases with investigation of the immunological criteria for intrauterine infection. In one case (Paryani & Arvin, 1986) with maternal varicella at WG 12, IgM was negative after birth, the CMI response was negative at 1 month of age and death occurred at 6 months; no test for IgG antibody was performed. In the second report (Grose, 1989) with maternal VZV at WG 20, IgM was positive in the FAMA and the RIA test, CMI response was negative at age of 7 days and death occurred early postpartum. In the third report (Schuster et al., 1994) maternal varicella occurred at WG 11. IgM at birth was negative in the ELISA test, CMI response was positive several times between the ages of 3 months and over 3 years and IgG antibody were found by ELISA in high titers and in lower titers by IFA. No zoster episode had occurred and the infant was in fair health at age of 3 years and over.

In cases without the most typical clinical manifestations, such as skin scarring and limb hypoplasia, the value of the immunological markers is very limited for determining whether intrauterine VZV infection has occurred. VZV DNA detection is also limited for identifying VZV in fluid samples or skin biopsies. In contrast, DNA detection in tissues of various organs is the method of choice in cases of early death. In surviving infants, the most reliable immunological marker for intrauterine VZV infection appears to be the persistence of IgG antibodies beyond 7 months of age, when measured by ELISA. If persisting IgG antibodies are absent, CVS is unlikely but cannot be absolutely excluded. To this end, long-term follow-up observations, e.g. for zoster in early childhood, are needed.

Management of varicella in pregnancy

Passive prophylaxis with immunoglobulin

Passive antibody prophylaxis with a standardized preparation of VZIG (CDC, 1996; Department of Health, 1996a) is recommended for exposed susceptible pregnant women to prevent or attenuate varicella (Chapter 21). To the extent that VZIG completely blocks infection, it has the theoretical potential to provide the most benefit for mothers who are exposed during the first half of pregnancy, when the fetus is at risk of CVS. VZIG prophylaxis for women exposed later in pregnancy is recommended to

Table 16.7 Outcome of varicella exposure of 212 seronegative pregnant women following administration of VZIG

VZIG administration following days after exposure	Total (*n*)	Outcome					
		No infection		Subclinical infection		Modified/ normal varicella	
		(*n*)	(%)	(*n*)	(%)	(*n*)	(%)
1–2–3 days	153	83	(54)	7	(5)	63	(41)
4–5 days	46	27	(59)	1	(2)	18	(39)
6–10 days	13	4	(31)	3	(23)	6	(46)
Total	212	114	(54)	11	(5)	87	(41)

Notes: In this study the IgG and IgM antibodies were determined with the ELISA Enzygnost Anti VZV IgG-/IgM in the initial and follow up sera (Enders et al., 1994, 1997; Enders, unpublished data 1999).

reduce the risk of maternal pneumonia (Enders, 1984, 1985; Greenspoon & Masaki, 1988; Prober et al., 1990; Gilbert, 1993; Miller et al., 1993a; Enders et al., 1994).

Two preparations of VZIG for intramuscular administration are produced in Scotland and the UK, and are distributed by the Public Health Laboratory Service. These preparations have a relatively low IgG antibody concentration of about 700 IU per adult dose. Their effect is to attenuate disease rather than to prevent infection (Miller et al., 1993). VZIG preparations for intramuscular use are also available in the USA (CDC 1996) and Scandinavian countries. Subclinical infection occurs in a substantial proportion (15%) of high-risk nonimmune infants and children exposed to varicella and given UK VZIG as detected by testing for seroconversion in blood taken 1 month post-exposure (Evans et al., 1980).

In Germany, two commercial preparations of VZV immunoglobulins with defined antibody concentrations are available, one for intramuscular (i.m.) and the other for intravenous (i.v.) administration, with an IgG antibody concentration of approx. 2000 IU per adult dose. According to our experience in 212 seronegative pregnancies, about half of the mothers have clinical varicella even if VZIG is given in the recommended dosage i.m. or i.v., within 1–3 days of significant exposure, with modified or normal disease, and a further 5% have subclinical infection (Table 16.7). The dosage, for example in Germany for a 70 kg woman, is 20 ml Varicellon i.m. = 2000 IU or 70 ml Varitect i.v. = 1750 IU). Varitect is the only varicella-specific hyperimmunoglobulin which can be given i.v., not to be confused with intravenous IgG (IVIG), which has only average VZV IgG antibody titers. In the UK, the recommended dosage of VZIG is as vials/age (1 vial: 0–5 years; 2 vials: 6–10 years;

3 vials: 11–15 years; 4 vials: ≥16 years); in the USA, recommendation for dosage is 1 vial = 125 U/10 kg body weight up to maximum of 625 U i.m.

Whether VZIG has any benefit in prevention of CVS is not known. Comparing experiences with VZIG administration during pregnancy is difficult because the quality (i.e. IgG antibody concentration) of the various VZIG preparations that are used worldwide (e.g. UK, Scandinavia, France, Germany, USA) is different. Furthermore, it should be realized that immunoglobulin preparations should be measured for neutralizing antibody titers instead of determining the IgG concentration by ELISA. The nature of the maternal exposure is difficult to evaluate. No studies have been done with cohorts of pregnant women who had proven susceptibility to varicella, confirmed close contact and included population sample sizes that are adequate to provide data about efficacy (Brunell, 1992; Enders et al., 1994). In one prospective study, there were no CVS cases in 97 pregnancies in which maternal varicella occurred despite VZIG prophylaxis, and IgM antibodies were detectable at birth in only one infant (maternal varicella in WG 36) (Enders et al., 1994). This study has now been expanded to a total of 108 women, with known outcome of the pregnancy, who had varicella despite VZIG prophylaxis. About 80% of these pregnant women had received VZIG in the first and second trimester; no cases of CVS or infants with positive IgM antibodies at birth as evidence for intrauterine VZV infection have been observed (Enders et al., 1994) (G. Enders, unpublished data 1999). A total of eight infants were tested for persistent IgG, all of whom were negative. In another prospective study, Pastuszak et al. (1994) investigated 106 women with acute maternal varicella up to WG 20 and 106 age-matched control women. No attempts at prenatal diagnosis by virus and IgM antibody detection in the fetus were made. Five percent of the women with varicella had received VZIG after exposure; four infants were born with congenital defects. The mother of one of these infants was exposed to varicella at WG 11; VZIG was administered approximately 4 days after the exposure, although the type of preparation and dosage were not stated. The mother developed mild varicella in WG13 and ultrasound scanning in the 17th/18th WG revealed intrauterine growth retardation. This infant was born prematurely in the 35th week by cesarean section and had punctate lesions that resembled old chickenpox scars. The infant developed progressive respiratory distress and hepatic failure and died at the 22nd day of life. Ascitic fluid was positive for VZV IgG antibodies, which is not relevant for diagnosis because maternal IgG antibodies would have crossed the placenta by the time of birth; virus DNA was negative by PCR. The serum was not investigated for IgM antibodies. Postmortem examination revealed hepatic fibrosis and syncytial giant cell formation. The clinical features of the neonate and the histopathological findings are compatible with CVS. This case demonstrates that a very large prospective study would be needed to clarify whether passive prophylaxis is beneficial for the prevention of CVS.

Recommendations in Germany and the UK

Postexposure prophylaxis with VZIG is recommended only for seronegative pregnant women with significant exposure to an acute case of varicella (e.g. household contact, face-to-face contact). As a consequence of the findings in the prospective study (Enders et al., 1994), that no case of CVS occurred in maternal varicella after WG 20, VZIG is no longer recommended for women exposed after WG 22. In the case of exposure to zoster, passive prophylaxis is only recommended for seronegative women up to WG 21 intimately exposed to persons with extensive zoster lesions. In the UK, VZIG is only issued for women exposed after WG 20 if national supplies permit (Department of Health, 1996a). These recommendations differ from the US recommendation, which includes all susceptible pregnant women with close contact, because of maternal complications of varicella (Chapter 21). It is clear that seropositive women do not need VZIG. These women can be assured that they are unlikely to be infected as a result of exposure.

Active prophylaxis with vaccine

Pregnancy is a contraindication for administration of live attenuated varicella vaccine. However, because the virulence of the vaccine virus is less than wild-type VZV, the risk to the fetus, if any, should be even lower, and inadvertent administration is not considered a reason for termination of pregnancy. As a precaution, however, nonpregnant women who are vaccinated should avoid becoming pregnant for one month following each injection. In the US, universal vaccination of children, adolescents and young adults including all nonpregnant women of childbearing age with no history of varicella has been recommended since 1996 (CDC, 1996, 1999). In most West European countries, the introduction of universal vaccination is still controversial. In Germany, however, there are plans to introduce the universal varicella vaccination in the near future according to the US recommendations. In the meantime, selective vaccination is increasing in the groups of persons who have proved to be susceptible either by lacking history of varicella or a negative immunity status. These groups include women of childbearing age, particularly those who are planning a pregnancy.

Our own experience with vaccination of nonpregnant seronegative women ($n = 66$) indicates that the two recommended dosages of Varilrix vaccine given 4–8 weeks apart induces IgG antibodies in approximately 95% at medium level, which persist 3–6 years. In the case of exposure to varicella, significant IgG titer rises without IgM antibody detection or clinical symptoms were observed (Enders, unpublished). As in the case of naturally acquired immunity, these observations of asymptomatic boosting are not considered a matter of concern in pregnancy.

Varicella vaccine may be effective as postexposure prophylaxis (Asano et al., 1977; Arbeter et al., 1986; Salzman & Garcia, 1998), but active prophylaxis is not

Table 16.8 Acyclovir exposure during pregnancy, earliest trimester of exposure and outcome (June 1, 1984–July 31, 1998)

	Earliest trimester of exposure				
Outcome	First	Second	Third	Unknown	Total
Birth defects	19	2	7	0	28
No birth defects					
Live births	562[a]	185[b]	269[c]	1	1017
Spontaneous fetal loss	76*	0	2*	0	78
Legal induced abortions	82	1	0	1	84
Total	739	188	278	2	1207

Notes:

Patients who received topic treatment with acyclovir (Zovirax ointment) were excluded.

[a] includes 7 sets of twins, [b] includes 2 sets of twins, [c] includes 3 sets of twins, * includes 1 stillbirth.

advocated for women in early pregnancy because the vaccine contains live, infectious virus.

Antiviral therapy

Although acyclovir (ACV) is generally contraindicated in pregnancy, in varicella complicated by pneumonia, or other signs of dissemination, early treatment with intravenous ACV at any stage of pregnancy is essential and life-saving. Pregnant women with clinical signs of VZV pneumonitis should be admitted to hospital and monitored for hypoxemia by pulse oxymetry or other methods because reduced oxygen saturation may be documented well before the patient has X-ray signs of pneumonia or dyspnea (Nathwani et al., 1998). Although ACV in intravenous and oral form is not licenced for use in pregnancy, the prospective follow-up of a total of 1207 women treated during the first, second and third trimester did not show an increase in the number of birth defects when compared with those expected in the general population, or any consistent pattern of defects (Table 16.8). Similar data have been reported on the outcome of 94 pregnancies in which oral valacyclovir had been given (1st January 1995 to 31st July 1998).

Practices and recommendations for treatment of varicella in adults, including pregnant women, with oral ACV, differ in various countries. In contrast to Germany, in the UK and Australia oral ACV is offered to persons presenting within 24 h of onset of rash and given for 7 days; pregnant women over 20 weeks of gestation with varicella are treated with this regimen, which is known to reduce the disease manifestations in adolescents and adults, which is more severe than in

children (Balfour et al., 1992; Wallace et al., 1992). Oral ACV has been given during the incubation period (Suga et al., 1993; Lin et al., 1997), but its use is not established for pregnant women. Should oral ACV, or valacyclovir with its higher bioavailability for oral application, no longer be contraindicated for use in early pregnancy, one may hypothesize that it could reduce the risk of intrauterine infection and CVS by limiting viral replication during maternal viremia, and thereby the risk of transplacental passage of VZV (Haddad et al., 1987).

Management of the neonate

For newborns whose mothers have varicella around the time of delivery, prophylaxis with VZIG is recommended to prevent neonatal infection or to modify the disease. In the US and Germany, prophylaxis is only recommended for neonates whose mothers develop varicella between 5 days before to 2 days after delivery (CDC, 1996) while in the UK, prophylaxis is recommended for any infant whose mother develops varicella in the 14 day period centred on delivery (Department of Health, 1996a). The rationale for the extended risk period in the UK is the observation that neonates whose mothers develop varicella up to 7 days before delivery may be VZV antibody negative at birth (Miller et al., 1989), and a few case reports of severe disseminated varicella in infants exposed within the first week of life (Rubin et al., 1986).

In the UK, VZIG is also recommended for infants with nonmaternal postnatal exposure during the first 7 days of life and who lack VZV antibody (Department of Health, 1996a). Susceptible infants can be identified by testing a stored antenatal blood sample from mothers with a negative history of chickenpox. In the US and Germany, prophylaxis for postnatal exposure is restricted to premature infants. Because transfer of maternal IgG antibodies may be inadequate before the third trimester, it is recommended that exposed infants born before 28 weeks or weighing less than 1000 g should be given VZIG, regardless of a positive maternal history of varicella (CDC, 1996); although in practice many such infants will be VZV antibody positive (Patou et al., 1990; Gold et al., 1993). Moreover, some infants with a positive maternal history and more than 28 weeks gestation at birth may be VZV antibody negative (Gold et al., 1993) particularly if they are more than 60 days old (Patou et al., 1990) or have had repeated blood sampling with replacement by packed red blood cell infusion (Ng et al., 1996). Serologic testing to determine VZV antibody status of neonates with nosocomial exposure is therefore recommended rather than reliance on birth weight, gestational age and maternal history (Gold et al., 1993).

If severe varicella develops in the infant despite VZIG prophlyaxis, high-dose intravenous ACV should be given (Reynolds et al., 1999). Fatal outcomes have been reported despite VZIG prophylaxis and treatment with ACV (Holland et al., 1986; King et al., 1986). All were in infants whose mothers developed varicella within the period 4 days before to 2 days after delivery. Early treatment with intravenous ACV

of neonates infected in this high risk period is therefore recommended (Nathwani et al., 1998; Reynolds et al., 1999). Prophylactic use of intravenous ACV in this group has also been advocated (Sills et al., 1987) but there is no evidence of efficacy.

At present, there is no convincing information about whether mothers who develop varicella in the high risk period around the time of delivery should breastfeed or be isolated from their infants. Not unexpectedly, VZV DNA has been detected by PCR in breast milk (Yoshida et al., 1992) but whether VZV was transmitted to the newborn via breast milk could not be determined because the mother was breastfeeding in the 24 hours prior to rash onset. The close face-to-face contact in this period explains why the infant developed varicella but the older child did not. Furthermore, neonates whose mothers have varicella shortly before delivery may have already been infected transplacentally at the time of birth. For those infants whose mothers develop varicella in the puerperium, transmission during the infectious period before rash onset is likely. The wisdom of recommending isolation of the infant from the mother (Hanshaw et al., 1985) has therefore been questioned (Trompeter et al., 1986; Stephenson, 1993).

Management of nosocomial exposure

Despite the concern raised when varicella exposure occurs in an antenatal clinic or neonatal intensive care unit, documented nosocomial transmissions in these groups are rare. This is probably due to the low intensity of exposure, particularly in neonatal units. Two cases of neonatal infection acquired in an intensive care nursery have been reported, both in infants who had an intimate exposure to an adult health care worker (Gustafson et al., 1984). Both infants' mothers were VZV antibody positive and one infant had received VZIG. Other control measures which have been advocated to prevent transmission in a neonatal intensive care unit include cohorting of infants by exposure and VZV antibody status (Stover et al., 1988). The index case is often a health care worker and control measures may require extensive testing of patients and staff contacts (Meurisse et al., 1990). Administration of VZIG to antenatal patients on the basis of a negative history of chickenpox without VZV antibody testing can result in four out of five women being given VZIG unnecessarily (Meurisse et al., 1990). Postexposure vaccination of susceptible health care workers is now recommended in the USA (CDC, 1999).

Zoster

Incidence of zoster

Based on consultation rates for herpes zoster in the 15–44 year age group in primary care in England (Miller et al., 1993a), the risk of an attack in pregnancy

Table 16.9 Fetal outcome after maternal herpes zoster according to stage of gestation of maternal infection: prospective study

Maternal zoster (WG)	Lancet study[1] 1980–93 ($n = 366$)	Update Stgt. 1994–99 ($n = 108$)	Lancet + Stgt.[2] 1980–99 ($n = 474$)
0–12	119	26	145 (30.6%)
13–24	117	42	159 (33.5%)
25–36	130	40	170 (35.9%)
Spontaneous abortion	5	—	5 in WG: 9, 2 × 10, 12, 13
Therapeutic abortion	2	1	3 in WG: 8, 10, 13
Live-born infants	359	107	466
CVS*	0	0	0
Zoster in early infancy up to 1 year	0	0	0

Notes:

* Two infants had other abnormalities – one with achondroplasia (IgM neg, PCR neg. at birth), the other, with a cleft lip and palate, not blood tested.

[1] Enders et al. (1994).

[2] Enders et al. (1994); Enders, unpublished data (1999).

is likely to be around 2 per 1000. Unlike varicella, there is no evidence that the incidence in adults has changed over the last few decades. The annual incidence in the 15–44 year age group over the period 1995–7 in England was 209 per 100 000 compared to 219 between 1967 and 1969. As mild immunosuppression occurs in late pregnancy it is possible that the incidence in pregnant women is higher than in the general adult population but there are no data on this question (Table 16.9).

Diagnosis of maternal zoster

Because zoster occurs in immune individuals, VZV IgG antibody is detectable at the onset of zoster, but titers rise to high levels after 4 days and gradually decline over 6–8 months. In our experience, IgA antibodies become detectable in 93% of patients within 4–5 days following onset of zoster and may reach high titers (1:512–8000), particularly in patients with extensive lesions and delayed therapy. Positive IgA titers remain detectable in approximately 70–80% of patients up to 4–7 months in medium titers (1:512–1:1024). IgM antibodies are also detectable in about 40% of zoster patients within 5–31 days and in about 20% during week 5–12 in medium level ($\geq$ 1:160), but are no longer detectable ($<$ 1:40) 4–7 months after onset of zoster.

Table 16.10 Herpes zoster in pregnancy: serology in newborns and infants

	Lancet study[1] 1980–93 ($n = 366$)	Update Stgt. 1994–99 ($n = 108$)	Lancet + Stgt.[2] 1980–99 ($n = 474$)
IgM at birth			
tested	43/359	25/106	68/465
positive	0/43	0/25	0/68
Persistent IgG			
tested	34/359	not tested	34/466
positive	0/34		0/34

Notes:

[1] Enders et al. (1994).

[2] Enders et al. (1994); Enders, unpublished data (1999).

Outcome of zoster in pregnancy and the puerperium

Women who develop localized zoster in pregnancy should be reassured that the risk to the fetus is negligible. Localized zoster in pregnancy could theoretically result in fetal infection, if the dermatomes involved were T10 to L1, as sensory nerves to the uterus originate from these segments; but no such cases have been documented (Miller et al., 1993a). Various congenital defects have been reported after maternal zoster but only one infant with characteristic limb hypoplasia and skin scarring has been described (Higa et al., 1987). The case can be explained because it followed disseminated maternal zoster at WG 12, with presumed maternal viraemia. In a large prospective study, Enders et al. (1994) found no clinical or serological evidence of intrauterine infection in the infants of 366 pregnant women who developed localized zoster before 37 WG. Follow-up of another 108 women by the Stuttgart laboratory since 1994 has confirmed these findings (Table 16.9 and 16.10). Similarly, Miller et al. (1989) found no clinical or serological evidence of VZV infection in 25 infants whose mothers had perinatal zoster; 18 were exposed during the last 16 days of pregnancy and seven between 1 and 18 days after delivery. Passive immunization of neonates whose mothers develop perinatal zoster is therefore not indicated. The low risk of post-neonatal infection from maternal zoster cases is due in part to the lower infectivity of zoster compared to chickenpox. Furthermore, if the onset of maternal zoster is ≥ 4 days before delivery, the infant passively acquires high titers of VZV IgG antibody from the mother, whose pre-existing immunity is reinforced as a result of the reactivation.

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Part IV

Laboratory Diagnosis

17

Laboratory diagnosis of infection

Bagher Forghani

The laboratory diagnosis of varicella-zoster virus (VZV) infections has been improved by the development of techniques and production of new reagents including use of specific monoclonal antibodies (mAbs) to detect VZV viral antigens directly from vesicle smears by immunofluorescence antibody (IFA) staining, eliminating cross-reactivity with other herpesviruses; production of VZV DNA probes for dot-blot hybridization; and in situ hybridization and polymerase chain reaction (PCR) methods to detect VZV nucleic acid in clinical samples. Serological tests have been developed to measure VZV IgA, IgM, and IgG antibodies, such as enzyme immunoassays (EIAs), which are useful for rapid serodiagnosis and suitable for small or large scale epidemiological studies. These new techniques provide the clinician and diagnostician with an increasing array of tests for rapid, accurate and early diagnosis of VZV infections. These approaches can help both in the management of the individual patient and in instituting appropriate public health measures in response to epidemics (Gershon & Forghani, 1995).

Collection and preparation of clinical specimens

A key requisite for the successful laboratory diagnosis of VZV infection is to obtain a proper clinical sample, collected at the right time in the clinical course, from the right site, and transported to the laboratory in good condition. Since clinical samples are usually in very limited quantity and a large number of assays is available, careful selection of the appropriate test is necessary to ensure a proper viral diagnosis. Collection of clinical samples should be goal-oriented, depending on whether the sample is intended to be tested for direct detection of viral antigens by IFA staining, nucleic acid amplification by PCR, or virus isolation, since each method has different conditions and criteria for specimen collection. The recommended approach is a "triage" using information on patient disease condition (e.g., zoster, encephalitis, etc.), the clinician's presumptive diagnosis, and the diagnostic tests to be performed by the laboratory (Garcia-Kennedy, 1995).

Skin scrapings and vesicular fluid

Because the major target organ of VZV is the skin, the virus is usually accessible for laboratory testing, and laboratory diagnosis is best accomplished by examining material obtained from skin lesions. Specimens to be examined include scrapings from the base of vesicular skin lesions and vesicular fluid. Smears are made from skin scrapings from the base of vesicular lesions and are used for IFA staining to demonstrate VZV antigens in infected epithelial cells. The smears must contain skin cells rather than just vesicular fluid. The sensitivity of IFA staining decreases if fewer than 20 epithelial (parabasal) cells are present (Weller, 1979).

Prior to sample collection, skin lesions should be cleansed with alcohol, allowed to dry, and then ruptured with a sterile lancet. The lesion should then be rubbed with a sterile cotton swab, taking care to exert enough pressure to obtain skin cells. Calcium alginate swabs inhibit VZV infectivity and should not be used for specimen collection. The swab should be rubbed onto an area of about 0.5 cm diameter on a clean microscope slide, to be used for IFA staining. Vesicle fluid can be aspirated with a capillary tube or with a 26-gauge needle attached to a tuberculin syringe and the aspirated fluid used as virus transport medium for virus isolation, for electron microscopy (EM) or PCR testing. Transport medium containing swabs of vesicular lesions may also be used for virus isolation. In general, lesion crusts do not yield infectious virus and are not suitable for IFA staining but may be used for EM and PCR testing.

Biopsy and autopsy samples

Autopsy tissues can be used to prepare smears for IFA staining by excising pieces of tissue about 10 mm diameter and gently pressing the cut surface to the clean area of a slide. Three or four slides should be prepared from each tissue. The slides are allowed to dry at room temperature and are fixed in acetone for 10 minutes and stained by IFA. Suspensions of cells recovered from fresh or frozen tissue may also be prepared and used for virus isolation and IFA staining. Formalin-fixed sections of punch biopsies from maculopapular skin lesions, or autopsy tissues, are also suitable for IFA staining. Enough samples should be prepared to permit staining with antibodies to both VZV and herpes simplex viruses (HSVs).

Samples for virus isolation

For successful virus isolation of VZV from skin lesions, vesicular fluid from lesions should be obtained as early in the course of varicella or herpes zoster as possible. By 4–5 days after onset of varicella, this fluid is no longer likely to contain infectious virus, although viable VZV may be present in zoster lesions for a longer period of time, particularly in immunocompromised patients. VZV cannot be isolated from skin lesions that have become pustular or dried. VZV may sometimes

be isolated from autopsy samples or cerebrospinal fluid (CSF) (Andiman et al.,1982) and, rarely, from throat, pharyngeal, and conjunctival specimens.

Sampling for PCR

Vesicle swabs and infected tissues for PCR testing should be placed in a sterile container without additional fluid. CSF and other body fluids should be collected aseptically in a sterile container and delivered to the laboratory promptly.

Serum and CSF for serologic tests

Serologic diagnosis requires taking an acute-phase blood specimen as soon as possible after onset of symptoms, to be subsequently tested in parallel with a convalescent-phase specimen, collected 10–14 days later. Acute and convalescent sera are used to demonstrate a diagnostically significant increase in VZV antibody titer and/or the presence of specific IgM, or IgA. VZV immunity status is determined by testing a single blood specimen for specific IgG collected before, or as soon as possible after, exposure to VZV infection.

Storage and transport of specimens

Specimens for virus isolation should be inoculated into suitable cell cultures as soon as possible after collection. If transport medium is used, only a small volume should be employed to avoid compromising infectivity by dilution of the specimen. If inoculation must be delayed, specimens may be held at refrigerator temperature for only 12 hours. For longer storage, dry-ice temperatures (−70 °C) are required. Smears to be examined for viral antigen by IFA staining or by cytology for intranuclear inclusions do not require refrigeration. Samples from vesicular lesions should be considered infectious, and suitable protective precautions must be taken in packing, shipping, and storage. Specimens for PCR may be stored 1–2 days at 4°C, but for longer storage time, −70°C is recommended. All clinical samples should be transported to the laboratory on dry ice, if transport will take more than several hours.

Direct examination of clinical specimens

Direct and indirect immunofluorescence antibody staining

Immunofluorescence staining methods are preferred for laboratory diagnosis of varicella and herpes zoster because they are rapid, sensitive, and highly specific. Direct immunofluorescence antibody (DFA) or indirect (IFA) staining is more sensitive for VZV detection than virus isolation (Schmidt et al., 1965; Peters et al.,1979; Drew & Mintz, 1980; Emmons, 1980; Sadick et al., 1987; Coffin & Hodinka, 1995). Direct fluorescent staining is the simplest and most reliable of methods, with fewer

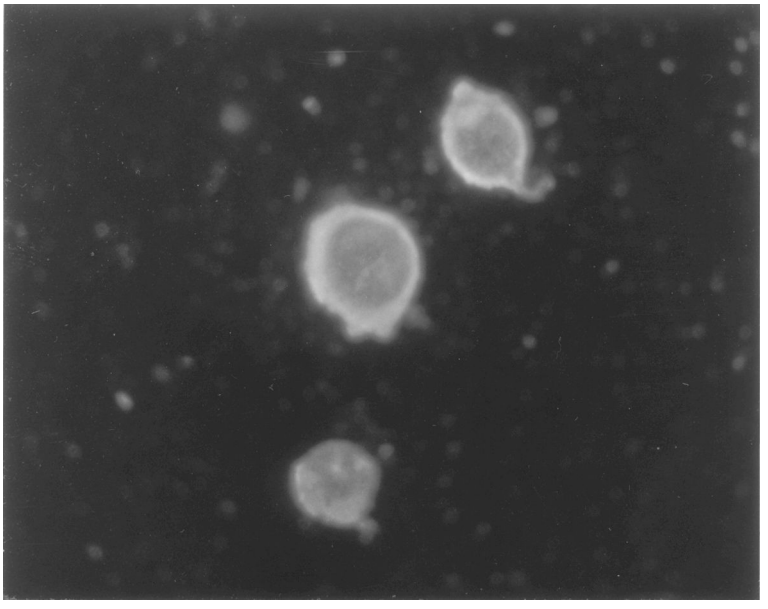

Figure 17.1 Immunofluorescent antibody staining of skin lesion from a child with varicella. Note the bright cytoplasmic and nuclear fluorescence stain of epithelial cells with large nuclei reacted with pooled mAbs against VZV proteins.

nonspecific reactions. Pretitrated fluorescein-labeled mAbs (monoclonal antibodies) (available from several manufacturers) are applied directly to the slide specimen being examined (skin scraping, tissue smear, etc., which has been acetone-fixed for 10 minutes), and incubated for about 30–40 minutes at 37°C. The unbound antibody is then removed and the stained preparation is washed, mounted in Elvanol solution containing 2.5% (wt/vol) 1,4-diazabicyclo(2.2.2.) octane to prevent fading, and examined with a fluorescence microscope (Forghani & Hagen, 1995). Estimates of the intensity of fluorescence are scored 1+ to 4+ to indicate relative intensity of staining from low to high, respectively. Demonstration of the presence of VZV-positive staining in the cytoplasm and nucleus of the cell is highly suggestive of VZV infection. An example is shown in Figure 17.1.

The indirect staining procedure is more sensitive than the direct method because two layers of antibody produce amplification but problems of nonspecificity may be increased. In the IFA method, the clinical specimen is first incubated with pretitrated mAbs (preferably pooled mAbs of several gene products) for 30–40 minutes. The sample is then washed to remove unbound antibody and incubated with conjugate (e.g., fluorescein-labeled goat anti-mouse immunoglobulins). The preparations are washed again, mounted and examined for immunofluorescence.

Currently, most commercial conjugates for DFA staining are pooled mAbs

against several VZV proteins and are packaged in convenient plastic dropper bottles at working dilutions (Gleaves et al., 1988; Rawlinson et al., 1989; Schirm et al., 1989). Examination of positive samples with a fluorescence microscope should reveal specific staining of 1+ to 4+ intensity, associated with the cytoplasm and/or nucleus of the epithelial cells stained with the VZV conjugate and little or no staining of cells treated with HSV conjugates. Vesicular lesion specimens containing too few epithelial cells for definitive examination should be reported as unsatisfactory rather than negative.

Cytological examination

The Tzanck preparation is a rapid test for cytological examination of cellular material collected from the base of vesicular lesions. Smears are fixed with methanol and stained with buffered Giemsa, Wright's, and/or Sedi (Becton Dickenson, Parsipany, NJ) stain. Microscopic examination reveals the presence of multinucleated giant cells with characteristic intranuclear inclusions (Cowdry type A). The test is not specific because these cells are typical of infection with either VZV or HSV (Nahass et al., 1992; Sadick et al., 1987).

Electron microscopy (EM)

EM provides the capability for early presumptive diagnosis by the rapid identification of viral particles from scrapings of skin lesions, vesicular fluids and biopsy specimens. Specimens should be examined promptly to avoid bacterial growth. Demonstration of virus with typical morphology of herpesviruses identifies the etiologic agent as a member of this group, but it cannot provide a specific diagnosis of VZV infection. Vesicular fluid may be examined directly or crusts from lesions may be ground in 1 or 2 drops of distilled water. Alternatively, smears of fluid and/or crusts may be prepared on glass microscope slides and air dried for shipment; material from these smears is then reconstituted in a drop of water for examination. A drop of the specimen is placed on a grid, blotted with filter paper, and a drop of 2% buffered phosphotungstic acid is then added and blotted (Oshiro & Miller, 1992).

EM has limited diagnostic utility, because only major diagnostic and reference laboratories have electron microscopes and it requires a highly trained electron microscopist. Although smallpox, with which varicella often used to be confused, has been eliminated from the world, the new issue of bioterrorism may make it necessary to rule out smallpox by this method.

Virus isolation and identification

In general, VZV isolation is relatively slow and less sensitive than DFA staining or PCR. This difference is in part because infectious virus persists only for a short

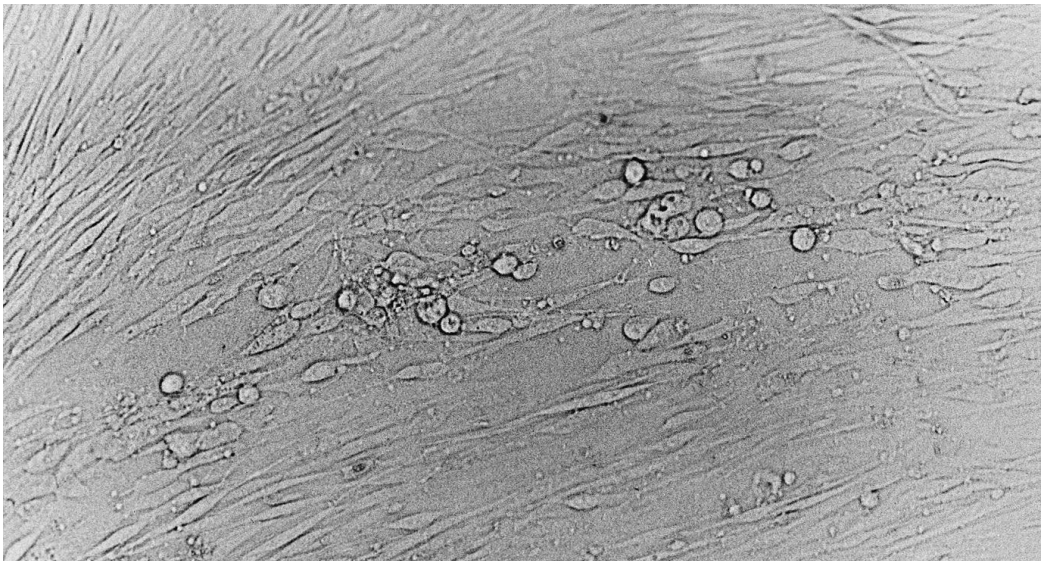

Figure 17.2 Cytopathic effects of human fetal diploid lung cell monolayer 72 hours after inoculation with cell-free varicella-zoster virus. Note the Small foci of rounded and swollen refractile cells, resulting from spindle-shaped viral plaques and surrounded with elongated uninfected fibroblast cells.

period of time in VZV vesicles, even though they may contain a large number of noninfectious virus particles.

Although a number of cell lines of human and simian origin have been used, cell lines of human origin are the most sensitive cells for virus isolation and growth (Weller, 1979; Forghani, 1986; Westenfeld & Washington, 1994). In our laboratory, human fetal diploid lung (HFDL) cells and/or human fetal diploid kidney (HFDK) cells are used in parallel for isolation of suspected VZV. Over the years, more viruses have been recovered from HFDK cells than HFDL cells (Forghani, unpublished observation). Swabs or vesicular fluids are diluted in cell culture medium and inoculated directly into tissue culture tubes containing cell monolayers. Cultures are incubated at 36°C and observed daily for development of cytopathic effects (CPE). The spread of infection can be accelerated by blind passage, dispersing cells in the infected culture with trypsin and replanting them in growth medium in the same culture vessel (Weller et al., 1958; Weinberg et al., 1996).

Presumptive identification of VZV may be made on the basis of typical CPE, which is focal in nature and progresses more slowly than does that of HSV. VZV produces a unique CPE with rounded, refractile cells and spindle shaped plaques in susceptible cells (Weller et al., 1958), as illustrated in Figure 17.2. VZV may be

differentiated from HSV by its failure to produce CPE in rabbit or hamster kidney cell cultures. The source of the specimen, epidemiologic factors, and clinical manifestations of the illness usually eliminate confusion between VZV and human CMV. However, if isolations are made from tissue specimens, there may be a need to distinguish between these herpesviruses. VZV grows well in epithelial cells, whereas CMV generally does not. In contrast to VZV, CMV strains fail to produce CPE in primary monkey kidney cells and development of CPE caused by CMV is generally slower.

Specific identification of VZV can be accomplished by direct or indirect immunofluorescence staining using polyclonal animal antisera or mAbs to VZV proteins. The assay was initially developed in this laboratory using polyclonal animal antisera (Schmidt et al., 1965). The use of commercial mAbs to VZV is recommended. Cell cultures showing CPE may be used to prepare slides from trypsinized cell suspensions. Cells are placed as small drops on microscope slides or by using a cytospin apparatus, and then dried and fixed with acetone. Smears are tested for VZV by direct or indirect IFA staining, with HSV-1 and -2 conjugates tested in parallel. Smears from uninfected cells should be prepared similarly, and included along with known positive VZV and HSV control slides in each assay. Positive results are based upon staining of 1+ to 4+ intensity with the VZV conjugate, little or no staining with HSV conjugates, and no staining of the uninfected control cells. The pattern of staining depends on what type of mAb has been used. Figure 17.3 shows intense fluorescence staining in the cytoplasm, cytoplasmic membrane and Golgi apparatus of a cell with multiple nuclei, using mAb to VZV gC.

A commercial kit, involving virus isolation in shell vials and early specific identification of virus with mAb prior to development of CPE, is a convenient means for identification of VZV in clinical specimens (Schirm et al., 1989). Shell vials contain coverslips on which cell monolayers have been propagated. Swabs or vesicular fluids are inoculated into each of six shell vials and centrifuged at low speed. The vials are incubated at 35°C. After 24 hours, the coverslip monolayers of three infected vials and one control vial are rinsed, fixed in cold acetone, and examined immediately by DFA. If all theee vials are negative after 24 hours of incubation, the three remaining inoculated vials are examined similarly after 72 hours of incubation. Isolation of VZV by shell vial was more sensitive than routine virus isolation (Weinberg et al., 1996).

In the past, other methods have been used to identify VZV, such as virus neutralization, complement fixation, immune adherence, or countercurrent immunoelectrophoresis. These methods are time-consuming and cumbersome to perform and do not offer any advantages over immunofluorescence staining.

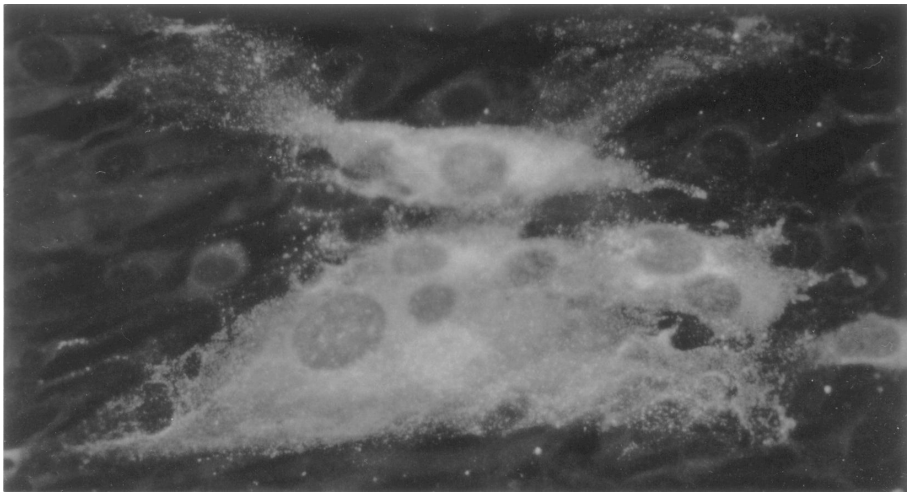

Figure 17.3 Indirect immunofluorescent staining of acetone fixed human fetal diploid lung cells 48 hours after inoculation with cell-free varicella-zoster virus; reacted with mAb against VZV gC. Note the Intense specific green fluorescence staining in the cytoplasm, cytoplasmic membrane, and Golgi apparatus of a cell with multiple nuclei. The reddish-brown cells are uninfected cells counter-stained with Evan's blue.

VZV antigen retrieval test

Paraffin-embedded, formalin-fixed tissues are generally used in immunohistochemistry due to excellent preservation of cell and tissue structures for microscopic observation. However, these types of tissues have been less frequently used in virology due to lack of specific and sensitive immune reagents developed for this purpose. With the use of our mAbs, retrieval of VZV antigen can be done with fixed tissues.

For the VZV antigen retrieval test, 4–5 micron sections of tissue are cut and transferred to microscope slides that have been previously treated with 3-amino propyl triethoxysilane to prevent loss of tissue during the assay. The tissue sections are deparaffinized by submerging sequentially in a solution of xylene, xylene/ethanol, and ethanol and air-dried. The slides are then transferred to a plastic jar containing citrate buffer, boiled for 10 minutes and used immediately for indirect IFA staining using pooled mAbs against several VZV proteins. Using our library of mAbs, we have observed that mAbs only react in immunoblotting assays which use sodium dodecyl sulfate-denatured antigens (Forghani et al., 1990; Forghani & Sanchez, 1997). The test has potential value either with recent biopsied and autopsied tissues or with archived formalin-fixed tissues. Figure 17.4 shows IFA staining of kidney, lung, liver, and spleen autopsied tissue from an AIDS patient with VZV infection.

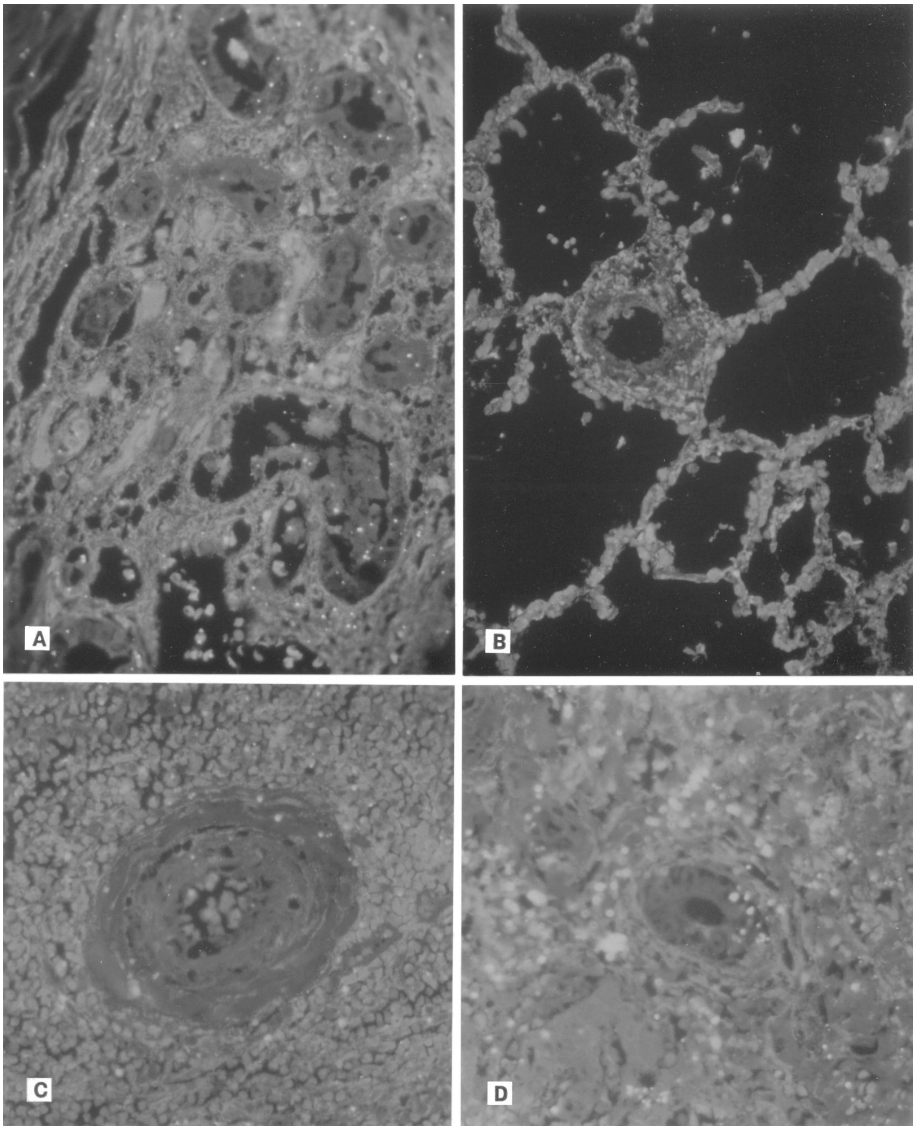

Figure 17.4 Indirect immunofluorescent staining of kidney, liver, lung and spleen of formalin-fixed paraffin-embedded tissue sections from an AIDS patient, reacted with pooled mAbs against several VZV gene products and counter stained with Evan's blue. (A) Strong focal fluorescent staining is seen in the interstitium of kidney tissue sections, while no staining is observed in the tubules. (B) Strong fluorescent staining is seen in the blood vessel within the interstitium and the endothelium of the vessel, and the interstitial compartment of alveolar sacs and the pneumocytes. (C) Positive fluorescent staining is seen in the interstitium of portal triads of the liver with focal staining of the bile duct cells. (D) Positive fluorescence staining is seen in a splenic artery surrounded by white pulp, smooth muscle, endothelium and the surrounding interstitium.

Hybridization for VZV DNA and RNA detection

The principle of nucleic acid hybridization (base-pairing) is hybrid formation between a labeled (e.g., radioisotope [^{32}P] phosphorus) strand of specific DNA or RNA, known as a probe (produced in vitro) and an unlabeled strand to be tested (target), which is derived from the patient's specimen. Base-pairing occurs between adenine (A) and thymine (T), and cytosine (C) and guanine (G) in two complementary strands of DNA, a strand of DNA and one of RNA, or two strands of RNA. The chemical reaction of hybridization between two strands of DNA is electrostatic non-covalent hydrogen bond and therefore reversible. Most hybridization procedures involve the following steps: (1) denaturation (dissociating double-stranded DNA) is usually done by heating the DNA specimen to 95°C so that single-stranded target nucleic acid is available for hybridization to the labeled probe; (2) hybridization at lower temperatures to allow adherence between target and probe; (3) exhaustive washing to remove the unhybridized probe. (4) The new hybrid is detected as a signal from a label attached to the probe. Signal-generating systems may include: radioactivity, biotin–avidin affinity, enzyme conjugation, fluorescent conjugation, and digoxigenin–antidigoxigenin probes. However, most hybridization procedures use a nonisotopic biotin–avidin complex attached to an enzyme (Forghani et al., 1991; Forghani & Erdman, 1995). Hybridization is performed by Southern blotting, dot-blotting and in situ hybridization (ISH) methods.

For Southern blotting (Southern, 1975), the sample DNA is cleaved with a restriction enzyme, the DNA fragments are separated by agarose gel electrophoresis, and electro-blotted to a nitrocellulose or nylon membrane. The membrane is then hybridized with a probe. Northern blotting detects RNA. Partially purified RNA of a clinical sample is separated and transferred to membrane prior to hybridization. This assay was used to map VZV RNA transcripts (Ostrove et al., 1985).

A modified procedure for nucleic acid blotting is dot-blot or slot-blot hybridization. This method involves spotting partially purified DNA from the sample directly on a membrane, or applying the DNA under vacuum so the membrane serves as a DNA retaining filter. The membrane is then hybridized with a probe. The major advantage of dot-blotting over Southern blotting is that it is less labor intensive because it eliminates electrophoresis and transfer steps; however, the main disadvantage of dot-blotting is that the size of target DNA cannot be identified and it is more subject to nonspecificity than Southern blotting (Seidlin et al., 1984; Cuthbertson & Grose, 1988; Forghani et al., 1991).

In situ hybridization (ISH) provides exquisite sensitivity and specificity for detecting VZV DNA in clinical tissues or cells. The assay has been extremely valuable for delineating the site of VZV latency in human ganglia (Gilden et al., 1987; Croen et al., 1988; Vafai et al., 1988; Mahalingam et al., 1990; Croen & Straus, 1991; Lungu et al., 1995; Kennedy et al., 1998). Briefly, ISH utilizes histological pro-

cedures to immobilize intact cells or tissue sections on a microscopic glass slide. Hybridization is then done by adding the labeled probe directly to the tissue section fixed on the slide, which hybridizes to those cells that contain VZV DNA. After proper incubation time, the unhybridized probe is removed by washing and cells containing VZV DNA are identified by nuclear stain (Vonsover et al., 1987; Koropchek et al., 1989; Forghani et al., 1991; Annunziato et al., 1997).

Polymerase chain reaction (PCR)

PCR methodology has revolutionized the detection of viral nucleic acids. The test is very versatile and many modifications and variations of PCR have been applied in all fields of molecular biology (Mullis & Faloona 1987). The principle of PCR is based on three successive reactions: (1) denaturing double-stranded DNA into single-stranded DNA at high temperature; (2) annealing (hybridizing) two specific primers (synthetic oligonucleotides) to each strand at 5′–3′ termini at a lower temperature; and (3) enzymatic extension (incorporation of nuleotides) to synthesize new complementary DNA strands. These three steps represent one cycle, and the number of DNA molecules double at the end of each cycle. In practice, 30–40 cycles of amplification are recommended.

PCR is the most sensitive method for detecting VZV DNA in clinical samples and has markedly improved the diagnosis of VZV infection (Dlugosh et al., 1991; Koropchak et al., 1991; LaRussa et al., 1992). Primers have been described which amplify different regions of the VZV genome (Mahalingam et al., 1993). VZV-PCR is the test of choice for diagnosing VZV encephalitis and/or other related central nervous system (CNS) infections (Puchhammer-Stockl et al., 1991; Echevarria et al., 1994; Ito et al., 1995; Cinque et al., 1997; Koskiniemi et al., 1997; Kleinschmidt-DeMasters et al., 1998).

The utility of PCR for amplifying DNA from CSF has been improved with the introduction of "herpes consensus primers," so-called "Stair Primers" (Coliman et al., 1996) by Argene Biosoft (North Massapequa, New York). These primers allow simultaneous amplification of six herpesviruses, CMV, EBV, HSV-1, HSV-2, herpes 6 and VZV, in a single tube with high efficiency. The resultant amplicon is analyzed by hybridization in a microtiter plate with the six different biotinylated oligonucleotide probes specific for each of the viruses. After hybridization, the unbound probes are removed by washing and the signal is detected by alkaline phosphatase with *p*-nitrophenyl diphosphate as substrate. The enzymatic activity is measured by a spectrophotometer and the result for each amplicon is compared to positive and negative controls in the test. The amount of DNA in the sample is directly proportional to the enzyme activity detected in the microtiter plate wells.

Although there appears to be only one strain of VZV, there are minor variations in the DNA and peptides of viruses isolated in different areas of the world (Straus

et al., 1983; Kinchington & Turse 1995; Takayama et al., 1997). The DNA of VZV isolated in the United States and in Japan (including the Oka vaccine virus) are somewhat different. This observation has been useful in the United States for distinguishing between vaccine and wild-type VZV. By the use of PCR and the treatment of the amplicon with restriction enzymes *Bgl*-I and *Pst*-I (the restriction fragment length polymorphisms (RFLP) method), it is possible to differentiate between the vaccine virus and the natural wild-type virus (Gelb et al., 1990; LaRussa et al., 1992, 1998). The RFLP pattern differences, however, are not due to attenuation of the virus but represent differences between circulating VZVs in the United States and Japan, where the Oka strain originated. The molecular basis for attenuation of VZV is not known; the possibility that VZV gC may play a role has not been confirmed but replication of gC negative mutants is restricted in skin (Kinchington et al., 1990; Cohen & Seidel, 1994; Moffat et al., 1998). Additionally, PCR has proven useful to identify whether rashes resembling varicella or zoster are caused by vaccine or wild-type VZV and to investigate cases of possible transmission of vaccine and wild-type VZV to susceptible contacts (Hughes et al., 1994; Feder et al.,1997; Salzman et al., 1997; LaRussa et al., 1997).

One of the most promising new approaches to nucleic acids amplification is in situ PCR. The assay combines the sensitivity and specificity of PCR and unique feature of in situ hybridization. This extraordinary sensitivity arises because the amplified viral DNA is concentrated in the cell, giving rise to a microscopically small area with an enhanced signal. Three approaches have emerged for in situ PCR. In the indirect procedure, amplification is accomplished by adding the standard PCR mixture directly to the fixed cells or tissue sections, and amplified in a thermocycler. The cell bound amplified DNA is detected by the conventional in situ hybridization method. In the direct amplification in a standard PCR reaction mixture, either the concentration of one of dNTPs is reduced and replaced with a modified dDNTP (e.g., biotin-dUTP), or the standard primers are replaced by labeled primers (e.g. biotin) amplified. The amplified DNA is then detected by alkaline phosphatase labeled avidin. The assay may have limited diagnostic value, but it is a valuable research tool for VZV latency and pathogenesis studies (Dueland et al., 1995; Mahaingam et al., 1995; Nahass et al. 1996; Kennedy et al., 1998).

Serological methods

Serologic assays have historically played an important role in clinical evaluation and epidemiological investigations of VZV infections. Although the emphasis has shifted in recent years to direct detection of VZV antigen and nucleic acid by hybridization and PCR, serological assays still remain powerful tools in the diagnosis of acute infection with varicella or zoster and to determine immune status in

order to control VZV transmission in hospitals and other institutions. A study was conducted by the National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention (CDC), to determine the VZV seroprevalence on over 25000 sera collected for the National Health and Nutrition Examination Survey (NHANES-III) among US residents aged 6 years and older from 1988 to 1994. By EIA, over 95% had detectable VZV antibody and were presumed to have already experienced VZV infection (Kilgore et al., 2000). Serodiagnosis is particularly useful for evaluating healthy adults who have no prior history of varicella; those who are varicella-susceptible need immunization. Serology can also be used to measure the presence of antibodies to VZV in CSF, which have been shown to be increased in patients with some neurological disorders due to VZV infection. Additionally, serological assays are important tools to assess the immunogenicity of the varicella vaccine (Arvin & Gershon, 1996).

Insensitive classical serological assays, such as complement fixation tests, have been replaced by more sensitive methods that use highly specific reagents and sensitive indicator systems, such as many variations of enzyme immunoassays (Forghani, 1992). The application of these new assays to automated technologies for large-scale testing has broadened their usefulness as clinical diagnostic aids and research tools for VZV epidemiologic studies. A wide variety of serological assays to measure VZV IgG, IgM and IgA antibodies have been described. A general overview of the basic principles of the more commonly used serological assays for determination of VZV antibody follows.

Serological assays for determination of IgG, IgM and IgA antibody

Solid-phase enzyme immunoassay (EIA)

The most commonly used assay for determination of VZV antibody is a solid-phase EIA. The EIA is simple to perform, can be adapted to automation, and is suitable for small- or large-scale testing. The assay can be easily modified by changing conjugates to detect VZV-specific IgA, IgG and IgM antibodies. In addition, for satisfactory sensitivity and specificity, the antigen used in EIA need not be extremely pure. Purified VZV antigen is necessary only when high sensitivity is desired. The purer the antigen, the higher the serum titer that can be demonstrated under the same assay conditions (Forghani & Schmidt, 1979).

In the EIA test, viral antigen and control antigen (e.g., human fetal diploid lung cells in which VZV has been propagated) are adsorbed to microtiter plate wells, and the unreacted binding sites are blocked with bovine serum albumin or casein, etc. This step is essential because it prevents the attachment of other immunoreactants into the later steps of the assay and reduces nonspecific binding. Next, dilutions of sera are added to the proper well. Incubation is followed by washing. The specific antibody bound to the antigen is detected by an enzyme (e.g., alkaline

phosphatase)-conjugated anti-human IgG, IgA or IgM ((Forghani et al., 1978; Forghani, 1986; Hacham et al., 1980; Gershon et al., 1981; Levy & Sarov, 1981; Sundqvist, 1982; Shehab & Brunell, 1983; Demmler et al., 1988). The quantity of enzyme which has been bound onto each well is measured by reaction with appropriate substrate (e.g., *p*-nitrophenylphosphate). The amount of VZV IgG antibody in serum is directly related to the enzyme activity detected in each microtiter plate well. In practice, the absorbances of each well are read with a dual wavelength automatic EIA reader and the absorbance values are calculated and recorded electronically.

In our laboratory, the EIA result reported to the clinician is the optical density index (ODI). The ODI is calculated in the following way: the absorbance value of the control antigen well is subtracted from the absorbance value of the VZV antigen well and divided by the cut-off value (defined as the mean absorbance plus 3 standard deviations (SD) of a large number of sera that have tested negative by anticomplement immunofluorescence assay (ACIF) and plaque-reduction virus neutralization test (PRNT)). An ODI of greater than or equal to 1.0 is considered positive for the presence of antibody and an ODI less than 1.0 is negative for antibody. For quality assurance, all tests are run with control sera including a high and low VZV-positive serum and a VZV-negative serum. A run is rejected if the SD value of one of the control wells is greater than 4.0 (Forghani, 1986; Kilgore et al., 2000).

For serodiagnosis of VZV infection, acute- and convalescent-phase sera must be assayed in the same test-run. In assays such as CF, PRNT and ACIF, a fourfold or greater increase in antibody titer between acute- and convalescent-phase serum is considered diagnostic of recent varicella infection. However, in EIAs, a simpler approach has been to use a single dilution for the serum samples and determine the ODI of acute- and convalescent-phase of each serum. A ratio is then calculated by dividing the ODI value of the acute serum into the ODI value of the convalescent serum. In our experience, for a large number of viruses including VZV, a ratio of >1.50 is significant for current or recent infection.

A number of commercial tests for measurement of VZV antibody by EIA are available and have been compared with each other and to standardized assays (Demmler et al., 1988; LaRussa et al., 1987). These comparison studies have shown that some commercial tests lack sensitivity and others lack specificity. Lack of sensitivity is more commonly encountered and, therefore, some individuals with immunity to varicella may be misidentified as susceptible. These methods often fail to detect antibodies in individuals who have been immunized with live attenuated varicella vaccine (Balfour et al., 1988). An EIA that uses glycoproteins (gps) of VZV for antigen (known as gp-ELISA) has been reported to be highly sensitive (Wasmuth & Miller, 1990), but positive antibody titers have been detected by this

method in some 2-year-old children prior to developing varicella, raising questions about the sensitivity and specificity of the assay. A comparison study of commercial kits from five manufacturers to our in-house EIA for determination of VZV IgG or IgM antibody in a panel of well characterized sera showed that the commercial assays often perform poorly (Forghani, unpublished data).

In our initial description of the development of EIA for several viruses including VZV, we reported that antigen requirements are central factors for determining the sensitivity and specificity of the assay (Forghani & Schmidt, 1979). In our experience, solubilization of VZV-infected cells with a nonionic detergent such as NP40 and deoxycholate in the presence of protease inhibitors such as EDTA and aprotinin results in the most satisfactory and stable viral antigens for EIA. A detailed method to prepare such VZV and control antigens from HFDL suitable for EIA has been described elsewhere (Forghani, 1986).

Antibody class capture assay

Determination and interpretation of class-specific antibodies to VZV is fraught with difficulties. Problems in the assay of IgM antibody can be attributed, in large part, to the presence of and competition by large amounts of IgG in test sera. Use of a separation method is time-consuming, costly, and requires ultracentrifugation, column chromatography, or absorption with *Staphylococcus aureus* protein A (Langone, 1982), or *Streptococcus* sp. protein G (Kronvall et al., 1979). These methods may result in the loss of specific IgM antibody and fail to remove some of subclass IgG antibody.

To overcome these difficulties, an alternative approach to the indirect EIA assay for determination of VZV IgA or IgM antibody is the antibody class-capture assay (Forghani et al., 1984). In this system, anti-human immunoglobulin class-specific antibody (e.g., μ-chain specific for IgM and α-chain specific for IgA) is first adsorbed onto wells of a microtiter plate, and the unreacted sites are blocked. Antibodies in the serum of appropriate class are bound by the capture phase, including VZV- specific antibodies if present. After a proper incubation time, the unbound antibodies are removed by washing, followed by another blocking step. Next, VZV and control antigen are added to the proper wells, followed by pooled mAbs to VZV proteins, enzyme-labeled anti-mouse γ globulin and substrate solution, with a washing step between each step. The plate is then read by an EIA-reader and the ODI is calculated as described above for IgG.

The assay detects VZV IgM antibody with high titers in most patients with acute varicella and in 50% of zoster patients. VZV-specific IgA antibodies detected in varicella or zoster are variable and are not reliable markers of active VZV infection (Levy & Sarov, 1981; Forghani et al., 1984).

Complement fixation test

The complement fixation test is a classical serological test for diagnosis of viral infection and still performed worldwide (Schmidt et al.; 1964, Forghani et al., 1978). Currently the test is performed in a microtiter plate format and is done in two stages. First, diluted serum and pretitrated viral antigen are mixed in the presence of pretitrated guinea pig complement. If the serum antibody and viral antigen react and form antigen–antibody complexes, the complement will bind to the complexes and will be depleted or "fixed". In the absence of specific antibody the complexes will not form, and the complement will remain free in the reaction mixture. In the second stage, sheep erythrocytes coated with anti-sheep erythrocyte antibody (hemolysin) are added to the reaction mixture. If complement remains from the first stage, the erythrocytes are lysed (hemolysis). Conversely, "fixation" of complement by specific antigen–antibody–complement complexes prevents hemolysis and the erythrocytes settle at the bottom of the microtiter well, giving a characteristic button. Antibody determined by the CF test tends to be more broadly reactive than antibody measured by other assays. All subclasses of IgG bind to complement, some more efficiently than others. In general, complement binds more efficiently to IgM than IgG. CF antibody rises slowly following primary chickenpox and declines sooner than antibody detected by other assays. Therefore, the CF test is less sensitive than other assays and should not be used for immune status.

Immune adherence hemagglutination assay (IAHA)

The IAHA is a variation of the CF test in which agglutination rather than lysis of erythrocytes is measured. In the IAHA, the antigen–antibody–complement complexes bind to C′3b receptors on primate erythrocytes (e.g., human O cells). Agglutination of the erythrocytes indicates that specific antibodies are present in the test serum. The IAHA is more sensitive, rapid, and especially less subject to interference from anti-complementary reactions than CF. Reagents for the IAHA are similar to those needed for CF test, but the assay may actually be too sensitive and produce false positive reactions (Gershon et al., 1976; Forghani et al., 1978; Wong et al., 1978; Yamada et al., 1979).

Fluorescent antibody membrane assay (FAMA)

FAMA is a form of indirect immunofluorescence antibody testing. The assay uses unfixed VZV-infected cells instead of acetone-fixed cells, which are traditionally used for other viruses (Williams et al., 1974). The sensitivity of the FAMA assay is comparable to neutralization, probably because live virus-infected cells are used, which preserves the conformational structure of VZV glycoprotein antigens on the surface of infected cells. This structure is probably altered by fixation, which leads to decreased sensitivity and specificity of antibody binding. The main disadvantage

of the assay is that it requires trypsinized infected cells, two transfers to a new vessel, results in the loss of cells during several washing steps, and requires working with live virus. To overcome most of these difficulties, we have adapted the assay to a microtiter plate format, using an inverted fluorescence microscope (e.g., AXIOVERT, Zeiss, Germany) for reading, which improves specificity and sensitivity (Forghani, unpublished data).

Briefly, to prepare antigen for the assay, cell monolayers of HFDL or other susceptible cells are prepared in a microtiter plate and the cell monolayers are infected with a pre-titrated cell-free virus to give 15–20 plaques 48–72 hours post-infection. For the test, the culture medium is removed, and serial dilutions of test serum starting at 1:2 are added to proper wells with a multichannel pipetter. After incubation the unbound antibodies are removed by washing and fluorescent labeled anti-human IgG is added to each well. Again, the unbound conjugate is removed and the microtiter plate is examined microscopically. The presence of green fluorescent plaques indicates the presence of specific VZV antibody in serum being tested. The assay may also be used to determine specific VZV IgM or IgA antibody, in which case a fluorescein labeled anti-human IgM or IgA conjugate is applied. A modified FAMA assay in which cells are briefly fixed in dilute glutaraldehyde using the Raji cell line has been described (Zaia & Oxman, 1977; Iltis et al., 1982).

Anti-complement immunofluorescence assay (ACIF)

The ACIF is a modification of IFA for detection of CF antibody and is based on the addition of complement during or after exposure of VZV- infected cells to a diluted serum sample. The bound complement is then detected with fluorescein conjugated anti-C´3. The ACIF avoids the nonspecific staining often associated with IFA staining of Fc receptors (Yao et al., 1993) on VZV-infected cells, a form of nonspecific binding of IgG. The ACIF reactions are not affected because nonspecifically bound IgG does not fix complement. Furthermore, the assay is more sensitive than IFA because of the amplifying effect of many C´3 molecules with a single IgG.

In the test, smears of VZV-infected and uninfected cells are reacted with dilutions of heat-inactivated test serum, diluted in buffer containing calcium and magnesium ions; unbound antibodies are washed off; pretitrated guinea pig complement is added to each well and incubated at 37°C; the unbound complement is washed off, then fluorescein-conjugated anti-guinea pig complement is added. After incubation the slides are washed again, mounted and examined by a fluorescence microscope. Studies in this laboratory have shown that the ACIF test yields results comparable to those of FAMA tests and is more convenient to perform (Gallo & Schmidt, 1981; Preissner et al., 1982). The assay may also be used to determine specific VZV IgM or IgA antibody, in which case a fluorescein-labeled anti-human IgM or IgA conjugate is applied.

Latex agglutination (LA) assay for detection of antibody

The latex agglutination assay is used for rapid detection of viral antigen or antibody. This test is based on agglutination of antigen-coated microspherical latex particles in the presence of specific viral antibody. Viral antigens are either bound by covalent linkage or adsorbed passively to the particles. The most widely used latex microparticles of nanometer size are made of polystyrene, polyacrylate, polyacrolein or polyacrylamide. In the test, antigen-coated latex particles are mixed with diluted serum on a microscope slide. The appearance of agglutination is read visually within 10–15 min (Gershon et al., 1994).

There is a high degree of correlation between the presence of antibodies to VZV measured either by FAMA or LA and protection from varicella after natural infection and after immunization (Gershon et al., 1984). One disadvantage of the LA assay is that in 1–5% of antibody-positive serum samples, there is a prozone effect that yields apparent negative results at lower serum dilutions. This problem can be avoided by retesting sera that are negative using higher dilutions. Although apparent false negative reactions can occur with the LA assay, false positive results are unusual (Landry & Ferguson, 1993).

Plaque reduction virus neutralization test

Virus neutralization assays are considered the gold standard of all serologic assays for VZV because they provide both a sensitive and specific correlate of host immunity against reinfection. In general, neutralizing antibodies persist well beyond the initial illness and correlate well with host protection. The principle of virus neutralization is based on the binding of antibodies to particular antigenic determinants (epitopes) on the surface proteins of the virus, which block attachment of the virus to susceptible cell receptors or prevent penetration. As a consequence the virus becomes noninfectious. Virus neutralization can be performed in different formats, but the plaque reduction neutralization test (PRNT) is the most sensitive for determining VZV-specific antibodies. Although PRNT can be used for diagnosing both acute varicella and zoster, its primary use has been when managing outbreaks and in vaccine efficacy trials. Difficulties in obtaining cell-free virus has hampered the use of the PRNT in diagnostic or investigational studies. However, the utility of VZV PRNT can be improved by the production of high-yield cell-free virus by sonication (Schmidt & Lennette,1976), the demonstration of enhancement of neutralization by the incorporation of complement in the test as described from this laboratory (Schmidt & Lennette, 1975; Forghani et al., 1982) and rapid visualization by immunoperoxidase staining (Gerna & Chambers, 1976).

In the test, a pretitrated number of plaque-forming units of cell-free virus are mixed with serial dilutions of serum and incubated at 37 °C. The mixture is transferred to cell monolayers in a microtiter plate for virus adsorption; the unadsorbed

virus is removed and the cultures are rinsed. The monolayers are then supplemented with fresh tissue culture medium. After 72 hours incubation, the culture medium is removed and the plates fixed with acetone. For visualization of plaques, pre-titrated pooled mAbs to VZV are added, unbound mAbs are removed by washing, and peroxidase-conjugated anti-mouse γ-globulin and 3-aminoethylcarbazole substrate solution is added. A reduction in the number of reddish-brown plaques by the test serum compared to positive virus controls indicates neutralization. The PRNT titer is expressed as the highest serum dilution that reduces the plaque count more than 50% (Amad et al., 1997).

Assays for antibodies to VZV proteins

Antibody responses to individual VZV proteins can be determined by radioimmune precipitation assay (RIPA) and immunoblotting (IB). These assays combine the ability of gel electrophoresis to separate complex viral antigens into individual polypeptide bands with the sensitivity of radiolabeled or enzyme indicator systems to detect a low level of VZV-specific antibody in a serum or CSF sample. Although protein-specific assays are ideal for determining a low level of specific antibodies, they are not particularly well suited for quantification of antibody. These assays are time-consuming and are analytical tools in research but may be applied for confirmation of screening antibody tests in certain clinical conditions.

The radioimmune precipitation assay (RIPA) is the most sensitive and potentially highly sensitive method for determination of antibody to individual VZV proteins. An important feature of RIPA is that by using different radioisotopes, different viral proteins such as glycoprotein, phosphoprotein and lipoprotein can be identified. In practice, VZV-infected and -uninfected cells are labeled with [^{35}S]-methionine or [^{14}C]-glucosamine. After proper labeling time the labeled cells are harvested, washed and solubilized. The unsolubilized component is removed by high-speed centrifugation and the solubilized component serves as a source of radiolabeled antigen. A sample of antigen is first allowed to react with diluted test serum and, if specific antibody is present, an antigen and antibody complex will form. The immune complexes are bound to *Staphylococcus aureus* protein-coated Sepharose beads by several washings to remove unbound reactants. The immune complexes are disrupted with reducing agent (e.g., 2-mercaptoethanol), the polypeptides resolved by 10% SDS-PAGE (sodium dodecyl sulfate-polyacylanide gel electrophoresis), then the gels are exposed to X-ray film for autoradiography and specific viral bands (polypeptides) are identified. The RIPA was used to differentiate antibody responses to individual VZV proteins in sera of varicella and zoster patients and the elderly. Most major VZV glycoproteins and nonglycosylated proteins were detected in all sera with no significant differences group. However,

the prominent proteins were gB, and the capsid proteins of 150 kDa and 32–36 kDa (Zweerik & Neff, 1981; Grose, 1983; Weigle & Grose 1984; Larkin et al., 1985; Vafai et al., 1988).

Antibody responses to individual VZV proteins can be determined by immunoblotting. Virus is partially purified on a sucrose density gradient, solubilized, and disrupted to individual polypeptides as a source of antigen. Control antigen from uninfected cells must be prepared in the same manner. The polypeptides are separated by SDS-PAGE under reducing conditions on a 10% gel and electroblotted onto a nitrocellulose membrane (Burnette, 1981). After blotting, the membrane is treated with blocking agents (e.g., casein), washed and cut into 3–4 mm strips. For testing, diluted sera are reacted with each strip, incubated, and the unbound antibody is removed by washing. The membrane is reacted with enzyme (e.g., peroxidase), labeled goat anti-human IgG. Unbound labeled antibody is removed by washing and followed by substrate solution. Specific color bands in the viral antigen strip are compared to those in the control antigen strip to indicate the presence of antibody. The assay was used to differentiate antibody responses to VZV proteins in sera of varicella and zoster patients. Small differences were observed, but did not allow reliable differentiation of the two types of infection (Palumbo et al., 1984; Harper et al., 1988; Harper & Grose, 1989; Dubey et al., 1989).

Interpretation, limitations and communication of laboratory results

Detection of viral antigens and nucleic acids

Rapid laboratory confirmation of VZV infections can be accomplished by detection of viral antigens by direct IFA and/or nucleic acid amplification by PCR. These assays can yield a definite diagnosis within 3–6 hours after receiving clinical samples in the laboratory, which may help the physicians in the management of patients, especially as several anti-VZV drugs are available for treatment. All herpesviruses, including VZV, establish and maintain latency, but VZV does not cause persistent active infection or asymptomatic shedding. Therefore, detection of virus or viral antigens in biopsy or autopsy samples indicates active VZV infection. Since virus isolation is time consuming and less sensitive than direct detection methods, PCR has been a valuable complementary test for diagnosis of VZV in CNS and other infections.

Even using our library of mAbs to various gene products of VZV against many VZV isolates, no serotypes of VZV have been identified. However, recently an isolate of VZV mutated at the ectodomain of gE and several mutants induced after treatment of AIDS patients with anti-VZV drugs have been described (Boivin et al., 1994; Santos et al., 1998). Molecular epidemiology by PCR is important since live varicella vaccine is licensed for general use in the United States and many other

countries. The RFLP pattern of VZV can identify whether rashes resembling varicella or herpes zoster are caused by vaccine or wild-type virus. The virus from individuals who have been previously vaccinated but develop a clinically significant localized or generalized rash should be typed by RFLP to determine whether it is a vaccine or wild-type strain of VZV (Gelb et al., 1990; LaRussa et al., 1998).

Serodiagnosis of VZV

Serological assays can be used to diagnose VZV infections. Quantitation of specific VZV antibody is required for determining the significant rise of an antibody level between an acute- and convalescent-phase of infection. Historically, the most common approach has been to serially dilute a serum and report an endpoint titer derived from an assay such as CF, PRNT or FAMA. By definition, a fourfold or greater rise in titer was considered evidence of current or recent infection. However, with newer methods like EIA, titration of antibody is labor intensive, not practical, and does not generate more information than single-dilution testing. A simpler approach has been to select a single-screening dilution and determine the ODI for the acute- and convalescent-phase of each serum and calculate the ratio. A ratio of 1.50 or greater between acute and convalescent ODI is considered a current or recent infection (de Savigny & Voller, 1980).

An important consideration for serodiagnosis of VZV infection is cross-reactive antibodies to other herpesviruses and nonspecific increases in titers due to immune activation (Schmidt, 1982; Shiraki et al., 1982; Cremer et al., 1984). DNA sequence analyses have shown that some homology exists within all hepesviruses, especially within glycoproteins of the alpha-herpesviruses (McGeoch et al., 1993). These similarities explain simultaneous increases in antibody titer to VZV and HSV observed in patients with HSV infections who have previously had varicella and vice versa.

In some cases, the determination of other classes of antibody such as IgM and IgA may help in diagnosis of VZV infection. However, these assays are difficult technically and should not be relied upon unless performed by laboratories that have experience with VZV serologic diagnosis. During acute varicella infection, IgM antibody usually appears within the first few day after the onset of symptoms and is at its highest concentration by 6–12 days. It gradually declines to an undetectable level over the next 3–4 weeks, with the resolution of the infection. However, detection of IgM antibody in the serum does not distinguish varicella from herpes zoster or varicella immunization. Most importantly, VZV IgM antibody is detectable in VZV immune individuals without clinical manifestations (Gershon et al., 1981; Sundqvist, 1982; Forghani et al., 1984; Schmidt & Arvin, 1986). This may be a specific antibody increase; however an important cause of false positivity is rheumatoid factor (RF).The RF is an antibody, usually of the IgM class (but can be

also of IgG or IgA class), with specificity for the Fc portion of IgG (Vejtorp, 1980). It is produced in certain autoimmune diseases and also in several viral infections, including VZV. Preferably therefore sera or CSFs for IgM testing should be absorbed to remove or reduce RF prior to testing (Forghani et al., 1984).

The usefulness of specific VZV serum IgA antibody detection as an adjunct to IgM and IgG testing has been explored. In general, IgA antibody persists much longer than IgM antibody. In our experience, VZV IgA antibodies detected in patients with varicella and herpes zoster were variable and could not be considered to be reliable markers for active VZV infection (Levy & Sarov, 1981; Forghani et al., 1984). For this reason, tests for VZV IgA antibody usually are not done routinely in the diagnostic laboratory but can be very helpful in certain clinical situations. Detection of VZV IgM and IgA antibody in cord blood or in the blood of infants in the immediate postnatal period suggests the diagnosis of congenital infection (Cuthbertson & Grose, 1988; Grose et al., 1989) Subclass distribution of IgG antibody to VZV also has been explored for determination of seroconversion and differentiation of varicella from zoster. Compared to other serological assays its diagnostic significance proved to be limited (Mathiesen et al., 1989; Echevarria et al., 1990).

Serologic tests of CSF have also been used for diagnosis of VZV infection. Presence of antibodies in CSF may indicate VZV infection in the CNS because of either damage to the blood–brain barrier and leakage of serum antibodies to CNS, or because of antibody production within the CNS (Forghani et al., 1980).To differentiate between the two conditions, determination of the CSF/serum ratio of albumin and the CSF/serum ratio of IgG and their indices have been very helpful (Tibbling et al., 1977; Tourtellotte & Booe, 1978; Reiber & Lang, 1991). Under normal conditions, the ratios of albumin and IgG remain fairly constant between CSF and serum. With blood–brain barrier damage, each ratio increases, but the quotient between the two ratios remains constant. In CNS infection, IgG and/or IgM synthesized within CNS and the increased IgG index may indicate local antibody production. With intact blood–brain barrier and in the absence of CSF antibodies to other viruses (e.g., HSV), a high level of CSF antibody to VZV is considered evidence of VZV infection in CNS (Andiman et al., 1982; Jemsek et al., 1983; Echevarria et al., 1987, 1990; Mathiesen et al., 1989; Mustonen et al., 1998). In our testing algorithm for suspected HSV or VZV infection, all CSFs are tested simultaneously for both viruses. A recent study has determined that the presence of VZV-specific antibody in CSF was valuable for diagnosis of VZV in CNS infection (Gilden et al., 1998).

Determination of immune status

The minimum immunity titer of antibody that correlates with immunity to varicella has not been determined for any commercially available VZV antibody test.

Serologic testing has become more important since development of varicella vaccine because it is useful to identify adults who need to be vaccinated and for control of spread of varicella in hospitals. The specificity and sensitivity of various VZV antibody assays varies considerably, and many commercial tests have not been systematically evaluated in clinical situations with regard to exposure to VZV and subsequent protection from illness. It is hoped that more sensitive and specific serologic tests for VZV may become commercially available. A history of past varicella is usually predictive of immunity (Ross et al., 1962; LaRussa et al., 1985)

The ideal serologic assay for determination of immunity is the virus neutralization assay, because it would clearly identify susceptible individuals to be immunized. However, this assay is extremely difficult to perform and available in very few research settings.

The presence of VZV antibodies measured by FAMA (titer $>$ 1:4) is highly correlative (96%) with protection from varicella after close exposure to VZV in healthy vaccinees or after natural infection (Gershon et al., 1994). The presence of VZV FAMA antibody in serum, however, does not predict immunity in all circumstances since occasional second attacks of varicella occur even in seropositive healthy individuals (Gershon et al., 1984; LaRussa et al., 1985).

The CF test is not adequately sensitive for measurement of immunity and sera with anticomplement reactions remain an insoluble problem. ACIF is currently used in our laboratory and appears to be an excellent means for confirmation of immune status. The LA test, which is rapid and easy to perform, has also been used for determination of immune status to varicella. In one study a high correlation between the presence of antibody measured by LA and protection was reported (Gershon et al., 1994). There was also a high degree of correlation of FAMA and LA.

EIAs are sensitive, specific, and more practical and well suited for large scale testing than other tests. Detection of VZV antibody by EIA is highly likely to indicate prior varicella. Although EIA results are quantifiable, specific EIA values (e.g., ODI) that should be used to define protection have not been determined (Forghani, 1992).

In immunocompromised patients, the presence of detectable VZV antibodies using any serologic method, even FAMA, correlates less well with clinical protection from varicella than in healthy persons. It is therefore preferable to base management of immunocompromised patients with a close exposure to varicella on historical information rather than on serologic testing (Gershon & Forghani, 1995).

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Part V

Treatment and Prevention

18

Treatment of varicella

Richard J. Whitley

This chapter emphasizes the controlled clinical trials that support the use of antiviral interventions for the treatment of varicella. Since varicella represents a primary infection, with varicella-zoster virus (VZV), the viral load is high and there is a documented viremic phase. While varicella is usually a mild and self-limiting infection in the normal host, life-threatening disease can occur in the immunocompromised host.

Therapeutic agents

Antiviral therapy was first shown to be efficacious for the management of varicella in the late 1970s and early 1980s using the nonspecific inhibitors of viral replication, vidarabine and interferon. Acyclovir, which is a second generation, specific inhibitor of VZV replication was then proven useful and has become the drug of choice. The prodrugs of acyclovir (valaciclovir) and penciclovir (famciclovir) have been licensed for the treatment of herpes zoster; but data are not yet available to prove the efficacy of these drugs in the management of varicella. Additionally, foscarnet, a pyrophosphate analog, is available for intravenous therapy of varicella infections in high-risk immunocompromised hosts who are presumed to have an infection caused by an acyclovir or penciclovir resistant virus.

Acyclovir and valaciclovir

Acyclovir has become the most widely prescribed and clinically effective antiviral drug available to date for the management of herpesvirus infection. Valaciclovir, the L-valine ester oral prodrug of acyclovir (Valtrex®), was developed to improve the oral bioavailability of acyclovir.

Chemistry, mechanism of action and antiviral activity

Acyclovir (9-{2-hydroxyethoxymethyl}guanine) is a synthetic acyclic purine nucleoside analog that is a selective inhibitor of herpes simplex virus (HSV types 1 and 2) and VZV replication (Elion et al., 1977; Schaeffer et al., 1978). Acyclovir is con-

verted by a virus-encoded thymidine kinase (TK) to its monophosphate derivative, an event that does not occur to any significant extent in uninfected cells (Fyfe et al., 1978). Subsequent di- and tri-phosphorylation is catalyzed by cellular enzymes, resulting in acyclovir-triphosphate concentrations 40 to 100 times higher in HSV- and VZV-infected than in uninfected cells. Acyclovir triphosphate inhibits viral DNA synthesis by competing with the deoxyguanosine triphosphate as a substrate for viral DNA polymerase (Derse et al., 1981). Because acyclovir triphosphate lacks the 3′ hydroxyl group required for DNA chain elongation, viral DNA synthesis is terminated. Viral DNA polymerase is tightly associated with the terminated DNA chain and is functionally inactivated (Furman et al., 1984). In addition, the viral polymerase has greater affinity for acyclovir triphosphate than does cellular DNA polymerase, resulting in little incorporation of acyclovir into cellular DNA. In vitro, acyclovir is most active against HSV-1 ($EC_{50} = 0.04\,\mu g/ml$), HSV-2 (0.10 μg/ml), and VZV (0.50 μg/ml) (Collins & Bauer, 1979).

Valaciclovir is cleaved to acyclovir by valine hydrolase which, is then metabolized in infected cells to the active triphosphate of acyclovir. Because it is metabolized to acyclovir, valaciclovir has the same in vitro spectrum of activity.

Absorption, distribution and elimination

Acyclovir is available in topical, oral, and intravenous preparations. In the treatment of varicella, physicians should only consider use of the medication in either oral (immune competent host only) or intravenous formulation (immunocompromised host). The oral formulations include a 200 mg capsule, a 800 mg tablet, and a suspension (200 mg/5 ml). Absorption of acyclovir after oral administration is slow and incomplete, with oral bioavailability of about 20% (deMiranda & Blum, 1983). After multidose oral administration of 200 mg or 800 mg of acyclovir, the mean steady-state plasma concentrations are about 0.57 and 1.57 μg/ml, respectively (Laskin, 1984). Much higher plasma acyclovir levels can be achieved with intravenous administration. Steady-state peak acyclovir concentrations following intravenous doses of 5 mg/kg or 10 mg/kg every 8 hours are about 9.9 and 20.0 μg/ml, respectively. Acyclovir penetrates most body tissues well, including the brain. The terminal plasma half-life is 2 to 3 hours in adults with normal renal function. Acyclovir is minimally metabolized and about 85% is excreted unchanged in the urine via renal tubular secretion and glomerular filtration. Acyclovir dosage adjustment is required in patients with impaired renal function. In patients with creatinine clearance (CrCl) of >50 ml/min, 100% of the recommended intravenous dose is given at 8 hour intervals. For a CrCl of 25–50 or 10–25 ml/min, the dosing interval is extended to 12 or 24 hours, respectively. If the CrCl is <10 ml/min, the standard intravenous dose is reduced by 50% and given every 24 hours. Recommendations for use in renal impairment appear in Table

Table 18.1 Dosage adjustment for intravenous acyclovir in patients with impaired renal function (Whitley 1998)

Creatinine clearance (ml/min/1.73 m^2)	Percentage of standard dose	Dosing interval (hours)
>50	100	8
25–50	100	12
10–25	100	24
0–10*	50	24

Note: * Administered after hemodialysis

18.1. For patients with severe renal failure (CrCl < 10 ml/min), the dose of oral acyclovir for VZV infections should be reduced to 800 mg every 24 hours. Acyclovir is readily removed by hemodialysis, but not by peritoneal dialysis.

Valaciclovir is only available as a tablet formulation. It is metabolized nearly completely to acyclovir within minutes after absorption. The subsequent oral bioavailability of acyclovir is about 65%. Notably, plasma levels of acyclovir achieved after 1 g of valaciclovir given orally, three times a day, are approximately equivalent to 5 mg/kg administered every 8 hours intravenously. Dosage adjustments are not required unless the CrCl is < 25 ml/min, in which case dosing frequency should be decreased to twice daily.

Foscarnet

Chemistry, mechanism of action and antiviral activity

Foscarnet, a pyrophosphate analog of phosphonoacetic acid (PAA), has potent in vitro and in vivo activity against herpesviruses. Unacceptable toxicity was demonstrated with PAA (disposition in bone), but foscarnet is less toxic. These drugs inhibit the DNA polymerase of all human herpesviruses by blocking the pyrophosphate binding site, thus inhibiting the formation of the 3'-5'-phosphodiester bond between primer and substrate and preventing chain elongation. Unlike acyclovir, which requires activation by a virus-specific TK, foscarnet acts directly on the virus DNA polymerase. Thymidine-kinase deficient, acyclovir-resistant herpesviruses remain sensitive to foscarnet. Foscarnet has been used successfully to treat severe infections due to acyclovir-resistant HSV-2 (Chatis et al., 1989) and VZV.

Absorption, distribution and elimination

Foscarnet is administered by the intravenous route because oral bioavailability is extremely poor. An intravenous infusion of 60 mg/kg every 8 hours results in

peak and trough plasma concentrations that are approximately 450–575 and 80–150 μm, respectively. The cerebrospinal fluid concentration of foscarnet is approximately two-thirds of the plasma level.

Renal excretion is the primary route of clearance of foscarnet with >80% of the dose appearing in the urine. Bone sequestration also occurs, resulting in complex plasma elimination.

Penciclovir and famciclovir

Chemistry, mechanism of action, and antiviral activity

Famciclovir is a new member of the guanine nucleoside family of Famvir®. It is the prodrug of penciclovir, which does not have significant oral bioavailability (less than 5%). In contrast, famciclovir provides excellent oral bioavailability (approximately 77%) and has a good therapeutic index for the treatment of both HSV and VZV infections (Fowles et al., 1992). Famciclovir is the diacetyl ester of 6-deoxypenciclovir. When administered orally, it is rapidly converted to penciclovir. The spectrum of activity of penciclovir and, therefore, famciclovir against human herpes viruses is similar to that of acyclovir (Sutton & Kern, 1993). Penciclovir is phosphorylated more efficiently than acyclovir in HSV and VZV infected cells. Host cell kinases phosphorylate both penciclovir and acyclovir to a small but comparable extent. The preferential metabolism in HSV and VZV infected cells is the major determinant of its antiviral activity. Penciclovir-triphosphate has, on average, a 10-fold longer intracellular half-life than acyclovir-triphosphate in HSV-1, HSV-2, and VZV infected cells after drug removal. Penciclovir-triphosphate is formed at concentrations sufficient to be an effective inhibitor of viral DNA polymerase, albeit at a lower K_i than that of acyclovir-triphosphate. In virus yield reduction assays, inhibition of VZV replication in MRC 5 cells occurs at concentrations between 3.0 and 5.1 μg/ml, values virtually identical to acyclovir. The majority of acyclovir resistant VZV clinical isolates are also cross-resistant to penciclovir; however, a few acyclovir-resistant strains have shown sensitivity to penciclovir.

Absorption, distribution and elimination

The major metabolic route of famciclovir is de-acetylation of one ester group as the prodrug crosses the duodenal barrier of the gastrointestinal tract. The drug is transported to the liver via the portal vein where the remaining ester group is removed and oxidation occurs, resulting in penciclovir, the active drug. Famciclovir is absorbed rapidly and extensively following oral administration. The first metabolite that appears in the plasma is almost entirely the de-acetylated compound with little or no parent drug detected. Pharmacokinetic parameters for penciclovir are linear over famciclovir oral dose ranges of 125–750 mg. Penciclovir

is eliminated rapidly and almost unchanged by active renal tubular secretion and glomerular filtration (Fowles et al., 1992). The $t_{1/2\ elimination}$ in healthy subjects is approximately 2 hours. Food appears to slow the rate of conversion of famciclovir to penciclovir, but has no effects on the ultimate availability of penciclovir (The International Valaciclovir HSV Study Group et al., 1993). Dosages used for therapy include 125, 250, 500, and 750 mg/dose, administered 1, 2, or 3 times per day.

Treatment of varicella

Immune competent host

Varicella, even in the immune competent host, can be associated with significant morbidity. Medical management involves preventing avoidable complications, especially secondary bacterial infection of the skin. These infections can be averted by meticulous care of the skin lesions and by measures to decrease pruritus. *Streptococcus pyogenes*, particularly group A β-hemolytic streptococci, and *Staphylococcus aureus* are the most common causes of secondary bacterial infection in patients with chickenpox. Pruritus can be significantly lessened with topical dressings of calamine lotion or the oral administration of Benadryl or Atarax at appropriate dosages. Furthermore, maintaining closely cropped fingernails will prevent excoriation of the skin and associated secondary infection. Administration of antipyretics should be approached with care in the child suffering from chickenpox because of the association between aspirin and its derivatives and the development of Reye's syndrome. Acetaminophen appears to be safe for children with varicella who are febrile.

Oral acyclovir therapy accelerates the events of cutaneous healing in the immunocompetent host but only when therapy is instituted within the first 24 hours. For children between 2 and 12 years of age, the total and mean number of lesions were significantly decreased in a large, placebo-controlled trial (Dunkle et al., 1991). Similar benefit was evident in adolescents and adults. The dosage of oral acyclovir is 20 mg/kg (up to 800 mg) either four (adolescents) or five (adults) times daily for 5–7 days.

The outcome of treatment of chickenpox in the immune competent child with oral acyclovir can be summarized by the following observations:

1. The maximum number of lesions is significantly decreased in acyclovir recipients; by approximately 75, based upon counting a maximum of 500 lesions.
2. The time to cessation of new lesion formations and time to appearance of maximum number of lesions were significantly shorter for acyclovir than placebo recipients. For the cessation of lesion formation, the mean was 2.7 days for the acyclovir group and 3.2 days for the placebo group.

Table 18.2 Efficacy of oral acyclovir in adult varicella (Wallace et al., 1992)

	Early group (<24 hours)			Late group (25–72 hours)		
	Acyclovir ($n=38$)	Placebo ($n=38$)	P value	Acyclovir ($n=36$)	Placebo ($n=36$)	P value
Time to maximum number of skin lesion (days)	1.5	2.1	0.002	1.3	1.2	0.86
Days of new lesion formation	2.7	3.3	0.03	3.0	2.3	0.03
Time to onset of cutaneous healing (days)	2.6	3.3	<0.001	2.4	2.3	0.79
Time to 100% crusting (days)	5.6	7.4	0.001	7.0	6.8	0.96
Maximum number of lesions	268	500	0.04	233	158	0.03

3. The acyclovir treated children healed faster than those receiving the placebo, as measured by days to 5% decrease in the maximum number of lesions (2.9 versus 4.1 days for acyclovir and placebo recipients, respectively).

For children ages 2 to 12 years, Dunkle et al. found that acyclovir was well tolerated and significantly accelerated resolution for all cutaneous events (Dunkle et al., 1991). Systemic symptoms (fever and mean constitutional illness scores) for treated compared to placebo recipients were also reduced. Approximately 20% of immune competent children with varicella who received the placebo continued to form new lesions 6 days after the onset of disease as compared to 3 days for the children who received acyclovir.

For adolescents between 13 and 18 years of age, Balfour et al., reported that acyclovir, administered at 800 mg four times daily for five days, significantly reduced the time to cessation of new lesion formation, days to maximum number of lesions, and maximum number of lesions, by about one day as compared to the placebo recipients. As with children and adults, evidence regarding the effect of acyclovir in otherwise healthy adolescents on the incidence of complications is inconclusive (Dunkle et al., 1991; Balfour et al., 1992; Wallace et al., 1992).

In the management of chickenpox in adults, intravenous acyclovir will shorten the course of uncomplicated varicella but parenteral antiviral therapy is expensive and is neither practical nor desirable (Al-Nakib et al., 1993). Oral acyclovir, on the other hand, is beneficial for management of varicella in the adult population. In a study of 148 naval recruits with uncomplicated varicella, oral acyclovir (800 mg, five times daily for seven days) administered within 24 hours of rash onset, reduced the duration of varicella and the severity of symptoms as compared to placebo. These benefits are summarized in Table 18.2. If therapy was administered after 24 hours, no appreciable treatment benefit was detected.

Because of marginal clinical benefit, as noted from the data above, the American

Table 18.3 Target populations to consider for treatment of varicella (Feldman 1993)

Target Groups	Specific high-risk underlying diseases
Individuals over 18 years of age	Congenital/neonatal infections
Pregnant women	Cardiopulmonary disease (cystic fibrosis, congenital heart disease)
Secondary or tertiary household exposures	
Children receiving steroid therapy (asthma)	Diabetes
All immunocompromised individuals*	Chronic or severe skin disorders (atopic dermatitis, pustular psoriasis, burns)
	Any significant chronic illness

Note: * Intravenous therapy only.

Academy of Pediatrics, through the Committee on Infectious Diseases, does not recommend the routine therapy of all immune competent children with chickenpox, in spite of highly significant statistical assessments. However, populations that could be considered for treatment are identified in Table 18.3. Assuredly, therapy of adolescents and adults as well as pregnant women is currently the standard of care in most communities in the United States.

Immunocompromised host

The data from three successful antiviral trials of varicella in the immunocompromised child are summarized in Table 18.4. The first successful antiviral chemotherapeutic for the management of chickenpox in the immunocompromised host was vidarabine (adenine arabinoside) (Whitley et al., 1982) However, this drug is no longer available in a parenteral formulation. Interferon-alpha was also beneficial (Arvin et al., 1982).

In placebo-controlled trials, treatment with intravenous acyclovir improved the outcome of varicella, as evidenced by a reduction of VZV pneumonitis from 45 to less than 5% (Prober et al., 1982; Nyerges et al., 1988). No significant toxicity was reported. In spite of the lack of large-scale controlled studies, the safety of acyclovir and its efficacy for other VZV infections has led to its preferential use in this disease. The dose is 500 mg/m^2/i.v. q. 8 h for 5–7 days. Oral therapy is *not* recommended at this time for varicella in the immunocompromised host.

Valaciclovir and famciclovir

No data are available to assess the newly available prodrugs of acyclovir and penciclovir for varicella in the normal or immunocompromised host. As a consequence,

Table 18.4 Therapy for chickenpox in immunocompromised patients[a]

Demographics	Vidarabine ($n=19$)	Leukocyte interferon ($n=23$)	ACV ($n=8$)	Placebo ($n=48$)
ALL or AML	12 (63%)	18 (78%)	6 (75%)	37 (77%)
Disease duration (days ± SE)	1.7 ± 0.8	1.61 ± 0.50	2.2 ± 1.6	1.94 ± 1.08
Healing, time to lesion cessation (days ± SE)	3.8 ± 1.1	3.8 ± 1.89	NR	5.45 ± 2.16
Visceral complications				
Postenrollment pneumonia	1 (5%)	3 (13%)	0	13 (27%)
Encephalitis	0	0	0	3 (6%)
Hepatitis	0	5 (22%)	0	9 (19%)
Overall	0	8 (35%)	0	25 (52%)
Mortality	0	2 (9%)	0	8 (17%)

Notes:

[a] Data from Takahashi et al. (1975); Balfour et al. (1983); Ashley et al. (1988).

ALL, acute lymphoblastic; AML, acute myelocytic leukemia; NR, not reported.

specific recommendations regarding their use in high-risk patient populations can not be made at this time. For therapy of disease in high-risk populations, intravenous acyclovir therapy should be considered the treatment of choice at the present time. However, it must be recognized that, at least for valaciclovir, significantly higher plasma levels are achieved than following the administration of oral acyclovir. Based upon this information, valaciclovir therapy should be efficacious. It is hoped that orally bioavailable prodrugs will be available in formulations for children, which will permit their evaluation in high-risk patient populations.

Resistant VZV infections

In children with human immunodeficiency virus (HIV) infection, varicella can be chronic in nature with the appearance of verrucous-like lesions. In this population, as well as in bone marrow transplant recipients, therapy has been associated with the development of resistance to all the drugs that have been used. Resistance to acyclovir usually occurs because of a deficiency in VZV-thymidine kinase function. However, VZV-TK with an altered substrate specificity for acyclovir as well as mutations in DNA polymerase have been identified in individuals with persistent

VZV infections.(Kost et al., 1993; Kimberlin et al., 1995) These isolates remain susceptible to foscarnet. As a consequence, foscarnet can be used in the treatment of children who have apparent acyclovir-resistant isolates.

Summary

Antiviral therapy is effective for varicella in both normal and immunocompromised hosts. Acyclovir has proved the most successful of the antiviral drugs used to date. While no clinical trials have evaluated valaciclovir or famciclovir for the management of chickenpox in either patient population, these drugs will probably be efficacious because of the high plasma levels that can be achieved. Recognizing that such clinical trials will be difficult to perform, it is reasonable to consider the use of these drugs for the management of chickenpox in adult patients who are otherwise normal hosts.

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19

Treatment of herpes zoster

Martin Dedicoat and Martin Wood

Introduction

Herpes zoster in the intact (otherwise immunocompetent) host is generally a short-duration inconvenience with local rash and pain the only problems. In certain cases the dermatome affected may be associated with an increased risk of complications (e.g. ocular problems with ophthalmic zoster or motor nerve paralysis in cervical or sacral involvement) and in the elderly there is a risk of chronic pain. On the other hand, in the immunocompromised host, specifically patients with profoundly depressed cell mediated immunity, the disease is not only more extensive and prolonged but there is also a risk of viraemia with widespread cutaneous and visceral dissemination. The aims of treatment in these two groups of patients are somewhat different.

In the intact host the aims are, firstly, to reduce the severity and duration of the acute symptoms by speeding the healing of the rash but, secondly, particularly in the elderly and others at high risk, to reduce the likelihood and duration of complications such as chronic neuralgia and ocular damage. These benefits are also of some importance in the immunocompromised patient but in these individuals the prime consideration is to prevent the morbidity and mortality associated with visceral dissemination of varicella-zoster virus (VZV).

Intact hosts

Drugs of choice

Since the manifestations of acute herpes zoster are caused by replication of VZV, inhibition of such replication is the most logical way of reducing disease severity. VZV is susceptible to several antiviral drugs and, at present, there are a number of orally active nucleoside analogs that are clinically effective in the treatment of herpes zoster in immunocompetent patients, provided they are started within 72 h of onset of the rash.

Acyclovir

Acyclovir is approximately 10-fold less active against varicella-zoster virus (VZV) than herpes simplex viruses (HSV): the mean 50% inhibitory concentration (IC_{50}) for VZV strains ranges from <1 to $>15\,\mu$mol, depending upon the viral strain and the tissue culture system used. Such concentrations of acyclovir can easily be achieved in the serum after intravenous administration of 5–10 mg/kg acyclovir. After oral administration, however, the poor bioavailability of acyclovir (ca. 15%) means that, even at a dosage of 800 mg every 4 h, peak serum concentrations of acyclovir may be below the IC_{50} for some strains of VZV. Acyclovir is phosphorylated by the VZV thymidine kinase (TK) and then by cellular enzymes to its active triphosphate form, so that it is specifically activated in VZV-infected cells.

Several randomized, placebo-controlled, double-blind trials showed the efficacy of intravenous acyclovir, at a dose of 5–10 mg/kg 8-hourly for 5 days, for the treatment of herpes zoster in otherwise intact hosts (Wood, 1995). Some of these studies reported significant improvements in the rate of skin healing and all showed a reduction in acute pain, providing acyclovir was started within 72 h of the onset of the rash or within 96 h of the onset of the first symptoms. However, some of the acyclovir recipients reported a return of their pain on cessation of treatment and there was no difference between the two groups in the incidence of pain one month or more after the rash had healed.

In otherwise normal individuals intravenous acyclovir is somewhat impracticable for treating herpes zoster. Hence, studies were undertaken with oral acyclovir, at a higher dosage than that used for HSV infections. Initially 5 days' therapy with oral acyclovir 400 mg 5 times daily was compared with intravenous acyclovir 5 mg/kg 8-hourly (Peterslund et al., 1985). Although there were no significant differences between the two groups with regard to duration of the rash or the pain, this study did not include a placebo group. Other, placebo-controlled trials of oral acyclovir at a dose of 400 mg 5 times a day produced no statistically significant differences in resolution of the rash or the acute pain (Crooks et al., 1991; Wood, 1995).

The failure of the 400 mg dose to produce significant improvements in herpes zoster was thought to relate to the poor oral bioavailability and inadequate serum concentrations of acyclovir. This, together with the apparent rebound of pain after 5-days' intravenous therapy, led to a series of randomized, placebo-controlled trials using an acyclovir dose of 800 mg 5 times daily for 7 or 10 days for herpes zoster of less than 72 h rash duration in immunocompetent patients (Straus, 1993; Whitley & Straus, 1993; Wood, 1995). These studies showed that oral acyclovir reduced the duration of viral shedding and new lesion formation and significantly accelerated healing of the rash. In the largest of these studies (Wood et al., 1988), 7 days of acyclovir was associated with a 36 h reduction in the time to full crusting of the skin

lesions. There was also a smaller proportion of patients with cutaneous dissemination of lesions. Another large study from New Zealand (Morton & Thomson, 1989) showed acyclovir reduced the mean time to full healing of the rash by 6 days compared with placebo. Pain was assessed somewhat differently in the various studies but all showed some improvement in pain during the acute phase. On the other hand, the individual studies produced conflicting data in respect of the efficacy of acyclovir on the incidence of postherpetic neuralgia (PHN). The largest study, containing only patients aged over 60 years, found no reduction in the incidence of PHN (McKendrick et al., 1989) but trials reported from the USA (Huff et al., 1988) and New Zealand (Morton & Thomson, 1989) each showed a significant reduction in the incidence of PHN at 3 months in the acyclovir groups.

Since separate measurement of the duration of the acute pain accompanying herpes zoster and the pain persisting beyond rash healing is both clinically meaningless and potentially statistically biased (Wood & the Herpes Zoster Clinical Trials Consensus Group, 1995) the most appropriate and meaningful way to assess the pain associated with herpes zoster may be analysis of the duration of pain measured as a continuum. Recently, therefore, the primary data from the placebo-controlled trials of oral acyclovir 800 mg 5 times daily have been combined in a meta-analysis with the duration of the pain analysed in terms of the continuum of zoster-associated pain (ZAP) (Wood et al., 1996). This analysis has confirmed that the licensed dose of acyclovir does reduce the duration of pain caused by herpes zoster, however the pain is defined or assessed.

A further meta-analysis of the placebo-controlled trials, comparing the efficacy of early (started $<$ 48 h after the onset of rash) versus late (not started until 48–72 h after the onset of rash) acyclovir in immunocompetent patients with cutaneous herpes zoster has recently been reported (Wood et al., 1998). Median times to complete resolution of zoster-associated pain were statistically significantly reduced in the acyclovir recipients irrespective of when therapy was started within the first three days of the rash.

Extending acyclovir therapy beyond 7 days confers no additional benefit (Wood et al., 1994).

Penciclovir/famciclovir

Penciclovir is similar in structure, metabolism and mode of action to acyclovir, except that, since it has a hydroxymethyl group at the 3′ position of the acyclic side chain, it does not act as an obligate DNA chain terminator (Bacon, 1995). The in vitro inhibitory effect of penciclovir on VZV is virtually identical to that of acyclovir but the intracellular half-life of penciclovir triphosphate (the active drug) is much longer than the half-life of acyclovir triphosphate, suggesting the potential for once- or twice-daily administration. The oral bioavailability of penciclovir is,

however, even worse than that of acyclovir, and an oral preparation is unavailable. Famciclovir is the diacetyl-6-deoxy analogue of penciclovir. It is well absorbed and after oral administration is rapidly and virtually completely converted to penciclovir by deacetylation and oxidation within the gastrointestinal tract and liver (Gill & Wood, 1996).

Trials have indicated that famciclovir is broadly equivalent to oral acyclovir in the treatment of herpes zoster in immunocompetent patients. A placebo-controlled trial showed that famciclovir (500mg or 750 mg every 8 h) significantly reduced the duration of viral shedding and accelerated rash healing, particularly in patients recruited within 48 h of rash onset (Tyring et al., 1995). The effects on the acute pain were less convincing since, when all patients were considered, loss of pain was significantly faster than placebo for the 500 mg dose but not for the 750 mg dose. Another study compared 7 days' therapy with famciclovir, given 8-hourly at one of three different dosages (250 mg, 500 mg or 750 mg), with acyclovir (800 mg 5 times daily) in immunocompetent adults with acute herpes zoster. Similar rates of healing of rash, duration of pain during rash healing and duration of viral shedding were obtained (Degreef & Famciclovir Herpes Zoster Clinical Study Group, 1994). The data also revealed that, among patients treated within 48 h of the onset of rash, there was a significantly faster resolution of ZAP in the groups who received famciclovir (at any dose) compared with the acyclovir recipients.

Various dosages of famciclovir are licensed for the treatment of herpes zoster in different countries. The doses used in the USA (500 mg) and most of Europe (250 mg) given every 8 h for 7 days, have been assessed in trials with at least 6-month follow-up and seem at least as efficacious as oral acyclovir 800 mg 5 times daily for 7 days in reducing the burden of pain following herpes zoster. Other licensed doses (750 mg once daily or 500 mg every 12 h) have not, however, been assessed for their effects upon anything other than the acute disease.

Valaciclovir

Valaciclovir is the L-valine ester of acyclovir. It is administered orally and after ingestion is converted rapidly and almost completely to acyclovir by a hydrolase enzyme within the gastrointestinal tract and liver. Its oral bioavailability is three to five times that of acyclovir, producing serum concentrations of acyclovir after an oral dose of 1 g valaciclovir that approximate to those achieved after an intravenous dose of 5 mg/kg acyclovir (Weller et al., 1993).

There are no placebo-controlled trials of valaciclovir for the treatment of herpes zoster but a randomized, double-blind comparison with acyclovir has been performed in immunocompetent adults aged 50 or over (Beutner et al., 1995). This trial included 1141 patients with herpes zoster and compared the efficacy and safety of valaciclovir, 1000 mg 8 hourly for 7 or 14 days, with that of acyclovir 800 mg

5 times daily for 7 days. The intention-to-treat analysis showed that there were no differences in the rash parameters between the three groups but valaciclovir (for 7 or 14 days) significantly accelerated the resolution of ZAP compared with acyclovir. However, the incidence of pain at various time points after healing of the rash was not significantly reduced and pain intensity and quality of life measures were not different between the groups. A further analysis of these data has shown that the measurable benefit from valaciclovir compared with acyclovir was maintained whether the treatment was started within 48 h of the onset of their herpes zoster rash or between 48 and 72 h after rash onset (Wood et al., 1998).

In conclusion, the currently available data indicate that oral acyclovir, famciclovir and valaciclovir each provide similar clinical efficacy for the treatment of herpes zoster in the intact host and a choice between them may depend upon cost and convenience criteria (Tables 19.1, 19.2) (Smith & Roberts, 1998).

Other therapies

Corticosteroids

The use of corticosteroids in the treatment of acute herpes zoster is based on the premise that the pain experienced by patients primarily results from inflammation and necrosis of neurons and that reducing this with corticosteroids would reduce pain. Several older reports of uncontrolled studies appeared to confirm this hypothesis although there were equally compelling data from small studies showing no effect of steroids on PHN (Gill & Wood, 1994).

The effect of steroids in conjunction with acyclovir in preventing or reducing the pain of herpes zoster has now been examined in several controlled studies. The first of these studies compared prednisolone ($n=41$) with placebo ($n=37$), both groups also taking acyclovir in standard dosage. Although prednisolone recipients had less pain during the acute phase of the illness, the incidence of PHN was the same in both groups (Esmann et al., 1987). A second larger study ($n=400$) of normal individuals over 18 years of age with herpes zoster, also found that the addition of prednisolone to acyclovir conferred a slight benefit in reducing the incidence and severity of the pain during acute herpes zoster; again there was no additional benefit for long-term zoster-associated pain (Wood et al., 1994). Neither the time to first loss of pain nor the time to complete cessation of pain was different in the groups given prednisolone. Patients who received prednisolone, however, had more adverse events.

A more recent trial of acyclovir versus placebo (each with or without prednisolone) employed quality of life endpoints and suggested that corticosteroids had a quality of life benefit in the acute treatment of herpes zoster in otherwise healthy adults over the age of 50 years (Whitley et al., 1996). Pain resolved more quickly in those given acyclovir and prednisolone compared with placebo recipients and the

Table 19.1 Comparison of acyclovir, valaciclovir and famciclovir in the treatment of herpes zoster in immunocompetent adults

Parameter	Acyclovir	Valaciclovir	Famciclovir
Dosage	800 mg 5 times daily for 7 days	1000 mg 8-hourly for 7 days	250 mg[a] or 500 mg[b] 8-hourly[c] for 7 days
Rash healing	Shorter than placebo	Equivalent to acyclovir	Better than placebo. Equivalent to acyclovir
Duration of acute pain	Shorter than placebo in some studies	Not reported	Better than placebo (500 mg doses – 250 dose not studied). Equivalent to acyclovir
Incidence of postherpetic neuralgia (PHN)	Lower than placebo in some studies	Not reported	Lower than placebo (500 mg doses – 250 mg dose not studied)
Duration of zoster-associated pain	Shorter than placebo in meta-analysis	Shorter than acyclovir	Shorter than acyclovir (500 mg dose and for 250 mg dose in subset treated within 48 h of rash onset)
Incidence of ocular complications of ophthalmic zoster	Lower than placebo	Not studied[d]	Not studied
Cost of 7 day course in UK[e]	£96.57	£98.50	£118.08

Notes:

Unless otherwise stated data are from Huff et al., 1988; Wood et al., 1988, 1996; Cobo et al., 1986; Morton & Thomson, 1989; Harding & Porter, 1991; Beutner et al., 1995; Tyring et al., 1993; Degreef & Famciclovir Herpes Zoster Clinical Study Group, 1994; Dworkin et al., 1998.

[a] Licensed dose in UK and parts of Europe.

[b] Licensed dose in USA.

[c] Other doses are licensed in some parts of the world but have not been studied for effects beyond the first month of illness.

[d] Likely to be similar to acyclovir since drug is completely converted to acyclovir after oral administration.

[e] September 1998 (British National Formulary).

Table 19.2 Recommendations for treatment of herpes zoster in immunocompetent hosts

Type of infection	Suggested treatment	Duration of treatment
Uncomplicated herpes zoster	Oral acyclovir 800 mg 5 times daily	7 days
	or famciclovir 500 mg 8-hourly	7 days
	or valaciclovir 1 g 8-hourly	7 days
Ophthalmic zoster[a]	Oral acyclovir 800 mg 5 times daily	7–10 days
	or valaciclovir 1 g 8-hourly	7–10 days
Complicated herpes zoster, e.g., encephalitis, pneumonitis	iv acyclovir 10 mg/kg 8-hourly (500 mg/m^2 in children)	5–7 days (at least 3 days after the last new lesion)
Acute retinal necrosis due to VZV	Intravenous acyclovir 10 mg/kg 8-hourly	7–10 days
	followed by oral acyclovir 800 mg 8-hourly	3 months

Note: [a] All patients with ophthalmic zoster should be referred to an ophthalmologist.

group given combination therapy also had a quicker resumption of normal activity and uninterrupted sleep. At 6 months, however, the proportion of patients who were still suffering from postherpetic neuralgia was independent of the treatment given in the acute phase.

On the basis of this evidence steroids can not be recommended for routine use in herpes zoster but it may be appropriate to consider the use of prednisolone in combination with antiviral therapy for those individuals over the age of 50 years in whom corticosteroids are not contraindicated, i.e. those without hypertension, osteoporosis, diabetes or a history of peptic ulceration. Such an approach has been endorsed by the Infectious Diseases Society of America and some other groups.

Symptomatic therapy

The pain in acute herpes zoster may be severe. Aggressive control of pain during this phase is important, both to relieve the patient's symptoms and also to minimize the potential for chronic pain. Chronic post-herpetic pain is believed to be at least partially related to the degree of nociceptive barrage that reaches the spinal cord neurons at the time of the acute illness. Certainly investigations have shown that the likelihood of chronic pain is related to the severity of the acute pain (Whitley et al., 1999). The choice of analgesic depends upon the patient's pain severity and the presence of other conditions. In some patients, simple analgesics such as paracetamol (acetaminophen) are adequate, but others will need stronger analgesics such as codeine or opioids. Nonsteroidal anti-inflammatory drugs may also be used, although there is no evidence that they are better than straightforward analgesics.

Nerve blocks

Anecdotal reports of benefits from somatic nerve blocks in herpes zoster have not been confirmed by larger studies (Kost & Straus, 1996). Sympathetic blockade by means of ipsilateral ganglion block with local anaesthetic has been shown to rapidly (but only transiently) alleviate the pain associated with herpes zoster (Kost & Straus, 1996) but these benefits need to be weighed against potential complications such as transient recurrent laryngeal nerve palsy, which occurred in 40% of those treated in one study (Harding et al., 1986).

Antidepressants

Established PHN can be helped by the use of tricyclic antidepressants (for their analgesic rather than antidepressant activities). The drugs may work as a result of blocking the re-uptake of monoamine neurotransmitters released by descending axons from the brainstem. Since the benefits of tricyclic agents in patients with established PHN appear to be greatest when commenced early, there may be a benefit from their use during the acute illness, in an attempt to prevent the pathophysiological mechanisms that lead to the chronic pain. This hypothesis has been apparently successfully tested in a small controlled trial of amitriptyline use in acute herpes zoster (Bowsher, 1997) but further studies are needed to confirm these results.

Special situations

Ophthalmic zoster

Oral acyclovir has a significant benefit in ophthalmic zoster. If started within one week of the onset of the rash and given for 7 or 10 days it is associated with a reduction in the development of dendritic and stromal keratitis, anterior uveitis and episcleritis (Palestine, 1995). Topical use of acyclovir ophthalmic ointment is often also administered but is of little proven value in VZV keratitis. Topical corticosteroids (under the supervision of an ophthalmologist) are helpful in speeding the resolution of keratitis if used in conjunction with oral acyclovir.

Encephalitis

There are no controlled clinical trials but numerous case reports suggest that encephalitis associated with herpes zoster responds well to intravenous acyclovir (10 mg/kg 8-hourly for 14–21 days) and a review of these reports indicated a trend toward a lower mortality (Peterslund, 1988).

Acute retinal necrosis

There have been no randomized trials of therapy in acute retinal necrosis (ARN) but intravenous acyclovir treatment (10 mg/kg 8-hourly) for 7 to 10 days is

Table 19.3 Treatment recommendations for herpes zoster in the immunocompromised host

Type of infection	Suggested treatment	Duration of treatment
Localized herpes zoster in the severely immunocompromised or disseminated herpes zoster	Acyclovir i.v. 10 mg/kg 8-hourly (500 mg/m^2 for children)	5–10 days (at least 3 days without new lesions)
Localized herpes zoster in mildly immunocompromised patients	As above or oral acyclovir 800 mg 5 times daily or valaciclovir 1 g 8-hourly or famciclovir 500 mg 8-hourly	7 days (at least 3 days without new lesions)
Acyclovir-resistant VZV	Foscarnet 200 mg/kg per day i.v.	10 days

reported to speed the resolution of retinal lesions and decrease the risk of disease in the other eye. It does not, however, reduce the risk of retinal detachment or long term ocular complications (Palay et al., 1991). Initial intravenous treatment is usually followed by prolonged oral acyclovir therapy (800 mg 5 times daily for 3 months). Oral famciclovir for 3 months has also been reported to have been successful in treating a case of acute retinal necrosis that had failed to respond to acyclovir (Figueroa et al., 1997).

Immunocompromised hosts

Drug of choice

Acyclovir is the drug now consistently used for the highest risk patients with localized or disseminated herpes zoster. Four studies, one placebo-controlled and three comparative studies with vidarabine, have shown that progression of disease is reduced by intravenous acyclovir. The initial efficacy study of intravenous acyclovir (500 mg/m^2 8-hourly for 7 days) for the treatment of localized herpes zoster in immunocompromised patients was placebo-controlled (Balfour, Jr. et al., 1983). Patients were enrolled into the trial up to 45 days after the onset of symptoms as long as they still had new lesions forming. The study showed no significant effect on the healing of the rash or the resolution of acute pain although further potentially life-threatening cutaneous and visceral dissemination were prevented.

The studies comparing intravenous acyclovir with vidarabine in immunocompromised patients have shown that acyclovir not only produced more rapid resolution of the rash and less dissemination of disease but also was associated with far fewer side effects than vidarabine (Lekstrom-Himes 8 Straus, 1995). Intravenous acyclovir is now the treatment of choice for VZV infection, occurring within the 9 to 12 months following bone-marrow transplantation (BMT) and later

if the patient is still receiving immunosuppressive therapy for graft-versus-host-disease. There are no placebo-controlled clinical trials but anecdotal reports suggest that oral acyclovir, famciclovir and valaciclovir are effective therapy for herpes zoster in many solid-organ transplant recipients and for herpes zoster 12 months or more after BMT.

No studies of therapy have been performed specifically addressing therapy for herpes zoster in HIV-infected individuals but, by extrapolation from studies in other immunocompromised patients, intravenous acyclovir is generally given for the more severe cutaneous cases or those with visceral involvement. For less severely immunocompromised HIV-infected patients with herpes zoster, oral acyclovir, valaciclovir or famciclovir (in standard doses used for intact hosts – see above – but for 10–14 days) are usually adequate.

Other drugs

Vidarabine

Early trials showed that intravenous vidarabine was beneficial in immunosuppressed patients with herpes zoster but the studies described above showed acyclovir to be as effective but less toxic and easier to administer (Lekstrom-Himes & Straus, 1995).

Interferon

Another intervention that deserves brief mention is interferon-α. When given to immunocompromised patients with herpes zoster either human leukocyte interferon-α or recombinant interferon-2α reduced viral dissemination (Merigan et al., 1978; Winston et al., 1988). The effects on the duration of new lesions, rash healing or pain were not significant and side effects of interferon were common.

Sorivudine

Sorivudine or BVaraU (1-β-D-arabinofuranosyl-5-(2-bromovinyl)-uracil), a substituted pyrimidine nucleoside analog, was developed in Japan. It is an extremely potent inhibitor of VZV DNA polymerase and, therefore, of VZV replication: the IC_{50} is several thousand-fold lower than acyclovir. It is orally bioavailable and has a very long half-life, allowing once-daily administration. Unlike acyclovir, viral enzymes are responsible for the first two phosphorylation steps to sorivudine diphosphate. Open studies in Japan showed that higher doses of sorivudine (150 mg/day) administered to patients with haematological malignancies and herpes zoster produced better clinical responses than a 30 mg dose and were associated with few side effects in the trial setting (Hiraoka et al., 1991).

More recently, sorivudine has been compared with acyclovir for treatment of acute localized herpes zoster in HIV infected adults (Bodsworth et al., 1997; Gnann,

Jr. et al., 1998). In both studies, patients randomized to 7 or 10 days of 40 mg of sorivudine daily within 72 h of the onset of their rash had a significantly shorter median period of new vesicle formation than those given acyclovir 800 mg 5 times daily for 7 days. Fewer patients given sorivudine in one study also experienced herpes zoster recurrences but there were no differences in the incidence, severity, or duration of pain between the groups in either trial.

The major drawback of sorivudine is that one of its metabolites, bromovinyl uracil, interacts with the metabolism of other pyrimidine analogs such as 5-fluorouracil (5-FU) and leads to increased toxicity: there were at least 18 deaths in Japan secondary to myelotoxicity following co-administration of sorivudine and 5-FU. This interaction led to the drug being withdrawn from licensure in Japan and further development halted in many countries.

Special situations

ACV-resistant virus

Varicella-zoster virus can become resistant to nucleoside analogs by one of two means: reduced production of or changes in the enzyme thymidine kinase (TK), which is responsible for the intracellular monophosphylation of the two compounds; or by mutation of the DNA polymerase gene so that the triphosphate derivatives are not incorporated into the replicating viral DNA. Strains of VZV that are resistant to acyclovir as a result of TK gene mutation will usually be resistant to penciclovir, ganciclovir and sorivudine.

Acyclovir-resistant VZV as a clinical problem has only been encountered in HIV infected patients with chronic VZV infections for prolonged periods of time (Balfour, Jr. et al., 1994; Field & Biron, 1994). A phenotypic and genotypic analysis of 11 acyclovir-resistant VZV strains recovered from HIV infected individuals with chronic VZV lesions revealed that 10 of the 11 isolates had TK gene mutations (Boivin et al., 1994). All the isolates remained sensitive to foscarnet and treatment with intravenous foscarnet (40 mg /kg/day in divided doses, adjusted according to renal function) is recommended for such strains.

Foscarnet (trisodium phosphonoformate) forms complexes with viral DNA polymerase and prevents extension of the viral DNA chain. It does not require TK since it does not need to be phosphorylated to become active. Foscarnet has been shown to be effective treatment for acyclovir-resistant VZV in HIV-infected patients. In a recent review of 18 HIV-infected patients with chronic skin lesions caused by acyclovir-resistant VZV, 13 patients received intravenous foscarnet 200 mg/kg body weight/day (Breton et al., 1998). Ten patients (77%) experienced initial complete healing of their lesions, although herpes zoster relapsed after a mean of 110 days in five of these ten patients.

Foscarnet resistance in VZV has been described (Visse et al., 1998). In an HIV-

infected patient with VZV infection that was clinically unresponsive to foscarnet, two point mutations were found in the VZV DNA polymerase gene.

Cidofovir, a nucleotide analog that is also already phosphorylated, may also be of potential use for infections caused by foscarnet and nucleoside-resistant strains of VZV (De Clercq, 1993).

Progressive outer retinal necrosis in AIDS

Peripheral outer retinal necrosis in AIDS has a very poor prognosis for recovery of useful vision. Monotherapy with acyclovir or ganciclovir has little effect, although some success has been claimed for a combination of ganciclovir and foscarnet (Galindez et al., 1996).

Prophylaxis of herpes zoster in immunocompromised patients

Prolonged prophylaxis with acyclovir has been shown to prevent herpes zoster in allogeneic BMT recipients in two placebo-controlled trials: intravenous acyclovir (250 mg/m^2 t.d.s., starting 5 days before the transplant) for 5 weeks and followed by long-term (6 months) oral acyclovir (400 mg t.d.s.) prevented herpes zoster in allogeneic BMT patients (Lundgren et al., 1985) and similar results were obtained with higher doses of acyclovir, primarily given to prevent CMV disease (Prentice et al., 1994). Herpes zoster was prevented during the period of prophylaxis but once the acyclovir was stopped, herpes zoster frequently occurred. An earlier study (Selby et al., 1989), however, showed no overall reduction in herpes zoster in allogeneic BMT recipients given acyclovir for six months. In liver-transplant recipients given either intravenous ganciclovir or intravenous acyclovir followed by high dose oral acyclovir until day 100, there were no cases of VZV infection (Winston et al., 1995). High dose oral acyclovir (800 mg four times daily) given for 24 weeks after liver transplantation, as one arm of a trial comparing this with ganciclovir pre-emptive therapy for CMV disease, also prevented herpes zoster in the small number of patients studied, although there was only one case in the group given pre-emptive ganciclovir (Singh et al., 1994).

Given the ease with which reactivation of VZV can generally be treated, the use of long term prophylaxis specifically against VZV in immunocompromised patients seems unwarranted.

Secondary prophylaxis in HIV/AIDS

Patients with HIV who develop recurrent or progressive herpes zoster should be treated with intravenous acyclovir followed by life-long oral acyclovir prophylaxis (Wood, 1996). The efficacy of this in preventing cutaneous recurrences has been established but the advent of highly active antiretroviral therapy for HIV may make life-long prophylaxis unnecessary. It should also be noted that cases of VZV

neurological disease have been reported in HIV-infected patients receiving acyclovir for prophylaxis against herpes simplex, and use of an appropriate dosage of acyclovir to achieve anti-VZV activity is stressed.

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20

Management of postherpetic pain

Kathryn J. Elliott

Both varicella and zoster may result in an array of neurological complications. The most common neurological complication of zoster is the development of a neuropathic pain syndrome either acutely or chronically, as postherpetic neuralgia (PHN). Many approaches have been proposed to treat the pain of acute zoster, to proactively prevent the development of PHN, and to treat PHN. This chapter reviews the options for analgesia and prevention of zoster-associated pain.

Neuropathic pain and other neurologic complications of herpes zoster

Viral invasion of nervous system structures, in skin, peripheral nerve, dorsal root ganglion, and more rostral CNS structures, probably underlies the development of the VZV neuropathic pain syndromes (see Table 20.1). Cerebrospinal fluid (CSF) from 40–50% of patients with uncomplicated herpes zoster may manifest pleocytosis (Applebaum et al., 1962; Gold, 1966). Direct meningeal spread of virus may explain the more uncommon neurological complications of zoster including multiple cranial neuropathies, polyneuropathy, meningitis, meningoencephalitis/meningoradicultis, encephalitis, and myelitis (Elliott, 1994b). The syndrome of zoster sine herpete, or zoster without rash, and associated development of persistent neuropathic pain with this phenomenon in some individuals, further confirms the capability of viral invasion at multiple levels of the neural axis (Elliott, 1994b).

Pain is the most common symptom of acute zoster and may precede the skin eruption by hours, days or weeks, and may rarely be the only presentation (Gilden et al., 1991, 1992). Spontaneous symptoms of both acute zoster and PHN include aching pain or a more superficial burning pain in the dermatomal distribution of the affected skin, and provoked symptoms of severe discomfort that accompany the syndrome of neuropathic pain. The most common neuropathic symptom is characteristically provoked allodynia, whereby patients describe an unbearable slowly escalating painful sensation to a normally non-noxious stimulus such as a light brush of the skin in the region of the affected dermatome. This symptom is

Table 20.1 Neurological complications of herpes zoster

Acute herpetic neuralgia
Postherpetic neuralgia (PHN)
Peripheral motor neuropathy
Cranial nerve palsies
Myelitis
Encephalitis
Thrombotic cerebral vasculopathy
Acute ascending polyradiculitis
Aseptic meningitis
Zoster sine herpete

seen in both acute zoster and PHN and suggests a sensitized neuropathic pain state. Additional symptoms include spontaneous tingling or paresthesias, unpleasant tingling or dysesthesias and exaggerated pain response to normal pain sensation or hyperalgesia.

Prediction of how many individuals who develop acute zoster will be left with sustained PHN is difficult because the nature of zoster pain is both variable and improves over time. Additionally, there is no consensus about the definition of acute zoster pain and PHN. Most patients experience PHN as the continuation of acute zoster pain after the rash heals. Definitions of PHN include time frames of one month after onset of rash or varying times after the rash heals, such as 6 weeks, 3 months, or 6 months of persistent pain. Some authorities do not separate acute zoster pain from PHN but refer to the spectrum of pain symptoms as "ZAP" or zoster-associated pain (Whitley et al., 1999). Difficulties persist with all of these definitions, and the controversy has not led to improved diagnosis or treatment. Severity of pain, persistence of pain, and PHN are strongly correlated with advancing age (Hope-Simpson, 1975). The severity of acute zoster pain appears to be associated with the subsequent development of a more chronic pain syndrome (Dworkin et al., 1992, Whitley et al., 1999). While PHN, by any definition, is rare in the healthy young, patients over age 60 have a one in two chance of experiencing PHN following zoster (Watson et al., 1993, Kost & Straus, 1996, Herne et al., 1996). Additional risk factors for the development of both severe pain and PHN include the involvement of more than one dermatome, the involvement of the trigeminal ganglion, particularly the ophthalmic branch, and cervical dermatomal involvement. Recognition of the potential for intractability of untreated neuropathic pain should prompt early aggressive pain management.

Pain is a complex symptom that can be a major determinant of a patient's quality of life. Among individual patients, pain varies in intensity, duration, quality and

provoking factors. Pain associated with VZV can range from mild discomfort or itch to an incapacitating affliction. For patients who experience this severe, unrelenting pain, the pain becomes their disease. As in other pain syndromes, PHN pain is a personal subjective experience that varies dramatically among patients in response to what appears to be a similar external stimulus. Pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (Merskey & Bogduk, 1994), but pain is also an integrative experience modulated by cognitive, emotional, motivational, and environmental factors. The neuronal pathways and neurochemicals important in the transmission of nociception and the experience of pain are active areas of research and many new discoveries are leading to improvements in pain management.

Brain research has revealed that stimulation of selected brain regions produces pain relief, or antinociception, and that opioid analgesia is produced through activation of these same brain regions. Endogenous pain inhibitory pathways exist to dampen down pain signaling in similar brain pathways. This bidirectional flow of pain transmission is balanced with neurochemicals that are active at every level of the neuroaxis from the peripheral sensory receptors to the deep cortical brain regions. The balancing of pain inhibitory signaling with pain facilitating signals is effected by cognitive, emotional and stress-response systems, producing a complexity and uniqueness to an individual's response to pain. The end result of this diverse convergence of pain inhibition and pain facilitation is the patient's individual pain experience (Elliott, 1994a). The appreciation of the complexities of the pain experience as well as the individual variability in response to treatment intervention is central to the management of PHN.

Diverse brain regions are involved in pain processing. Studies in patients undergoing craniotomies for management of severe intractable pain have shown that pain can be induced by stimulating the normally non-noxious primary and secondary sensory cortex (Sweet, 1982; Woolsey et al., 1979). Some of these brain regions were far removed from the original Penfield cortical locations for pain in the sensorimotor cortex (Penfield & Boldrey, 1937). This may result from local neuronal excitation (most likely a result of overactivation of the *N*-methyl-D-aspartate receptor system with excitatory amino acid release, predominantly glutamate) or as a result of activation of more rostral corticothalamic pathways. Patients with zoster are presumed to have overstimulation of sensory afferents that reach the sensory processing pathways in the brain, possibly from more rostral neuronal excitation and/or loss of local pain inhibitory pathways. These observations suggest that severe pain produces alterations, or sensitization, throughout the CNS, even if the initial injury was localized to a region of painful scarring in the skin, such that more regions of the CNS than are normally involved in nonpainful sensory trans-

mission are recruited. The patient then experiences pain from a wide variety of sensations previously felt as normal, such as touch, brush, and temperature.

The new techniques of functional imaging with PET or fMRI enable the imaging of central nervous system (CNS) events (Jones, 1999). The benefit of functional imaging with PET or fMRI is that the integrated function of the whole brain can be studied at the same time. These studies support the concept of a neural matrix for pain processing. Results in volunteers given a noxious stimulus show a unique network of structures of peri-aqueductal gray, thalamus, lentiform nucleus, amygdala, hippocampus, insula, inferior parietal, primary and secondary somatosensory, prefrontal, premotor, and anterior cingulate cortices (Jones, 1999). Current evidence suggests that this pain matrix is activated by experimental pain as well as the types of acute and chronic neuropathic pain that patients with VZV pain syndromes experience (Jones, 1999).

The capsaicin model mirrors many of the abnormal sensations experienced by patients with VZV pain, including neuropathic and inflammatory, acute zoster and PHN. Subjects experience intense pain following intradermal injection of capsaicin, the active ingredient in chili peppers, and post-injection mechanical allodynia can be evoked with a light brush stroke of the injected area and the region surrounding it. A functional imaging study revealed that capsaicin produced activation in similar neuroanatomical pathways, confirming the physiological importance of the newly identified neuro/pain matrix or network (Iadarola et al., 1998).

Normal sensory function is perturbed during an attack of acute zoster and persists during the development of PHN. Nurmikko has shown, with careful detailed sensory evaluation, that abnormal pain, light touch and/or temperature sensation, as well as evoked allodynia, exists in most patients and sensory abnormalities associated with neuropathic pain extend beyond scarred regions of prior skin involvement. This suggests that sensitization processes may be occurring throughout the neural matrix (Nurmikko & Bowsher, 1990).

Pharmacotherapy

Non-opioid analgesics

For mild pain associated with either acute zoster or established PHN, the non-opioid analgesic, acetaminophen, and the nonsteroidal anti-inflammatory drugs (NSAIDS) are often prescribed. The analgesic action of acetominophen is most likely predominantly central in location (Carlsson & Jurna, 1987; Piletta et al., 1991). While acetominophen lacks gastrointestinal, hematopoietic, and renal side-effects seen with NSAIDS, hepatotoxicity is an infrequent but potentially fatal complication. Acetominophen is also less effective in inflammatory nociceptive

Table 20.2 Pharmacologic therapy for acute zoster (To prevent PHN)

Antiviral therapy
NSAIDS
Opioids
Tricyclic antidepressants
Consider gabapentin, ketamine, dextromethorphan, other adjuvants, anesthetic techniques (temporary nerve blocks, epidural catheter)

Table 20.3 Pharmacologic therapy for PHN

Gabapentin (has the best risk:benefit ratio in this population)
NSAIDS
Opioids (synergistic analgesia with gabapentin)
Tricyclic antidepressants
Consider trials of other adjuvant analgesics such as other anticonvulsants (esp. carbamazepine), topical agents (capsaicin, lidocaine), anesthetic and/or neurostimulatory techniques in intractable individual cases.

pain, such as occurs with acute zoster, than the NSAIDs. The classical NSAID is aspirin, which, as is typical of drugs in this class, has a ceiling effect so that increasing the dose beyond a certain level (800 mg–1200 mg per dose) produces no increase in peak analgesic effect. Tolerance and physical dependence do not occur with repeated administration. Aspirin and other NSAIDs have antipyretic, anti-inflammatory and anti-platelet actions in addition to their analgesic actions.

The analgesic effects of the NSAIDSs include their local effects, at the site of peripheral inflammation, that inhibit cyclo-oxygenase, thereby preventing the release of prostaglandin E2, by inhibiting leukotriene biosynthesis, by anti-bradykinin effects, and by stablizing lysosomal membranes. These chemical mediators of local tissue inflammation, such as prostaglandin E2, sensitize peripheral nociceptors in the presence of tissue injury (Elliott & Foley, 1993). Additionally, NSAIDs have more central analgesic properties. Spinal prostaglandin synthesis at the level of the spinal dorsal horn, a major convergence center for the relaying of pain signaling to the brain, is inhibited with a proposed analgesia produced by facilitating spinal inhibitory input (Malmberg & Yaksh, 1993). The major and most serious side effect of NSAID use is silent gastrointestinal perforation/bleeding, which is less common with the coadministration of the prostaglandin analog misoprostol (Maiden & Madhok, 1995).

Choosing which NSAID to utilize for the management of PHN depends most on

clinician knowledge and availability of the drug because there are no conclusive studies showing that a particular NSAID has more analgesic efficacy for VZV pain. In a controlled crossover study of eight NSAIDs and paracetamol used for the treatment of nociceptive pain, naproxen, diclofenac, and indomethacin appeared to be approximately equipotent for analgesia with the same side effect profile (Ventafridda et al., 1990). The agents in the NSAID class of analgesics differ from one another in their duration of analgesic action and their pharmacokinetic profiles. Ibuprofen and fenoprofen have short half-lives and the same duration of analgesic action as aspirin, while diflunisal and naproxyn have longer half-lives and longer duration of analgesia. Clinical experience shows that some patients respond better to one NSAID than to another; therefore each patient should receive an adequate trial of one drug before switching to another. If a patient fails to gain adequate pain relief with a non-opioid analgesic, the use of adjuvant analgesics should be considered.

Adjuvant analgesics

Tricyclic antidepressants

The tricyclic antidepressants possess established analgesic efficacy for PHN. Amitriptyline, nortriptyline, and desipramine are all first-line analgesics for PHN, and have shown benefit in double-blind controlled studies (Max et al., 1988). The analgesic mechanism(s) of the tricyclic antidepressants is hypothesized to be through their effects on inhibiting reuptake of norephinephrine and serotonin, thereby enhancing the endogenous descending inhibitory pathways that produce analgesia. Their analgesic properties are separate from their effects on mood, and these drugs are effective at dosages that are much lower than standard mood altering dosages for depression. Their analgesic properties have been observed in the laboratory in preclinical models in acute dosing studies. Patients often experience analgesic effects soon after starting the drug. Utility of these agents in the elderly for management of both acute zoster and PHN, however, is fraught with difficulty due to their side-effect profile and the sensitivity of elderly patients to these agents. Side effects that limit use are frequent and include dry mouth, drowsiness, confusion, exacerbation of delirium, urinary retention, cardiac conduction effects, and orthostatic hypotension. Some of these serious side effects are mediated through the anti-cholingeric effects of the tricyclic antidepressants. Starting therapy with a much lower dosage, such as 5 mg by mouth, rather than the 25 mg starting dosage that may be utilized in a younger age patient, allows slow dose escalation during the trial of a tricyclic in an elderly patient. Amitriptyline is the agent most often used for PHN; 5 mg at bedtime may be increased slowly every 3–5 days to an average daily dosage of 50–100 mg depending on effect, weight and sex of patient. Elderly female patients appear to be particularly susceptible to the side effects of

these drugs. Side effects do not appear to subside with time, and tolerance does not seem to develop, but introduction of low dose tricyclic is usually better tolerated by the patient and may also produce recognizable analgesia. Desipramine and nortriptyline have a better side effect profile than amitriptyline, with desipramine showing fewer anticholinergic effects and better analgesia in patients with PHN (Kishore-Kumar, 1990). Despite extensive study, ideal patient selection and follow-up provided in the controlled environment of the clinical trial, an average of only one in two patients receives analgesic benefit from tricyclics (Max, 1988; Watson et al., 1982, 1985, 1992). Anecdotal experience suggests that many more patients, who exceed the age limits of clinical trials, do not achieve an acceptable risk–benefit ratio, and experience more limiting side effects with these medications.

Anticonvulsants including gabapentin

The antiepileptic drugs are often used in the management of diverse neuropathic pain syndromes. Carbamazepine and phenytoin block voltage dependent sodium channels and in experimental models of nerve injury suppress the ectopic collection and activation of sodium channels at the site(s) of nerve injury, a possible explanation for their analgesic action. Carbamazepine is the clinical mainstay for the analgesic management of the intermittent shooting neuropathic pain syndrome of trigeminal neuralgia. However, the antiepileptic drug, gabapentin, has been used increasingly for severe PHN.

Gabapentin, a structural analog of gamma-aminobutyric acid (GABA) is a novel anticonvulsant suggested by case reports to have analgesic effects in both PHN (Segal & Rordorf, 1996) and reflex sympathetic dystrophy (Mellick & Mellicy, 1995). In controlled preclinical studies, gabapentin given subcutaneously or spinally to the rat, reduces formalin-induced nociceptive behaviors (Singh et al., 1996; Shimoyama et al., 1997a). Additional studies show enhanced antinociception in the tail flick assay, an opioid-sensitive test in rats, with subantinociceptive dosing of both morphine and gabapentin, suggesting the potential value of coadministering opioid and gabapentin to patients (Shimoyama et al., 1997b). Gabapentin shifts the dose response curve of morphine to the left, indicating a potentiation of the antinociceptive effects of morphine in this assay (Shimoyama et al., 1997b). This effect is completely reversed and blocked with naloxone, indicating that the antinociception is a mu opioid receptor mediated event. The mechanism(s) of the anticonvulsant/analgesic actions of gabapentin is still unclear. Gabapentin does not bind to any known neurotransmitter receptor, but binds to a novel site distributed uniquely in the CNS (Hill et al., 1993). Spinal gabapentin reduces nociceptive behaviors during the tonic phase of the formalin test at doses that are not antinociceptive in the tail-flick test (Shimoyama, 1997a). The tonic phase of the for-

malin pain test is thought to reflect central sensitization (Coderre et al., 1990), a process fundamental to the development of PHN. Thus, gabapentin may act directly or indirectly on the dorsal horn neuron to block its activation and thereby suppress central sensitization. The actions of the combination of spinal gabapentin and morphine may involve both presynaptic mu receptors and the possible actions of gabapentin on the dorsal horn neuron. Synergy between these two sites produces improved analgesia. Gabapentin has shown anecdotal efficacy in diverse types of neuropathic pain syndromes that are difficult to treat (Rosenberg, 1997), and patients with intractable mixed neuropathic pain syndromes from malignancy often experience pain relief (personal observation) from it. Gabapentin has a much better side effect profile than the tricyclic antidepressants, and is often used as first-line therapy for neuropathic pain, as in acute zoster and PHN. In a recent, randomized, controlled clinical trial, Rowbotham increased the gabapentin dose in 113 patients with PHN, over a four week period to a maximum of 3600 mg, and maintained therapy for an additional four weeks. Subjects receiving gabapentin had a significant reduction of pain scores from 6.3 points to 4.2 points, as compared with the placebo arm, which showed no change in pain scores (Rowbotham et al., 1998). Additional controlled data suggest that gabapentin is also effective in painful diabetic neuropathy, another neuropathic pain syndrome (Backonja et al., 1998). Gabapentin, 1800 mg to 3600 mg per day in three to four divided doses, is the range for analgesic treatment of PHN. By anecdotal report, the drug has been combined safely with other analgesics, such as the opioids and NSAIDs, in patients with either acute zoster or PHN (personal observation). Gabapentin's most significant side effect is drowsiness in the elderly. If the patient is still not obtaining satisfactory pain relief from gabapentin, a trial of an opioid and/or the addition of an opioid to gabapentin may be considered.

Opioids

An opioid should be considered in a patient with pain of either acute zoster or PHN who is not receiving adequate relief with the addition of an NSAID and/or an adjuvant analgesic. This combination provides an improvement in analgesia from the NSAID alone. Combination analgesics are most likely to produce added efficacy or synergy, as each agent works on different sites in the pain matrix/network . When 50 mg of codeine is administered with diclofenac, the analgesia is enhanced as compared with diclofenac alone (Stobel, 1992). NSAIDs have many putative sites of analgesic action, including the regions of skin and peripheral sensory receptors that are damaged by acute zoster, as well as the level of the spinal cord dorsal horn. Opioids exert multiple analgesic effects, predominantly in regions of the CNS that are active with stimulus induced analgesia, and may enhance inhibition throughout the neuropain network.

The introduction of oxycodone, in conjunction with an NSAID or acetominophen/paracetamol, is anecdotally effective for some patients with postherpetic pain. While there are no controlled confirmatory studies, there are data supporting the use of opioids, oxycodone in particular, in PHN. The opioid of first choice in the management of VZV pain syndromes is oxycodone. A single-dose opioid infusion study demonstrated significant analgesic effects in patients with established PHN (Rowbotham et al., 1991). Most importantly, in this small sample, subjects not only experienced a reduction in pain intensity but also a reduction in the development of brush evoked allodynia, an incapacitating symptom in most patients with established PHN and a most likely correlate for the development of a state of central sensitization. A recent controlled, randomized, double-blind crossover study evaluated the effects of oxycodone on the ongoing pain, spontaneous pain and evoked allodynia in patients with established PHN. Thirty-eight patients completed the study and received long-acting oxycodone 10 mg or placebo every 12 hours, with dosage increased weekly to a maximum of 30 mg every 12 hours for 4 weeks. Patients recorded pain intensity and relief daily, and steady pain, spontaneous pain and allodynia were assessed at weekly visits. The results were highly significant in favor of the oxycodone treatment arm. Oxycodone produced significant pain relief, and reductions in steady pain, allodynia and spontaneous pain compared with placebo (Watson & Babul, 1998). In clinical practice, oxycodone administration would most likely be administered until an effect on pain was achieved, or side effects were induced, rather than the artificial ceiling dose of 30 mg every 12 hours. Most of the side effects initially observed with introduction of opioid dosing can be managed as discussed below. Although the implications of any clinical study regimen for the management of the individual patient are limited, this study significantly enhances the justification for chronic opioid administration in patients with VZV pain syndromes (Watson & Babul, 1998). Oxycodone, 5.0 mg, is available in the US as a combination product with acetominophen. Administration four times daily in patients with PHN is a common starting dosage, to be escalated to effect or side-effect, with a maximum of eight tablets daily. Long acting preparations of oxycodone allow twice daily (every 12 hours) dosing. Separate dosing with acetominophen or an NSAID may be given with a titrated dose (to effect or side effect) of sustained release oxycodone.

Side effects associated with opioid analgesics include respiratory depression, sedation, nausea, vomiting, constipation, and myoclonus. Cognitive impairment and delirium are also commonly seen in elderly patients with VZV pain syndromes, and are associated with premorbid risk factors, in addition to opioid use (Elliott & Foley, 1993). Respiratory depression is the most serious adverse effect of opioids. The morphine-like agonists act directly on brainstem respiratory centers to produce a dose response depression that can proceed to apnea. At equianalgesic

dosages, all of the morphine-like opioid analgesics produce an equivalent degree of respiratory depression. Respiratory depression most commonly occurs in opioid naive patients after acute administration of an opioid and is accompanied by signs of CNS depression such as sleepiness and confusion. With repeated dose administration, tolerance to respiratory depression develops rapidly. However, it can recur in the opioid analgesic-tolerant patient with sudden dose escalation or when a trial of a subsequent opioid is instituted. If life-threatening respiratory depression occurs, it can be rapidly reversed with dilute (1:10) naloxone. An endotracheal tube should be placed prior to dilute naloxone if the patient is comatose, to prevent aspiration associated with salivation and bronchial spasm. Alternatively, if the patient is sedated but arousable, stimulation such as walking will often maintain minimal respiration while the opioid effects wear off.

Sedation is seen in the first days of dose initiation or with rapid dose escalation, and tolerance develops to this effect within days. In patients who feel excessively sedated a reduction in opioid dosage is preferable but the use of a mild stimulant such as caffeine, in the morning and before lunch, can be very efficacious. The routine use of stronger stimulants such as methylphenidate or dextroamphetamine, while often suggested with opioid use, is fraught with further risk of delirium in this mostly elderly population with VZV pain syndromes.

Nausea and vomiting result from interaction of opioid analgesic with opioid receptors in the brainstem medullary chemoreceptor trigger zone. As nausea occurs predominantly with movement and in ambulatory patients, a peripheral effect at the level of vestibular function is also present. Nausea and vomiting are unpredictable in the individual patient and may respond to rotation of opioid, with or without a reduction of dosage, or the addition of an antiemetic.

Constipation is the most common adverse side effect of the opioid analgesics and does not resolve with duration of opioid use. This symptom is best prevented with proactive use of a bowel regimen as it results almost universally with opioid usage and tolerance does not develop. Constipation results from opioid actions at multiple sites converging on the gastrointestinal tract to slow peristalsis and to decrease intestinal secretions. A regular bowel regimen of stool softeners, additional fiber, senna derivatives and cathartics tailored to the individual patient should be instituted with the start of opioid therapy.

Multifocal myoclonus occurs with rapid escalation in opioid dosage and with high doses of all the opioid analgesics. Initially recognized with repeated dosing of meperidine due to the toxic accumulation of the active metabolite, normeperidine, it can occur with dose escalation of any opioid. Patients are often in the early stages of delirium (often not clinically detected) when the multifocal myoclonic jerks develop. Additionally, patients may often report an exacerbation of night-time myoclonic jerks (a normal nocturnal event) prior to the development of full-blown

daytime myoclonus. While one approach may be to suppress myoclonus with anxiolytics, this may also exacerbate a developing delirium, and a preferable approach is to lower the dosage, to evaluate for alternative causes (such as electrolyte imbalances) and to consider switching to an alternate opioid. An under-recognized complication of oral opioid therapy is the exacerbation of cognitive dysfunction and/or the precipitation of a subtle delirium.

Delirium is an under-reported side effect of therapy for both acute VZV pain and PHN. Experience suggests that it is a major barrier to aggressive analgesic use, creating fears of opioid use in many physicians and limiting the utility of known analgesics in this patient population. Delirium is a common complication of inpatient hospital care especially in the elderly population. Inouye evaluated five targeted risk factors for the subsequent development of delirium: cognitive impairment, immobility, visual impairment, hearing impairment and dehydration. Following screening tests, the identification of these risk factors led to a structured intervention program, which was both intensive and rigorous in its controls and follow-up. This study was able to demonstrate a significant reduction in the prospective development of delirium (Inouye et al., 1999). Present clinical management of delirium in elderly patients hospitalized for VZV pain includes the rotation of analgesics, the addition of adjuvants, and the utilization of procedures that spare parenteral administration of psychoactive analgesics. Careful and meticulous attention to the interventions proposed in this study would proactively reduce the development of cognitive side effects that so adversely weigh against the use of psychoactive analgesics (tricyclic antidepressants and opioids) in the elderly population most at risk for PHN. This study strongly suggests that every patient with VZV pain would benefit from proactive identification and intervention into delirium risk factors as part of their treatment plan (Inouye et al., 1999).

Topical agents

A controlled trial of topical lidocaine demonstrated a significant short term analgesia in patients with PHN (Rowbotham et al., 1995). Commercially available EMLA cream, applied twice daily with occlusive dressing, has been found anecdotally to provide relief in some patients presenting with evoked allodynia or hyperalgesia. Because there are no data on the long term safety of this approach, this treatment must be monitored with regular serum lidocaine determinations to assure that there is no inadvertent systemic absorption of lidocaine. Capsaicin produces burning on application to affected skin and one controlled trial found a significant reduction in pain score compared with placebo after four weeks of topical application (Watson et al., 1993). However, most patients with severe PHN are not sufficiently able to tolerate the burning sensation to allow an adequate trial.

Others

A large anecdotal clinical experience with diverse analgesic agents and procedures exists for consideration in the patient who has failed to respond to the evidence-based therapy above. Many different types of anesthetic techniques, from local infiltration of skin, to peripheral nerve, paravertebral blocks, autonomic blocks, epidural catheters, dorsal root stimulators, dorsal root entry zone neurosurgical ablations and transcutaneous electrical nerve stimulation, may be considered for the severely impaired individual patient.

The future: preemptive therapy of acute zoster pain

There is little information about the beneficial effects of aggressive pain treatment of acute painful zoster on the risk of PHN. Some antiviral drug studies have shown a significant reduction in subsequent pain, usually classified as zoster-associated pain (ZAP). Benefits of antiviral therapy of acute zoster are most evident in the older population (Crooks et al., 1991). Preemptive therapy of acute zoster pain presumes that an adequately prescribed antiviral agent, such as acyclovir (800 mg 5 × daily for 7days), valacyclovir (1 g t.i.d. for 7 days), famciclovir (750 mg t.i.d. for 7 days) will be given promptly, in the right dosage for the correct duration of time in conjunction with a trial of adjunctive agents.

Amitriptyline

Bowsher evaluated the effect of a fixed dosage of 25 mg of amitriptyline in 72 patients over the age of 60 with acute zoster in a randomized, double-blind placebo controlled trial and found a 50% reduction in pain prevalence at six months in the treated arm (Bowsher, 1997). A major confounding problem with this small study is that antiviral therapy was not given according to a standard regimen. However, this study is the first attempt to evaluate aggressive analgesic management of acute zoster pain.

Gabapentin

Despite much anecdotal evidence and controlled data on established neuropathic pain syndromes, including PHN, no data exist for the preemptive use of gabapentin for acute zoster.

NMDA receptor antagonists–dextromethorphan and ketamine

Dextromethorphan may have a future role in the treatment of acute zoster, in conjunction with standard antiviral therapy and analgesics to prevent the development of PHN. As previously noted, the evolution to a chronic pain state is conceptualized as the development of a state of central sensitization, whereby the

pathways modulating sensation and pain become overcharged, responding to normally nonpainful stimuli and producing abnormally painful and sustained pain with light skin stroking or touch. The hyperresponsiveness thought to underlie the development of PHN is modulated by the *N*-methyl-D-aspartate (NMDA) receptor. Excitatory amino acids (EAAs) are important in nociceptive processing throughout the peripheral and central nervous system, and activation of the NMDA receptor produces abnormal pain states analogous to central sensitization in preclinical models (Elliott, 1994b; Elliot et al., 1995). Dextromethorphan, an antitussive agent available over the counter, is an NMDA receptor antagonist (Elliott et al., 1994b). There is emerging preclinical and clinical evidence that both ketamine and dextromethorphan are analgesic in models of central sensitization. However, their side effect profile, including ataxia, drowsiness, confusion, and dizziness, as well as a syndrome of psychosis at larger dosages, may preclude their long-term use in patients with PHN.

Use of ketamine and dextromethorphan during acute zoster, in order to prevent the development of PHN, has not been explored and requires controlled study. Dextromethorphan has demonstrated efficacy in reducing postoperative tonsillectomy pain and in reducing the severity of dental extraction pain (Kawamata et al., 1998; Gordon et al., 1999). A small study in patients with established PHN shows the efficacy of ketamine in reducing some of the correlates of central sensitization, such as brush-evoked allodynia and spontaneous pain, with acute parenteral administration (Eide et al., 1994). Although these drugs are inexpensive, controlled trials in acute zoster are difficult to fund because they are not under patent protection, except in combination with other agents, such as opioids.

One recent study from the National Institutes of Health further bolsters the hypothesis that NMDA receptor antagonists (either dextromethorphan or ketamine) may prevent PHN if given during therapy for acute zoster. The oral surgery model is used frequently to identify the clinical efficacy of newly identified putative analgesics. The activation of oral structures by tissue damaging stimulation, which has parallels to acute zoster, produces a neuronal barrage into the trigeminal brainstem and may be a model of central sensitization (Gordon et al., 1999). When dextromethorphan was administered in dosages of 60–120 mg by mouth on the day prior to surgery and continued for 48 hours, in a placebo-controlled trial of 75 outpatients, pain was reduced at 48 hours and patients self-administered fewer acetaminophen tablets for unrelieved pain over 24–48 hours postoperatively. These data suggest that dextromethorphan may be antagonizing NMDA receptors and preventing the hyperalgesia seen at 48 hours under these clinical conditions.

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21

Passive antibody prophylaxis

Philip A. Brunell

Historical background

Before antiviral drugs became available, the administration of passive antibodies to varicella-zoster virus (VZV) was evaluated for the prevention and treatment of varicella and herpes zoster. The cumulative observations made in these early studies identified specific indications to prevent and modify the severity of varicella, particularly in immunocompromised patients.

The first investigations of passive antibody preparations involved the transfusion of plasma obtained from patients convalescing from chickenpox to treat or prevent varicella and zoster. Plasma donors with recent VZV infections were used to ensure high titers of VZV IgG antibodies. Failure of high titer VZV immune plasma to impact the course of zoster might have been anticipated, as neither a delay in appearance of VZV antibodies nor their prompt appearance seemed to correlate with whether dissemination occurred (Miller & Brunell, 1970). In clinical studies, VZV immune plasma was ineffective for treatment of zoster (Groth et al., 1978) and in prevention of dissemination (Stevens & Merrigan, 1980). Moreover, the presence of VZV IgG antibodies in patients' plasma could be documented prior to onset of zoster (Miller & Brunell, 1970). It was apparent that the major use of passive immunization against VZV infections would be for the prevention of chickenpox.

With the development of immune serum globulin (ISG), the need to use plasma for prophylaxis of varicella diminished. Immune globulins have the advantage of delivering VZV antibodies in a smaller volume, with uniformity of titer and greater safety than plasma. Several reports about the use of ISG prophylaxis were published, but the landmark study was performed by Ross (1962). Children who had no history of chickenpox were given ISG prepared from varicella-immune donors. Doses ranged from 0.1 to 0.6 ml/lb; ISG was administered within three days of the occurrence of a case of chickenpox in the household. Chickenpox was not prevented, even with the maximal dose of ISG used, but there was a clear relationship between dose and modification of severity of disease. Modification was manifested

by a decrease in the number of skin lesions, and less fever and other symptoms. Subsequent anecdotal experience with the use of ISG in immunocompromised children suggested that ISG prophylaxis was less effective than in normal children. Therefore, it was anticipated that higher titer preparations of VZV immune globulin were needed for the increasing number of young children with leukemia who, with the advent of successful chemotherapy, were surviving longer and thus were more likely to be exposed to chickenpox.

The first high titer globulin, zoster immune globulin (ZIG), was extracted from plasma collected from healthy adults who were convalescing from zoster. Following zoster, VZV antibody titers are higher than following varicella. Globulin for the original studies of ZIG was prepared from sera obtained from patients convalescing from zoster who had a VZV antibody titer of 1:256 or greater by the complement fixation test (CFT).

A study was then performed in households where there were at least three young children, and in which one case of chickenpox had occurred in the family within the past 72 hours. It was not then possible to determine susceptibility to varicella accurately with any available serologic assay. However it was possible to study varicella in families with young children with no history of chickenpox; they were likely to be susceptible because the illness is so contagious and has such a high clinical attack rate. Therefore, on a double-blind basis, one of the exposed healthy children was given ZIG, and the other exposed child was given standard ISG. Ross (1962) had determined that 87% of these exposed children would have been expected to develop chickenpox if they had received ISG or had no intervention. When the first six families were studied, one child in each family had developed chickenpox and the other had not. All of the cases occurred in the placebo recipients, whereas those who received ZIG were completely protected. The volume of the protective dose of ZIG was one-tenth that of the highest dose of ISG that failed to prevent varicella in the previous trials (Ross, 1962). In a subsequent study, a quarter of the dose of ZIG that prevented varicella markedly reduced the number of vesicles in exposed susceptible children but did not prevent illness. Thus, the efficacy and dosage of ZIG for the prevention of varicella in normal children was established (Brunell et al., 1969).

ZIG was evaluated for use in immunocompromised children because varicella was associated with a 10% mortality rate in children with leukemia who developed chickenpox (Feldman et al., 1975). ZIG was prepared from plasma collected 7–28 days after onset of zoster; units of plasma with a titer of 1:128 or greater by CFT were included in the first lot, and units with a titer of 1:64 or greater were included in the second. Immunocompromised children, predominantly those with acute lymphocytic leukemia, were enrolled. Those who had household exposure to varicella were offered ZIG. The efficacy of passive immunization was somewhat less

impressive in these children. In this open label study, five of nine ZIG recipients were completely protected, and three had a very mild rash. Two of these cases occurred following a second household exposure for which the child was not reimmunized. These breakthrough illnesses probably resulted from a second exposure two weeks after ZIG was given. At this time VZV antibody would have been partially catabolized. One patient had a very severe case of chickenpox despite passive immunization. This child had received the less potent lot of ZIG (Brunell et al., 1972).

A more definitive report was published in which 10 of 15 seronegative immunocompromised patients who received ZIG developed varicella (Gershon et al., 1974). By this time it was possible to identify sero-susceptibility to varicella by indirect immunofluorescence, the fluorescent antibody to membrane antigen (FAMA) assay (Williams et al., 1974). Most of these cases of varicella were mild or even subclinical, although one child developed severe varicella but survived. The incubation period in these patients averaged 19 days, which was longer than the 14 days expected in unprotected children. The longest incubation period was 28 days following exposure. Six other ZIG recipients who did not become ill were excluded from the analysis because they were identified as seropositive by the FAMA assay. In two of the six, the antibody was transient and was believed to have resulted from recent prior passive immunization with ISG (Gershon et al., 1974). Thus ZIG was found to confer protection against severe varicella in immunocompromised children. The transient appearance of VZV antibodies in serum following the administration of blood products was confirmed subsequently (Taylor-Wiedeman et al., 1986).

After the efficacy of ZIG was documented, demand for the material increased to the point that the reliance on donors recovering from zoster as a source of plasma became impractical. As an alternative, plasma from routine blood donations was screened for VZV antibody, and IgG was extracted from pooled units with high VZV titers to prepare antibody preparations that were referred to as varicella-zoster immune globulin (VZIG). The VZV IgG titers of this material were similar to those found in ZIG (Zaia et al., 1978). In a large clinical trial, VZIG was shown to be an effective alternative to ZIG for protection of most immunocompromised children from severe varicella (Zaia et al., 1983).

Although serum titers of VZV IgG antibodies comparable to those achieved with VZIG were achieved with doses of intravenous immune globulin (IVIG) of 4–6 ml/kg (Paryani et al., 1984), titers declined by 4 weeks. Moreover, monthly administration of IVIG did not prevent the occurrence of varicella in HIV-infected children (Srugo et al., 1993).

Description of varicella-zoster immune globulin

VZIG is produced in the US by the United States Biologics Laboratory in Massachusetts, and is distributed by American Red Cross Distribution Centers. It is stocked in some hospital pharmacies. Several VZIG preparations are also produced in other countries, but comparisons of VZV antibody titer and clinical efficacy have not been performed.

IgG is extracted from plasma selected for high titers of VZV antibodies by the Cohn ethanol precipitation method. Glycerol is included as a stabilizer and thiomersol as a preservative. Recent lots have been solvent-detergent treated as an additional viral inactivation step. VZIG is distributed in vials of approximately 1.25 ml containing 125 units of antibody or larger volumes containing 625 units for use in adults. The recommended dose is 125 units per 10 kg of body weight with a maximum of 625 units. VZIG is administered intramuscularly. The cost of the preparation for the average adult is more than $400.

The half-life of IgG in VZIG is approximately three weeks. Thus, reimmunization is recommended if reexposure occurs more than three weeks following receipt of a dose. As pooled human plasma is used for the production of both VZIG and ISG and the production process is identical, VZIG contains essentially the same antibodies to many pathogens that one might expect to find in ISG. Therefore, live viral vaccines should not be given parenterally to recent recipients of VZIG. Inactivated vaccines can be given, since passively acquired antibodies do not produce interference. It is recommended that varicella and measles vaccines not be given for five months following globulin preparations; rubella vaccine should be deferred as well. VZIG should not be given to individuals recently immunized with live varicella vaccine nor given simultaneously with this vaccine. If VZIG must be given within three weeks following immunization with any live vaccine, reimmunization should be considered (Peter, 1997).

Indications for VZIG: general

VZIG is now recommended if the following four conditions are satisfied. The individual should: (1) be susceptible to varicella, (2) be able to receive VZIG within 72–96 hours of exposure, (3) have had an exposure that is likely to result in varicella, and (4) be at significant risk of increased morbidity and mortality from varicella.

Susceptibility

Establishing whether an individual is susceptible can be difficult, except in very young children with no history of chickenpox. Laboratory testing to establish

susceptibility to varicella is often not practical to perform before administering VZIG. For older children and adults, obtaining a detailed history of whether and when the individual had varicella, the occupation, and where they lived in childhood will often yield reasonable guidance as to whether passive immunization is indicated. More than 90% of adults with a negative or uncertain history of varicella will be seropositive and only about 1 of 200 with a positive history will be seronegative (Brunell & Wood, 1999). If serologic testing for varicella immune status is obtained, it is essential that sensitive and reliable methods be used. Several instances in which VZIG has been withheld from individuals who had been identified by laboratory testing as seropositive who subsequently developed chickenpox have been recorded (Le & Lipson, 1989; Gurevich et al., 1990; Oshiro et al., 1996). Further complicating the issue is that second attacks of varicella may on occasion occur in seropositive individuals (Gershon et al., 1984; Junker et al., 1991).

Timing

Although it is recommended that VZIG be given within 72–96 hours of exposure, it should be administered as soon as possible because antibodies to VZV must be absorbed from the site of injection. The effectiveness of VZIG more than 96 hours following exposure has not been evaluated. The British recommendation is that it be given within 72 hours following exposure (Tarlow & Walters, 1998). ZIG was given within 72 hours following exposure in the original studies (Brunell et al., 1969, 1972).

Exposure

Single or repeated household exposure will almost always result in infection of a person susceptible to varicella (Ross, 1962). Other types of exposure are less likely to result in varicella (Orenstein et al., 1981). In characterizing an individual exposure, the stage of disease in the source case as well as the circumstances of the exposure must be considered. The exact period of contagiousness has not been determined. Transmission occurs by aerosols and droplets from the respiratory tract and skin lesions. Transmission can occur before rash onset and was reported to be greatest in the day preceding rash in one study (Moore & Hopkins, 1991). In normal children, transmission has not been demonstrated more than five days after onset of rash (Thomson, 1919). In children with progressive varicella, the eruption may be more prolonged and so may be the period of contagiousness. The infectiousness of HIV infected children who have progressive or recurrent varicella has not been established (Srugo et al., 1993). The period of contagiousness of zoster has not been well studied but airborne spread has been documented from patients with localized zoster (Josephson & Gombert, 1988; Wreghitt et al., 1992).

Somewhat variable criteria have been established for nonhousehold exposures for which VZIG is recommended (Morbidity and Mortality Weekly Report, 1996; Peter, 1997). For children playing together, some experts recommend prophylaxis be given

if the exposure is as short as five minutes while others believe that at least an hour of this type of contact is significant. In a hospital situation, the duration of contact is similarly vague but it is recommended that a susceptible in an adjacent bed in a large ward or in a 2–4-bed room with a case of chickenpox would be a candidate for VZIG. For zoster, "intimate contact (e.g., touching or hugging) with a person deemed contagious" is considered exposure requiring passive immunization.

Definition of individuals at high risk for severe or fatal varicella

The most difficult consideration regarding use of VZIG, is usually whether an exposed individual is or is not at increased risk to develop severe varicella. Both the historical perspective and current recommendations will be discussed below.

Infants whose mothers had chickenpox in the perinatal period

Two observations suggested that passive immunization of infants born to mothers who developed chickenpox in the perinatal period might be beneficial. Infants born within 4 days prior to onset of maternal varicella appeared to be born without VZV antibody and developed varicella. However, those born subsequently had probably acquired maternal antibody and did not become ill (Brunell, 1966). A retrospective review of case reports revealed that four of 13 (30%) infants whose mothers developed varicella within the 4 days prior to delivery died of varicella (Meyers, 1974). Thus it seemed reasonable to recommend passive immunization of infants born to mothers with recent onset of varicella. VZIG is now recommended for infants at birth whose mothers have onset of varicella less than five days prior to or two days following delivery (Peter, 1997).

A recent review of the British experience, however, revealed that only one neonatal death per year had been reported over several decades, including a period when VZIG was not available (Miller et al., 1989). The authors concluded that the 30% mortality rate reported previously "was an overestimate based on selective reporting". Some of the deaths in the British study, moreover, were infants who had received VZIG (Miller et al., 1989). Although there have been no controlled studies to evaluate the efficacy of VZIG in reducing morbidity of infants born to mothers with varicella, the administration of VZIG has become standard care in the United States. A placebo-controlled study would now be considered unethical, given the demonstrated effectiveness of VZIG in healthy children (Miller et al., 1989).

However, a change in the US recommendation for VZIG prophylaxis has not been advised because of questions that were not resolved by the British study. Although there were no deaths attributable to varicella in 167 infants given VZIG who were born 7 days prior to and 7 days following delivery, this report did not focus on the group at greatest risk, those born between 4 days prior to and 2 days following delivery. A progressive decrease in serum VZV antibody at birth was

found in babies born from 7 to 3 days prior to delivery; babies born within 3 days of onset of maternal chickenpox were seronegative at birth. Following administration of VZIG to these seronegative infants, VZV antibody titers were generally restored to those of the 7–3 day group. It was difficult to demonstrate the efficacy of VZIG administration in these seronegative infants, however, as there was no difference in severity or outcome of seronegatives who received 100 as compared to 250 mg of VZIG antibody. There was also no difference in outcome in those VZIG recipients who had preexisting maternal antibody. The authors cited the occurrence of severe cases even in VZIG recipients and concluded that more effective measures are needed. Further complicating the picture was that some of these infants were given acyclovir for their varicella. There was also no effort to compare the potency of the British VZIG with that manufactured in the United States. It is possible that VZIG in the United States is more potent than that used in England, and the sensitivity of the antibody tests used on patients' sera is unclear. It has been demonstrated that VZIG with higher VZV antibody titers is more protective (Orenstein et al., 1981; Zaia et al., 1983).

A Swedish study (Hanngren et al., 1985) found no difference in the frequency of varicella in newborns given ZIG compared with historical controls. However, the authors postulated that the disease might have been modified, based on earlier historical data, although these data may have overestimated the risk.(Meyers, 1974). A near fatal case of varicella occurred in an infant who had been given plasma from a donor with a high titer of VZV antibody. Severe and even fatal varicella has been reported on rare occasions in American newborn VZIG recipients (Bakshi et al., 1986). Varicella has also occurred in newborns following receipt of VZIG who seroconverted prior to onset. Administering up to ten times the dose of VZIG that is recommended for children to premature infants did not produce VZV antibody levels approaching those found in normal adults (Lipton & Brunell, 1989). Hanngren et al. found that the severity of illness appeared to be unrelated to the dose of VZIG administered (Hanngren et al., 1985). However, the potency of the Swedish VZIG is unknown.

The incomplete protection afforded by VZIG should not be surprising. The cell-mediated immune response to VZV, a highly cell-associated virus, is of much greater importance in host defense than humoral immunity. Prior to the advent of vaccines against measles, mumps and rubella, cases of these diseases during the early months of life were much less common than were cases of varicella. This was not attributable to the failure of placental transfer of VZV antibody (Brunell, 1966) but probably was due to the relative protection afforded by antibody in these diseases. Three of 36 seropositive infants in the first 4 weeks of life who were given VZIG in the newborn period following exposure to a sibling developed chickenpox which, in one case, was described as rather severe (Miller et al., 1989). In the Swedish study, 12 of 20 infants who were exposed to maternal varicella three or more days postpartum and given ZIG developed mild disease (Hanngren et al., 1985).

Given the rarity of reports of severe or fatal varicella in newborn infants described above, it seems likely that these problems may be attributable to lack of potency in some VZIGs. In the United States, since administration of VZIG became standard of care, it has become extremely rare to observe a newborn with severe or fatal varicella. Thus, certainly in the United States, it seems reasonable to continue to conform to the recommendations of the Committee on Infectious Disease of the American Academy of Pediatrics with regard to use of VZIG in exposed newborn and premature infants (Peter, 1997).

VZIG is not indicated for full term infants exposed after the second day of life because VZV antibody titers in these infants are comparable to maternal antibody titers (Brunell et al., 1969). These naturally acquired VZV antibodies are present at greater levels than can be achieved by administration of VZIG at recommended doses to exposed newborns (Lipton & Brunell, 1989). Transmission of varicella in newborn nurseries is also rare.

Premature infants

In premature infants, VZV antibody levels may be low because the transfer of IgG is inefficient prior to 17–20 weeks gestation and reaches half of term levels at about 30 weeks of gestation (Lipton & Brunell, 1989). In addition, premature infants who are hospitalized often have blood for diagnostic tests taken routinely. This whole blood is commonly replaced with transfusions of red blood cells as needed. The receipt of numerous red blood cell transfusions, implying that much whole blood was removed, correlates with decreased VZV antibody titers (Lipton & Brunell, 1989). VZIG is recommended for infants who are exposed to varicella who are less than 1000 g and/or were born following less than 28 weeks gestation, or for any exposed premature infant whose mother has not had varicella (Peter, 1997).

Immunocompromised individuals

Although VZIG is recommended for exposed susceptible immunocompromised individuals, the definition of "immunocompromised" is not precise. Of 280 immunocompromised children who developed varicella from 1962 to 1986 at St. Jude's Research Hospital, 66% had acute lymphocytic leukemia (ALL), 18% had solid tumors, 5% had acute myelogenous leukemia and 3% each had non-Hodgkin's lymphoma, Histiocytosis X or Hodgkin's disease. Seven per cent of those who were being treated actively for their malignancy and did not receive antiviral therapy had a fatal outcome. All of these deaths were in patients being treated for ALL. The mortality in this group was 10%. Pneumonitis occurred in 32% of children with ALL and in 19% of the remaining patients. Thus patients being treated for ALL may be at greater risk from varicella than those with solid tumors or other types of

malignancies. Patients with an absolute lymphocyte count $<500/\mu L$ were at significantly greater risk of pneumonia; pneumonitis developed in 71% of those with counts <100. Acyclovir therapy had a dramatic effect on the outcome of all patients including some who had been given VZIG and nevertheless developed severe varicella (Feldman & Lott, 1987). Although some of these data were collected prior to the era of widespread use of antiviral therapy, the malignancies in some of these early cases may not have been treated as aggressively as they would be now. Thus on balance these data are likely to remain meaningful even today.

Although VZIG is recommended for all susceptible patients with malignancies who have significant exposures, it may not always prevent the occurrence of severe or even fatal illness in immunocompromised patients (Gershon et al., 1974; Feldman & Lott, 1987; Orenstein et al., 1981). "Immunocompromised" patients with malignancies developed varicella although they were given two and a half times the amount of ZIG that prevented varicella in normal children (Brunell et al., 1972). Thus, passive antibody may not completely repair the deficiency that is responsible for the severity of varicella in this group of patients. Also of particular concern with regard to increased risk from varicella are organ or bone marrow transplant recipients. Fatal varicella has also been reported in patients who have undergone renal (Feldhoff et al., 1981, Lynfield et al., 1991) and hepatic (McGregor et al., 1989) transplantation despite the administration of VZIG. Although it is difficult to determine the level or duration of immunosuppression that puts these children at increased risk, discontinuing azothioprine therapy (Feldhoff et al., 1981) or lower doses of cyclosporine (McGregor et al., 1989) have been associated with a better prognosis.

Adults

Although VZIG has not been formally evaluated in adults, it is recommended by some experts for susceptible adults exposed to chickenpox, because even immunocompetent adults suffer greater morbidity than do children from chickenpox. Most adults with a negative history of chickenpox are immune (Brunell & Wood, 1999). The dose of VZIG that is recommended for adults is 625 units. In an evaluation of pregnant German women who had received VZIG prepared in that country, only five of 25 exposed seronegative women developed chickenpox, which was mild in every case. In contrast, 16 of 18 women who did not receive prophylaxis developed varicella (Enders, 1984).

There is unanimity about the use of VZIG for exposed susceptible immunocompromised adults and it is recommended that even those receiving intravenous immune globulin (IVIG) regularly receive VZIG if exposed (Morbidity and Mortality Weekly Report, 1996). Bone marrow transplant recipients, moreover, should be considered susceptible unless they have had a history of VZV infection post-transplantation.

The use of VZIG in immunocompetent adults, particularly health care workers, is somewhat more complex. VZIG may not necessarily prevent varicella, and it may prolong the incubation period (Gershon et al., 1974, Orenstein et al., 1981). Thus administering VZIG to exposed health care workers will not necessarily prevent chickenpox and may delay their return to work. There was some concern about preventing rather than modifying varicella, rendering recipients susceptible to subsequent exposures to VZV (Zaia et al., 1983). With the advent of live attenuated varicella vaccine, however, health care workers who have received VZIG can be tested 2 months following passive immunization and, if they are seronegative and immunocompetent, they can be immunized with varicella vaccine. Alternatively they may be actively immunized without testing. The major deterrent to the use of VZIG is its cost, which is currently about $500 for one adult dose. In contrast the live vaccine costs less than $50 per dose, although two doses are required for adults.

There is equivocation concerning the use of VZIG in non-pregnant adults, but its use "should be strongly considered" in pregnancy, which is tantamount to its recommendation (Morbidity and Mortality Weekly Report, 1996). The main indication is to prevent morbidity in the mother. It remains unknown whether administration of VZIG to women who go on to develop varicella will prevent malformations in their offspring. Although no cases of the congenital varicella syndrome were observed in 97 women who received VZIG (Enders et al., 1994), there was a 1% rate of the syndrome in the remainder of the cohort. Thus the numbers studied were not large enough to conclude one way or the other with regard to prevention of the congenital syndrome. In another study, one infant whose mother received VZIG had a fatal outcome and may have had fetal varicella syndrome (Pastuszak et al., 1994). It is unknown whether VZIG is likely to impact the clinical course of a newborn delivered to women with chickenpox near term. Some period of time is required for the absorption of VZV antibody from the site of injection into the bloodstream and additional time is required for transport of the antibody across the placenta (Brunell, 1966). Passive immunization of the newborn at birth, which is the recommended approach, is a more effective and economical means of raising VZV antibody levels in the newborn.

Varicella can be a life threatening illness during pregnancy (Baren et al., 1996, Paryani & Arvin, 1986). The seriousness of the illness in pregnancy and the potential for possible beneficial effect on fetal outcome would indicate that VZIG prophylaxis should be given to exposed susceptible pregnant women.

Steroid recipients

The most frequent consideration as to whether exposed individuals are at increased risk from varicella arises when they are steroid recipients. The dose, route of administration, and duration of steroid therapy that should be indications for

VZIG have been hotly debated, as has the persistence of immunosuppression following cessation of steroids. The recommendations of the Committee on Infectious Diseases of the American Academy of Pediatrics provide guidelines for the use of live vaccines, which are often considered to be applicable to the indications for VZIG. These guidelines indicate that children who are receiving a daily dose of less than 2 mg/kg of prednisone, with a maximum of 20 mg, for conditions such as asthma or nephrosis, can receive live vaccines and presumably would not be candidates for VZIG. For individuals receiving higher doses, the recommendation is not to immunize them until at least one month after the steroids were stopped. By analogy, anyone who has received high doses of steroids within the past month might be considered a candidate for VZIG if exposed. These recommendations also suggest that the use of steroids intermittently and by inhalation is not thought to increase the risk of severe varicella (Peter, 1997).

The UK Advisory Committee (Tarlow & Walters, 1998) states that daily doses of more than 1 mg/kg of prednisone will place persons at increased risk of varicella. They conclude: "any patient receiving or having received systemic steroids within the preceding 3 months, regardless of dose, to be at increased risk of severe chicken pox." The literature is replete with letters and articles either documenting severe cases in individuals who did not fulfill the American recommendations or criticizing the frivolous use of VZIG if one follows the British guidelines. In addition, severe cases of varicella have been reported following the use of intranasal steroids on rare occasions (Abzug & Cotton, 1993), but so has fatal varicella in otherwise healthy children (Morbidity and Mortality Weekly Report, 1998).

The British Group indicates that steroids pose the greatest hazard when given during the incubation period. This is supported by a number of reports of children who had untoward outcomes after receiving steroids for even a short time immediately following exposure (Gershon et al., 1972; Silk et al., 1988; Dowell & Breese, 1993). In contrast, a group of children who had been maintained on relatively low doses of steroids for prolonged periods of time for control of asthma did not have severe varicella (Falliers & Ellis, 1965).

Although the data are inconclusive, it seems safest to be rather liberal in deciding whether a given exposed patient receiving steroids is immunocompromised and to err on the side of over-treatment rather than under-treatment with VZIG since it is no longer in scarce supply.

VZIG and prophylaxis with acyclovir

There have been a number of reports suggesting that acyclovir given 7 days following exposure, but not at the time of exposure, will prevent clinical varicella in most of the recipients (Asano et al., 1993). However, this is not a general recommendation because the experience involves small numbers of patients. When an exposure

of a high risk patient is known to have occurred, it is appropriate to instruct the patient or parent to monitor for the onset of varicella symptoms and to begin acyclovir therapy immediately, should these symptoms occur. Careful observation of this kind is indicated even when an immunocompromised person has received VZIG, because illness may not be modified by passive antibody administration in all cases.

Summary

Passive immunization with VZIG continues to be recommended at the time of exposure to prevent varicella in susceptible individuals who are believed to be at increased risk from chickenpox. The efficacy of VZIG prophylaxis was best demonstrated in healthy children and those with underlying leukemia.

For some other indications such as in other immunocompromised persons and adults, there are few data to support efficacy although it is the opinion of many physicians that VZIG is effective. It is anticipated that the introduction and widespread use of varicella vaccine will decrease the occasions when passive immunization against chickenpox might be indicated. In addition, prophylactic acyclovir, which can be started 7 days following exposure and does not have the time constraints of VZIG, is an alternative that may enjoy greater popularity.

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22

Development of the Oka vaccine

Michiaki Takahashi and Stanley A. Plotkin

Varicella vaccine, the first vaccine licensed against a human herpes virus, took many years to develop. A number of difficulties had to be overcome: skepticism about medical need, difficulties in consistency of production, and doubts about its safety and efficacy. However, it is a success story and is worth recounting. This chapter focuses on the conception of the vaccine by M. Takahashi, its developer, its characterization, the difficulties in overcoming American objections to the vaccine, and the first 10 to 12 years of development (1974–84).

The background to development of the Oka vaccine, as recounted by Takahashi

Experience of attenuation of measles and polioviruses

In 1959–1962, I worked on development of attenuated live measles vaccines. This virus was attenuated by passage in the amniotic cavity and chorioallantoic membrane of developing chick embryos. In addition to the work with measles virus, I worked on adaptation of poliovirus type 3 to chick embryo cells. As is well known, poliovirus type 2 grows well in developing chick embryo cells, but types 1 and 3 do not. Attempts to adapt type 3 poliovirus to chick embryo cells by alternate passage in chick embryo cells and monkey kidney cells failed. No continuous growth of poliovirus type 3 took place in chick embryo cells. However, after several alternate passages the virus was found to be remarkably thermosensitive, growing to low titers at 34°C and less neurovirulent.

These studies demonstrated that passage in foreign-species cells is a convenient and effective means by which to attenuate viruses for use as live virus vaccines.

Experience of malignant transformation experiments with conditional mutants of adenovirus and herpes simplex virus

I had long been interested in the possible causative relationship of human viruses to human cancer. In 1962, tumor formation by adenovirus type 12 was reported in newborn hamsters. Stimulated by that finding, I started in vitro

transformation experiments with adenovirus type 12; no viral growth or lytic viral infection was detectable in inoculated hamster embryo cells. In contrast, adenovirus type 5, which was classified as a nontumorigenic virus, caused lytic infection in hamster embryo cells. Both viruses are lytic to human embryo cells. Thus we tried to obtain conditional lethal mutants of adenovirus type 5 to ascertain whether such mutants could – like adenovirus type 12 – cause the transformation of hamster cells. We obtained temperature-sensitive mutants, which could not grow at 38.5°C, and host-dependent mutants, which caused lytic infection in human but not hamster embryo cells. Using an established hamster embryo cell line (Nil cells) that, unlike primary cultured cells, is readily transformed, we observed malignant transformation with both mutants. However, we detected no transformation of human embryo cells with these mutants. This finding was consistent with the lack of evidence of human adenoviruses as a cause of human cancer.

In 1971, Duff and Rapp reported that hamster embryo fibroblasts were transformed with ultraviolet-irradiated herpes simplex virus type 2 (HSV-2). We found their work interesting and attempted to transform hamster cells with temperature-sensitive mutants at a nonpermissive temperature. Approximately 2700 clones of HSV from mutagenized stock virus were isolated at 32°C, and 42 clones found to be nonpermissive at 38.5°C were examined for the ability to transform hamster and human embryo cells at 38.5°C (Takahashi & Yamanishi, 1974). Hamster embryo cells were transformed by three mutants. Transient transformation of human embryo fibroblasts was documented with one mutant, but resulted in the failure of serial passage of the cells so that the finding was not reproducible. Later on, we attempted repeatedly to transform human embryo fibroblasts with ultraviolet-irradiated human HSV-2, but were unsuccessful. Although human adenovirus and HSV induced malignant transformation of hamster and rat embryo fibroblasts (i.e., foreign-species cells) there was little or no relationship to oncogenesis in human cells (i.e., indigenous cells).

Motivations for and problems in the development of a live varicella vaccine

Chickenpox is usually a mild illness but occasionally manifests as a severe disease in children. After a member of my family had severe chickenpox in 1964, with high fever and widespread rash lasting for 3 days, I began to consider how this disease might be prevented by vaccination. Since live vaccines induced protective immunity against diseases such as measles and polio, my concept from the beginning of the study was to develop a live attenuated varicella vaccine.

Two major problems had to be considered. The first was the possible oncogenicity of varicella-zoster virus (VZV), which is a herpesvirus. The experiments with HSV showed that HSV is either minimally or totally unrelated to malignancy in

human cells. Although it was difficult to rule out VZV as a cause of malignancies, VZV had never been linked to any form of cancer. In 1980, Gelb et al. reported that fresh VZV isolates transformed hamster embryo cells morphologically, but they later reported that this observation was not reproducible (Gelb & Dohner, 1984). Thus, even in vitro, it seemed unlikely that VZV could induce malignant change. The second problem was the possibility that live varicella vaccine virus would become latent, perhaps resulting in later development of zoster. It was presumed that attenuated virus would have less capacity than wild-type virus to replicate in humans and thus to become latent. In addition, it was expected that symptoms of zoster caused by attenuated virus might be less severe than those of disease caused by wild-type viruses. Thus these two issues were not considered obstacles to the development of a live varicella vaccine.

Difficulties in preparing "cell-free" VZV

From the earliest studies on in vitro propagation of VZV, it was recognized that virus produced in cell cultures remains strongly cell associated; the inability to obtain cell-free infectious virus has hampered biological and immunological studies on this virus. Caunt (1963) and Caunt & Taylor-Robinson (1964) showed that infectious VZV could be isolated in a cell-free state following ultrasonic disruption of infected primary human thyroid cells. Shortly thereafter, Brunell (1967) reported the isolation of cell-free virus from infected human embryo lung fibroblasts. Based on these observations, we undertook studies to identify a suitable method for the isolation of cell-free virus from infected cultures and the composition of a suspension medium that would keep the infectivity of the virus as stable as possible. We reasoned that the following procedures would be likely to yield high-titered cell-free virus from infected cells: (1) use of cultured cells in the growth phase for inoculation of virus; (2) high-input multiplicity, with infected cells (rather that cell-free virus) used for inoculation because of the difficulty of obtaining a sufficient dose of cell-free virus; and (3) harvesting of the infected cell monolayer (by treatment with EDTA) before the appearance of advanced cytopathic changes with subsequent preparation of the infected cell suspension.

Because VZV is highly heat-labile, particular caution was required in the selection of a suspending medium that would preserve its infectivity. After comparison of various media, simple phosphate buffered saline (Ca^{2+}, Mg^{2+} free) was selected as the most suitable with sucrose (final concentration, 5%), sodium glutamate (0.1%), and fetal calf serum (10%, or 2.5% gelatin hydrolysate in the case of vaccine preparation) (Asano & Takahashi, 1978). With this medium, the decrease in infectivity during storage at −70°C was minimal; in fact, no decrease was detectable after 1 year.

Primary isolation of vaccine virus

Fluid was taken from the vesicles of a 3-year-old boy who had typical chickenpox but was otherwise healthy (Takahashi et al., 1974). The fluid was stored at −70°C until it was inoculated onto primary cultures of human embryonic lung (HEL) cells. At a temperature of 34°C, characteristic foci appeared after 7–10 days. The virus was designated as the Oka strain since this was the surname of the boy from whose vesicular fluid it was derived.

Rationale for and design of a live varicella vaccine

VZV spreads from cell to cell, forming distinct foci that are visible by microscopy even in unstained cell cultures and are clearly seen after methylene blue or fluorescent antibody staining. Cell-mediated immunity seems essential – or at least as important as humoral immunity – in preventing the spread of VZV in vivo. Since inactivated or subunit viral antigens are usually weak inducers of cell-mediated immunity, I reasoned that a live vaccine might be most useful for the prevention of varicella.

Demonstrating the pathogenicity of VZV in experimental animals is difficult. Therefore, it was anticipated that the attenuation of VZV would be proven only by extensive clinical trials, and that testing of only a limited number of candidate strains would be feasible. The classical empirical method of attenuation was used. Of the various kinds of nonprimate cultured cells tested for susceptibility to infection with the Oka strain of VZV, only guinea pig embryo fibroblasts (GPEF) were found susceptible, and fortunately, the Oka strain grew better in GPEF than several VZV strains tested. After the 11th passage of Oka-strain virus in HEL cells at 34°C, infected cells were trypsinized and inoculated onto GPEF. Characteristic CPE appeared in a few days, and the transfer of infected cells was repeated. Cell-free virus (1000 to 2000 PFU/mL) was extracted from infected cells by sonication. Passaged virus was identified as VZV by hemotoxylin–eosin staining, fluorescent antibody staining, and the neutralization test using HEL cells. Oka-strain VZV thus passaged 11 times in HEL cells and six times in GPEF was slightly more thermosensitive at 39°C than wild-type viruses and exhibited a greater capacity for growth in GPEF than the original or other wild-type strains. Safety testing of the vaccine revealed a lack of pathogenicity, even with intracerebral inoculation, in small nonprimate mammals and monkeys. The absence of C-type particles and of latent viruses, using methods available then, was confirmed morphologically and biochemically.

Early clinical trials: vaccination of healthy and hospitalized children

With the informed consent of their parents, healthy children who were living at home and had no history of varicella received various doses of Oka-strain virus

passaged six times in GPEF. A dose of 500 PFU elicited seroconversion in 19 of 20 children. Even at a dose of 200 PFU, an antibody response was detected in 11 of 12 children. No symptoms due to vaccination were detected in these children. Oka-strain virus passaged six times in GPEF was well tolerated and immunogenic.

The first clinical trial of the vaccine in hospitalized children was undertaken to terminate the spread of varicella among children with no history of the disease. In the hospital where the trial was conducted, chickenpox had frequently spread in the children's ward with severe cases on some occasions. In this protocol, children with no history of varicella were vaccinated immediately after the occurrence of a case of varicella. These children suffered from conditions including nephrotic syndrome, nephritis, purulent meningitis, and hepatitis. Twelve children had been receiving corticosteroid therapy. An antibody response was documented in all of the vaccinated children; within 10–14 days after vaccination six children developed a mild fever, and two of the six developed a mild rash. It was uncertain whether these reactions were due to vaccination or to naturally acquired infection modified by vaccination. No other clinical reactions or abnormalities of the blood or the urine were detected. Thus on this ward, the spread of varicella infection was prevented except in the case of a child who was not vaccinated because his mother mistakenly believed that he already had varicella and who became severely ill (Takahashi et al., 1974). This study offered the first proof that the Oka vaccine was well tolerated by patients receiving immunosuppressive therapy and stirred hopes that this vaccine would prove practical for the prevention of varicella.

Clinical trials with Oka strain vaccines prepared in human diploid cells

VZV yield from GPEF cells was considerably lower than that from human embryo fibroblasts. In addition, the level of viral infectivity was found to decrease to approximately one-third of the original level during lyophilization. Thus, cells that would yield more virus were sought. Because the human diploid cell line WI-38 had been widely used for vaccine production, we decided to cultivate the Oka strain of VZV in WI-38 cells. After 12 passages in GPEF, the virus was passaged several times in WI-38 cells. The virus thus obtained was subjected to the same safety testing described previously and was evaluated in clinical trials. When a shortage in the supply of WI-38 cells became a concern, MRC-5 cells were assessed. A master seed lot was prepared at the second passage level in MRC-5 cells after three passages in WI-38 cells, and vaccines were subsequently produced exclusively in MRC-5 cells.

In an examination of its protective efficacy, the resulting vaccine was given to susceptible household contacts immediately after exposure to varicella (Asano et al., 1977b). Twenty-six contacts (all children) from 21 families were vaccinated,

mostly within three days after exposure to the index cases. None of the vaccinated children developed symptoms of varicella. In contrast, all 19 unvaccinated contacts (from 15 families) exhibited typical varicella symptoms 10–20 days after the onset of the index cases. In three families where one sibling contact received vaccine and the other did not, none of the vaccinated children developed symptoms, whereas all unvaccinated controls exhibited typical symptoms. In general, the antibody titers after clinical varicella were 8–10 times higher than those after immunization. This study clearly demonstrated that vaccination soon after exposure was protective against clinical varicella.

In another clinical study, immunized children on a hospital ward were protected despite subsequent exposures to natural varicella and herpes zoster during the nine months after vaccination (Asano et al., 1977a). After two years of follow-up of 179 vaccinated children including 54 children who had been receiving steroid therapy, 50 of 51 (98%) remained seropositive in the neutralization test, and only one of 13 household contacts of cases manifested mild varicella (10 vesicles but no fever) (Asano & Takahashi, 1977).

In an institution for children less than two years old, prompt vaccination had a similar protective effect (Baba et al., 1978) (Table 22.1). Varicella developed in an 11-month-old infant on a ward for 86 children. A total of 33 children over 11 months of age were vaccinated; 43 children less than 11 months of age were not vaccinated, partly because they were expected to still possess maternal antibody. A small viral dose (80 PFU) was used for immunization. Of the vaccinated group, eight developed a mild rash and one of these eight had a mild fever (less than 38 °C) 2–4 weeks after vaccination. In contrast, typical varicella developed in all 43 unvaccinated children during the 10 weeks after onset of the index case. Symptoms were severe in 16 cases, with confluent vesicles and high fever; after recovery, scars remained in 13 of these 16 cases. These results suggested that vaccination with as little as 80 PFU frequently stopped the spread of varicella among children in close contact with one another.

Vaccination of children with malignant diseases

In the first vaccination trial in children with malignant diseases given virus doses of 200, 500, or 1500 PFU, chemotherapy was suspended for 1 week before and 1 week after vaccination (Hattori et al., 1976, Izawa et al., 1977). Of 12 immunized children with acute lymphocytic leukemia (ALL), three developed a mild rash; vaccination of high-risk children without suspension of chemotherapy, severe complication with no severe adverse reaction, was also reported (Ha et al., 1980). These results offered hope that a live varicella vaccine could be administered, with some precautions, to high-risk children.

Table 22.1 Relationship between severity of symptoms and age of children at outbreak of varicella

		Clinical symptoms		
Age (mo.)	No. of cases	Mild	Moderate	Severe
Unvaccinated				
1	4	4	0	0
2	4	1	0	2
4	1	1	1	0
5	3	0	0	2
6	4	1	1	2
7	6	0	1	2
8	5	1	4	1
9	1	1	3	0
10	2	0	0	2
11	3	0	0	3
12–24	10	3	5	2
Total	43	12	15	16
Vaccinated				
11	2	0	0	0
12–27	31	8	0	0
Total	33	8	0	0

Notes:
Mild: severe rash with no fever; moderate: countable rash with fever (37.5°C–39.9°C); severe: confluent rash with fever (38°C to ≧40°C).
Source: From Baba et al., 1978.

Development of varicella skin test to measure CMI (cell-mediated immunity) and its clinical application

A specific varicella skin test (a delayed-type hypersensitivity test against VZV antigen) was developed in the study of CMI to VZV (Kamiya et al., 1977). A VZV skin test antigen was prepared by harvesting the VZV-infected human diploid cells, washing the cells with phosphate buffered saline (PBS), and sonicating the cells, followed by centrifuging at 3000 rpm for 20 minutes. The supernatant was used as skin test antigen after heating at 56°C for 30 minutes, which destroyed infectivity but not antigenicity. The complement fixation antigen titer of this material was 1:64. A control antigen was prepared similarly. A volume of 0.1 ml of viral or control antigen was injected intracutaneously into the forearm. The reaction usually was read after 48 hours by measuring the diameters of erythema. Using the criterion of

5 mm of erythema as a positive result, 50 of 53 normal children with a history of varicella and VZV-neutralizing antibody had positive skin reactions. In 22 children without a history of varicella and no neutralizing antibody to VZV, skin tests were negative.

The skin test was applied prospectively to identify the susceptible subjects exposed to a varicella patient in an institution for mentally retarded children. The skin test was immediately performed on 65 patients. Twenty-four children with negative skin test results were vaccinated; those identified as skin test-positive were observed. As a result, no varicella occurred except for three vaccinated cases who developed varicella 2 weeks after the onset of the index case. These children probably were in the incubation period when the vaccine was given.

The VZV skin test was again used to identify varicella susceptibles rapidly when a case of varicella occurred in an institution housing 49 children (Baba et al., 1978). Among 20 children with negative results, 17 were vaccinated; the index case and two children with fever were not vaccinated. Only 1 of the 17 vaccinated children developed mild varicella within 2 weeks after vaccination; typical varicella developed in two unvaccinated children within 4 weeks after appearance of the index case. None of the 29 exposed children with positive skin tests developed varicella.

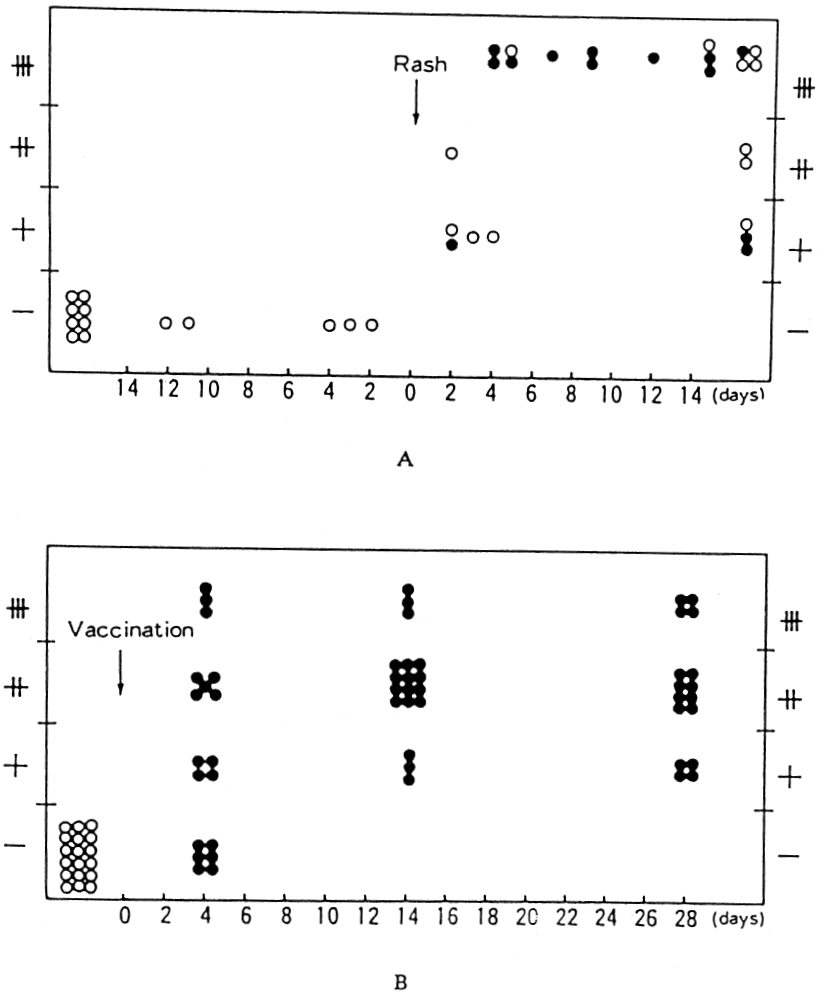
It was also found that when the VZV skin test was performed on vaccinees at sequential 5- to 7-day intervals, positive reactions were observed 4 to 6 days after vaccination, which was 7 to 9 days prior to detection of neutralizing antibody (Baba et al., 1978, Takahashi & Baba, 1984, Asano et al., 1985).

In natural varicella, skin test reactions were negative in all children before the appearance of a rash, whereas positive results were consistently obtained after the appearance of a rash (Takahashi & Baba, 1984) (Figure 22.1). Positive lymphoproliferative activity was observed a week after vaccination, which preceded the appearance of neutralizing antibody by 1 to 3 weeks (Kumagai et al., 1980). This early appearance of CMI after vaccination seems to be related closely to the protective effect of prompt vaccination of susceptible children, even shortly after their contact with varicella patients.

An improved VZV skin test antigen composed of viral glycoproteins, and free from viral particles and cell debris, was later prepared by using culture fluid of infected cells as starting material (Asano et al., 1981). It is more purified than the original crude VZV skin antigen and retains antigenicity comparable with the crude antigen. The improved skin antigen, composed mainly of two glycoproteins (gH and gI) has been licensed in Japan since 1990.

Biological and biophysical characteristics of the vaccine strain

The Oka vaccine virus was found to have various biological and biophysical attributes that can be used to distinguish it from wt (wild type) VZV. These include the following.



○ Indicates the first skin test

● Indicates the 2nd, 3rd and 4th skin tests

A : Skin test on 22 children with natural varicella

B : Skin test done 4 times on each of 18 vaccinated children

Figure 22.1 Comparison of the time of conversion of varicella skin reaction with natural varicella and with vaccination. (From Takahashi & Baba, 1984.)

Table 22.2 Infectivity of vaccine (Oka strain) and wild-type strains of VZV in GPEF (guinea pig embryo fibroblast) and HuEF (human embryo fibroblast)

Strain	Source	No. of passages in HEL cells	Viral titer in indicated cells (pfu/0.2 ml)		Infectivity ratio (GPEF/HuEF)*
			GPEF	HuEF	
Oka					
Vaccine	...	...	7.0×10^3	8.5×10^3	0.82
Parental	Varicella	10	2.8×10^2	6.8×10^3	0.041
Tsuchiyama	Varicella	5	5.2×10^1	1.5×10^3	0.035
Inoue	Varicella	7	6.6×10^2	1.3×10^4	0.051
Watanabe	Varicella	6	5.8×10^1	1.6×10^3	0.036
Wada	Varicella	8	3.8×10^2	6.2×10^3	0.061
Terada	Varicella	9	2.2×10^2	1.7×10^4	0.013
Morita	Zoster	2	1.2×10^2	6.5×10^3	0.018
Kato	Zoster	4	3.8×10^1	1.2×10^3	0.032
Takenaka	Zoster	4	1.5×10^2	7.0×10^3	0.021
Yamashita	Zoster	5	5.8×10^1	1.3×10^3	0.045
Yamaguchi	Zoster	7	1.2×10^2	1.9×10^3	0.063
Ellen	Varicella	...	8.0×10^1	2.1×10^3	0.038

Note: * The mean ratio ($\pm$ SD) except for vaccine virus was 0.038 ± 0.015.
Source: From Hayakawa et al., 1984.

Temperature sensitivity of the vaccine and wt strains

The Oka vaccine strain was found to be slightly temperature-sensitive at 39°C, unlike the wt strains. The foci of the vaccine strains were also smaller than those of wt strains at high temperatures but similar in size to those of the wt strains at lower temperatures (Takahashi et al., 1981; Hayakawa et al., 1984).

Difference in infectivities in GPEF and HuEF (human embryonic fibroblast) of the vaccine and wt strains

The infectivities of the vaccine strain and wt strains were assayed by plaque titration on GPEF and HuEF. The vaccine strain showed a higher ratio of infectivity in GPEF compared with HuEF than any of the wt strain (Takahashi et al., 1981; Hayakawa et al., 1984) (Table 22.2).

The immunogenicity of the vaccine virus is also far greater than that of other wt viruses in guinea pigs, which is probably related to the difference in the capacity of the vaccine virus to replicate in cultured guinea pig cells (Matsunaga et al., 1982). The growth of the vaccine virus is better than that of the original Oka strain and other strains in GPEF cultures, as determined by infectious center assay. In

addition, the adsorption rate of vaccine virus to cultured guinea pig cells as assessed by infectious center assay is higher than that of wt VZV. These results suggested that the vaccine virus is a variant of VZV at least with respect to thermosensitivity and host range.

DNA cleavage profile

Differences in the migration patterns of DNA fragments of the vaccine-type virus and other wild-type (wt) strains have been found after cleavage with restriction endonucleases. In a comparison of DNA from the VZV (Oka) and wt VZV strains, significant different cleavage patterns were seen using *Hpa*I, *Eco*RI (Hayakawa et al., 1984), and *Bam*HI and *Bgl*I (Martin et al., 1982) enzymes as well as *Pst*I (Brunell et al., 1987). Using *Hpa*I and *Eco*RI restriction endonuclease, DNA fragments (K and P) unique to VZV (Oka) were identified, and frequently used to distinguish the Oka vaccine strain from other clinical strains. However, because the technique requires considerable viral genomic DNA and is time-consuming, it was not suitable for routinely identifying the Oka vaccine strain. Also, because the Oka strain was one of several epidemic viruses during the early 1970s, there is a possibility that some currently circulating viruses may have *Hpa*I-K fragment identical in size to those in the Oka strain.

We and others have attempted to develop a more reliable method for identifying the Oka vaccine virus, and several other methods have been reported. These include finding a cutting site of *Pst*I in wt VZV in the *Pst*I site-less (PSL) region of Oka by PCR analysis (LaRussa et al., 1992) and analysis of three variable regions with repeated elements (termed R2, R4, R5) in the VZV genome (Takada et al., 1995).

Recently we have developed a new method to distinguish the Oka vaccine strain of VZV from other clinical isolates (Mori et al.,1998). The molecular characteristics of 52 clinical isolates from varicella or zoster patients with no history of VZV vaccination, and the Oka strain including vaccine and parental viruses, were analyzed by *Pst*I cleavage of the *Pst*I site-less (PSL) region. This was followed by single-strand conformational polymorphism (SSCP) after polymerase chain reaction of repeating region 2 (R2). Most of the clinical isolates tested, especially recent isolates, had a *Pst*I site in the PSL region, but the Oka strain did not. The SSCP patterns of R2 in Oka strain virus differed from those of other viruses. These results suggest that analysis of the *Pst*I followed by SSCP of R2 will be useful for identifying the Oka vaccine virus in isolates (Figure 22.2).

Gene level difference in vaccine, parental Oka strain and other clinical isolates

Even by SSCP patterns, it was difficult to distinguish between Oka vaccine and parental virus. It was reported that VZV (Oka) accumulates a low level of gC (gene14) polypeptides relative to wt VZV strains (Kinchington et al., 1990). The

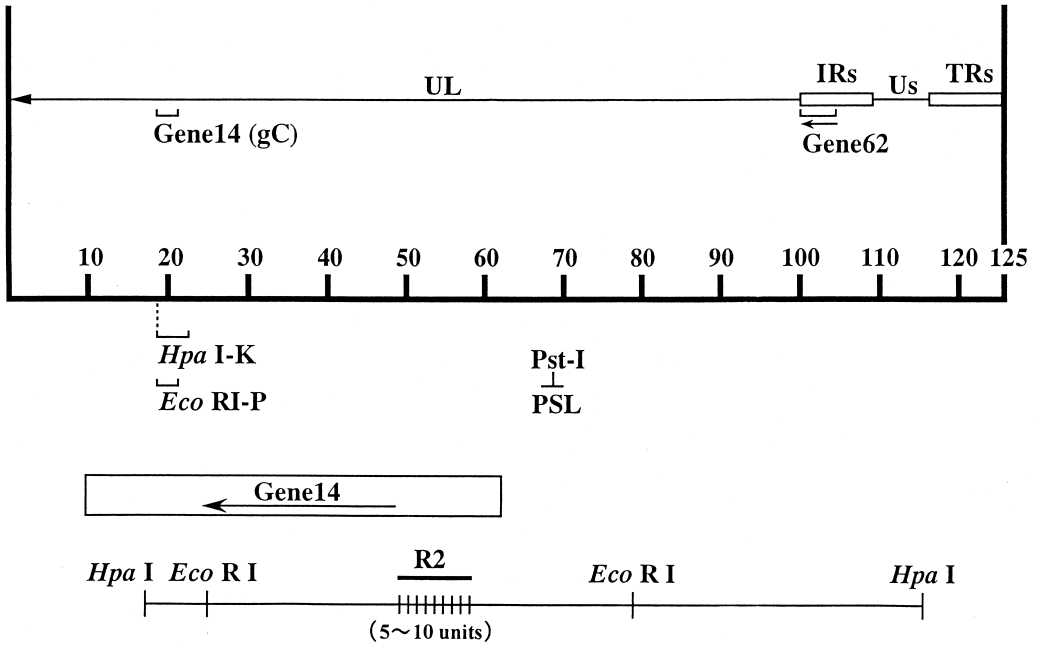


Figure 22.2 Genomic map of varicella-zoster virus (VZV) in relation to location of PSL (*Pst* I site-less) R2 regions, gene 14 (gC) and gene 62.

genome locus of gC where there are repetitive sequences corresponds to that of *Hpa*I-K and *Eco*RI-P fragments. A possible relationship between gC expression and attenuation of Oka vaccine virus was considered. However, no difference was noted between Oka vaccine virus and parental Oka virus in base sequence analysis. Therefore we examined immediate–early (IE) genes, which regulate expression of later genes.

When nucleotide sequences of Oka vaccine and its parental viruses were compared, a 15-nucleotide change was found in the immediate–early (IE) gene 62. Sequence analysis of DNA clones obtained by polymerase chain reaction amplification revealed that no complete identical sequence was found in gene 62 between Oka vaccine and its parental viruses. Furthermore, it was found that the vaccine virus contained mixed populations with variation in 15 nucleotides among 3929 base sequences in gene 62, while the Oka parental virus was composed of only a single clone. With the differentiation method using the simplified restriction enzyme (*Nae*I and *Bss*HII) fragment length polymorphism analysis, which was established based on the sequence analysis data, Oka vaccine virus could be distinguished not only from other clinical isolates but also its parental virus, suggesting that the gene 62 may have an important role for attenuation of VZV (Gomi et al., in press).

Early development of the Oka vaccine in the United States, as recounted by Plotkin

It was on the Boardwalk at Atlantic City during the 1976 ICAAC meeting that I recall introducing Maurice Hilleman to Michiaki Takahashi. Hilleman wanted to get the Oka strain for production by Merck, and that was the initial meeting that led to an agreement. At the time, Takahashi was also discussing rights to Oka in Europe with SmithKline Beecham.

However, the choice of Oka as the vaccine strain for worldwide development had not yet been made. At least two competing strains were developed, and both were tested in my laboratory. I had isolated a strain from the child of a family friend, named Webster. The Webster strain was passaged 77 times in WI-38 cells, and when tested clinically, it produced good seroconversions. However, one of nine exposed vaccinees developed varicella, and the strain was abandoned as being overattenuated (Hediard et al., 1983). In retrospect, the absence of virus passage in a nonhuman cell, such as guinea pig, probably resulted in more rapid attenuation. At the time we were obsessed with the need to keep viruses free of extraneous contaminating agents such as might be present in primary cells (Plotkin, 1971).

The second strain tested by us had been developed at Merck by Neff and coworkers (Neff et al., 1981; Arbeter et al., 1983). We conducted studies between 1979 and 1982 on this strain, named KMcC (Arbeter et al., 1983a,b). At the 40th passage in human diploid cells, the virus was insufficiently attenuated, whereas at the 60th passage, it was insufficiently immunogenic. At the 50th passage its properties were roughly equivalent to those of Oka manufactured either by Merck or SB (Table 22.3). However, the fact that KMcC could become rapidly overattenuated, and the growing experience with Oka, led Merck to abandon KMcC. Thus, by 1983 there was general agreement that Oka should be the vaccine strain for future development.

The first trial of the Oka strain in normal children in the United States was conducted in my laboratory (Arbeter et al., 1983b). This trial was conducted in 1978, subsequent to a trial started by Max Just in Switzerland in the previous year (Just et al., 1982). I believe that Just was the first investigator outside of Japan to test the Oka vaccine. In our phase 1 trial in Philadelphia about 20 seronegative children were inoculated, and we were able to confirm the safety and immunogenicity of the Oka strain.

However, not all believed that general varicella vaccination of children should be undertaken, and there were sharp exchanges of opinion on the subject during the early 1980s (Brunell, 1975a,b; Plotkin, 1975; Brunell, 1977a,b; Plotkin, 1977; Kempe & Gershon, 1977; Brunell, 1978a,b) Even Albert Sabin (1977) contributed to the debate, fortunately on the side of those who wanted to continue. The critics

Table 22.3 Antibody and cellular responses to varicella strains and to various lots of varicella vaccine

	Antibodies 6–7 wk after vaccination			Lymphocyte proliferation 6–7 wk after vaccination		
Vaccine	*n*	% > 1:2	Reciprocal GMT	*n*	% SI > 2.0	Mean SI
OKA-Biken 7710	18	100	29	15	93	12.2
OKA-Biken 7906	39	100	16	30	98	9.0
OKA-RIT Ag	22	59	21	22	86	7.4
OKA-RIT Ly	18	93	29	15	93	15.9
OKA-Merck – Ag	53	100	40	44	95	15.2
OKA-Merck – Ly	41	100	25	23	100	10.4
KMcC passage 40	26	100	35	23	96	9.2
KMcC passage 40	52	100	58	44	84	7.7
KMcC passage 50	17	100	26	11	100	8.6
KMcC passage 50	51	100	38	43	83	7.7

Notes: GMT, geometric mean titer; SI, stimulation index
Source: From Arbeter et al., 1984.

of the vaccine were principally concerned about the safety of the Oka live virus and the duration of immunity.

Nevertheless, between 1978 and 1982, a considerable amount of confirmatory data were collected in the United States and Europe showing that Oka was sufficiently attenuated in normal children (Arbeter et al., 1982, 1984 (Table 22.3)), leukemic children (Brunell et al., 1982; Gershon et al., 1984), normal adolescents and normal adults (Gershon, 1975, 1980). A low rate of zoster due to vaccine was noted, and encouraging immunologic data suggesting persistence of immunity were accumulated. The ability of Oka to protect children after exposure to varicella was demonstrated (Asano et al., 1977; Plotkin et al., 1985). Opposition to the vaccine faded, and the sentiment that normal children should be universally vaccinated began to grow.

This recommendation to vaccinate normal children was in some sense ratified by an international meeting held in Munich in 1984, the proceedings of which were published in the *Postgraduate Medical Journal* in 1985, and by a supplement to *Pediatrics* published in 1986. These two publications summarized studies on thousands of subjects, and essentially almost all of what we know about the clinical properties of the Oka strain as described. Safety, immunogenicity, protection against serious varicella and persistence of immunity were all documented, and I predicted that Oka varicella vaccine would be widely applied (Plotkin et al., 1985).

Nevertheless, the burden of natural varicella and the cost-effectiveness of varicella vaccination had to be demonstrated. Although some clinical observations had been published (Fleisher et al., 1982), it was the late Steve Preblud at CDC who contributed the first epidemiologic studies showing that varicella is an important cause of morbidity that is worth preventing (Preblud, 1986; Preblud et al., 1984, 1985). The importance of his efforts should be remembered.

Thus, within 10 years of its first clinical trial, the Oka strain developed by Takahashi was clearly on it way to licensure. In the United States, the final hurdle proved to be consistency of manufacture. Vaccine lots produced by slightly different methods gave different ratios of total protein to infectious virus, and the clinical result was varying efficacy against breakthrough natural infection (Bergen et al., 1990; Krause & Klinman, 1995; Johnson et al., 1997). Merck experienced difficulty in making the industrial process of vaccine production conform to the process that had resulted in the research lots. However, ultimately they succeeded, and current data on commercial vaccine show low breakthrough rates (Izurieta et al., 1997). The problem of using a vaccine that is not stable at 4°C was dealt with by setting up a distribution system for frozen product, although both Pasteur Mérieux Connaught and SmithKline Beecham have already developed stabilizers, and ultimately the Merck vaccine may also be stabilized at 4°C. Finally, the theoretical concerns about the long-term effects of vaccination (zoster, increasing susceptibility of adults, etc.) were dealt with by setting up a follow-up study to be conducted over 10 years.

I think it is probable that eventually a second dose of Oka will be routinely administered to children as part of an MMR-V for booster. When that happens, it will be possible to start thinking about the eradication of varicella, assuming that the rate of zoster from vaccination continues to be low. The original hope of Takahashi to prevent children from suffering chickenpox will then become real.

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23

Primary immunization against varicella

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Introduction

Live attenuated varicella vaccine was developed in the early 1970s by Takahashi and his colleagues in Japan (Takahashi et al., 1974). The first described use of this vaccine, an attempt to halt an epidemic of varicella in a children's hospital ward, was, perhaps surprisingly, not greeted with acclaim, but rather with skepticism and controversy. The vaccine however became more and more accepted over a period of about 15 years as ongoing clinical trials indicated that it was safe and effective not only in healthy children but also in certain immunocompromised patients who were at high risk of developing severe varicella (Gershon et al., 1996a). The vaccine was licensed for use in Japan in 1989, for immunizing healthy children. Paradoxically, varicella vaccine has not been used extensively in Japan, where, almost 10 years after licensure, only about 20% of healthy children were immunized (Asano, 1996). In other areas of the world outside of the United States, testing of varicella vaccine has been sporadic, but at present there continues to be interest in Europe and Australia (Varis & Vesikari, 1996). There is also significant interest in immunizing susceptible adults in Southeast Asia, since varicella in childhood is unusual in these tropical countries.

In the United States clinical trials of varicella vaccine progressed from studies in high-risk individuals, in particular immunocompromised children and healthy adults, to studies in healthy children. This approach was the opposite from the sequence of testing in Japan, which progressed from healthy to immunocompromised children. Decisions regarding populations for clinical trials were complicated by the lack of a practical animal model for varicella, a deficiency that persists even today. The safety and efficacy of varicella vaccine was first studied in immunocompromised children in the United States because at that time the risk–benefit ratio for vaccination was most appropriate for children with underlying leukemia. About 80% of these children were being cured of leukemia, but there was roughly a 10% mortality rate from varicella. Following the success of varicella vaccine in leukemic children, described below, large clinical trials of varicella

vaccine were undertaken in healthy children (Krause & Klinman, 1995; White, 1997). Several clinical trials in healthy children and adults were carried out during the 1980s in the United States (White, 1997). Major milestones occurred when the Food and Drug Administration (FDA) licensed the vaccine for general use and the American Academy of Pediatrics, the Public Health Service Advisory Committee on Immunization Practices (ACIP), and the American Association of Family Physicians recommended vaccination of all susceptible healthy children over the age of 1 year and healthy varicella-susceptible adults (Centers for Disease Control, 1991, 1996; Committee on Infectious Diseases, 1995; Marwick, 1995). In this chapter, we will focus on the record of varicella vaccine in healthy populations, followed by a summary of the experience concerning vaccination of immunocompromised children.

Varicella vaccine in healthy populations

Vaccines

Currently four pharmaceutical companies (Merck & Co., SmithKline Beecham Biologicals, Pasteur Merieux Connaught, and Biken) manufacture and market live attenuated varicella vaccine globally. Because most published experience concerns vaccines produced by Merck and SmithKline Beecham, this chapter concentrates on these vaccines. The Merck varicella vaccine (Varivax) is licensed for use in the United States and requires storage at −15°C. The SmithKline Beecham vaccine is licensed widely in Europe and Asia and requires storage at 2°C. The immunogenicity and efficacy of these vaccines in healthy children was demonstrated in blinded placebo-controlled studies and household contact studies (Weibel et al., 1984; Varis & Vesikari, 1996; White, 1997). Differences in their effectiveness and safety have not been recognized but subtle distinctions may exist. All the vaccines are derived from the original Takahashi attenuated Oka strain, but the vaccines vary in some ways such as passage number in human diploid cells, dose of virus (1000 to 10000 plaque-forming units, pfu), and stabilizers.

Vaccine efficacy

In the first double-blinded randomized placebo-controlled trial of varicella vaccine, 956 children aged 1 to 14 years were enrolled and followed for 2 years (Weibel et al., 1984; Kuter et al., 1991). Children were randomized to receive either Merck's vaccine or placebo, with half the children in a household receiving vaccine and half receiving placebo; 468 seronegative children were given vaccine and 446 seronegative children were given placebo (Weibel et al., 1984). Most of the families had 2–3 children enrolled, and the mean age of both groups was 4.7 years. A dose of 17000 pfu was administered (Gershon et al., 1999). The seroconversion rate,

measured by immune adherence agglutination, among the vaccine recipients was 94%; 97% of those 2–4 years old seroconverted and 90% of those 5–14 years old seroconverted. In the first year of surveillance, the vaccine was 100% effective in preventing chickenpox (Weibel et al., 1984). During the second year, vaccine efficacy was 96% (Kuter et al., 1991). Over 95% of vaccinees remained free of chickenpox during the following 7 years (Kuter et al., 1991).

Another double-blinded placebo-controlled trial was performed in Finland using the SmithKline vaccine (Varis & Vesikari, 1996). In this study, 513 healthy toddlers 10–30 months old were randomized to receive a high titer vaccine dose (10000 or 15850 pfu/dose), a low titer vaccine dose (630 or 1260 pfu/dose), or placebo. Ninety-four to 100% of previously seronegative children developed antibody to varicella, measured by indirect immunofluorescence, following vaccination. After an average observation time of 29 months, the attack rate of varicella in the high dose group was 3% (5 cases). In the low dose group, the attack rate was 11.4% (19 cases), and in the placebo group it was 26% (41 cases) ($P = < 0.005$ for each group).

Investigations of vaccine efficacy comparing breakthrough illness to natural rates of varicella for various ages of children and protection after household exposure have indicated approximately 90% protection against varicella and 100% protection against severe disease (Krause & Klinman, 1995; White, 1997). A case-control study of the effectiveness of varicella vaccine as it is used in clinical practice in the United States is currently underway, and in the first year of study has shown 85% efficacy (Shapiro et al., 1998). This is an important study since the currently licensed Merck product, which is stored lyophilized and frozen, is somewhat unstable, with loss of titer if not stored and reconstituted exactly according to the instructions of the manufacturer. Thus far the protection afforded by the vaccine in a clinical setting has been similar to that observed in a research setting.

Both vaccine dose and postvaccination antibody titer appear to correlate with protection against disease (Weibel et al., 1984, 1985; White et al., 1992; Varis & Vesikari, 1996). Vaccine efficacy in the first two years was 98% in the American placebo-controlled study in which a dose of 17000 pfu was administered. In the Finnish study an 88% vaccine efficacy was demonstrated in the high titer (10000 or 15850 pfu) recipients and a 55% efficacy in the low titer (6300 or 1260 pfu) recipients. These studies strongly implicate the dose of vaccine as important in the level of protection provided, the highest degree of protection being associated with the dose of 17000 pfu. Currently the Merck vaccine licensed for use in the United States provides a dose of approximately 2500 pfu. Better efficacy than the 85–90% currently observed with the licensed Merck product might be achieved by a higher dose of vaccine and deserves additional study.

Although a low titer vaccine elicits seroconversion at 6 weeks postvaccination

(Weibel et al., 1985; Varis & Vesikari, 1996), the mean geometric antibody titer attained is lower and vaccine efficacy is compromised compared with high titer vaccine (Varis & Vesikari, 1996). The absolute antibody titer 6 weeks following vaccination not only correlates with subsequent protection against varicella but is also inversely related to the severity of breakthrough varicella (White et al., 1992; Johnson et al., 1997). Vaccinees who attain a 6 week postvaccination glycoprotein (gp) ELISA titer greater than 5.0 units or a fluorescent antibody to membrane antigen (FAMA) titer greater than 8, are reported to be less likely to develop varicella than those with lower post-immunization titers (White et al., 1992; Johnson et al., 1997). However, there is no known gp ELISA value that guarantees protection against disease; the authors have observed a number of cases of breakthrough varicella in children who had a gp ELISA titer of 5 units (Gershon et al., unpublished).

Overall 2–3% of vaccinees in the United States and Japan have developed breakthrough varicella each year but the illness is generally a modified mild version of chickenpox (Watson et al., 1993; Asano et al., 1994; Asano, 1996; Johnson et al., 1997). Breakthrough varicella rashes tend to be maculopapular with a minimal number of vesicular lesions. Most reports describe fewer than 60 lesions and less than a third of patients have fever (Watson et al., 1993; Asano et al., 1994; Johnson et al., 1997). In one series of varicella cases among seroconverters following vaccination, the median number of lesions was only 18 and only 39% of the patients reported pruritus (Watson et al., 1993). Secondary household cases in vaccinees are not more severe (Watson et al., 1993). Observed breakthrough varicella in adults has also not been severe; no cases of pneumonia, which occurs with some frequency in unimmunized adults with varicella, have been described. There have been a few cases of severe varicella in vaccine recipients (B. Watson, personal communication). Presumably these unusual occurrences represent true vaccine failures.

Measurement of antibodies

A variety of serologic tests have been used to determine if vaccinees have responded to immunization. Practically, the most important of these assays include FAMA, gp ELISA, and commercial ELISA assays. The FAMA assay has been shown to correlate with clinical outcome in healthy individuals; presence of this antibody at the time of household exposure correlates with protection against disease. FAMA antibody titers are closely correlated with neutralization titers against VZV (Grose et al., 1979). In one study, sera from 86 healthy individuals who had either had varicella in the past ($n=41$), had been immunized ($n=26$) or had no history of varicella ($n=19$), were tested by FAMA at the time of a household exposure to chickenpox. Fifty-eight had positive FAMA titers and none became ill. In contrast,

of 28 individuals with FAMA titers of $<$1:2 upon household exposure, 9/9 healthy adult vaccinees and 19/19 of healthy unimmunized individuals developed varicella (Gershon et al., 1994). Over a period of several decades, only one positive healthy vaccinee with a positive VZV FAMA titer has been observed to develop clinical VZV infection after a household exposure; this woman developed only one vesicle and was otherwise not ill (Gershon et al., 1988). The unusual healthy individuals with a past history of disease and positive VZV FAMA titers who have developed varicella upon exposure are thought to represent second cases (Gershon et al., 1984; LaRussa et al., 1985; Junker et al., 1991).

In studies of vaccinated healthy children, 98% seroconverted by FAMA (Johnson et al., 1997), and in studies of vaccinated healthy adults, 94% seroconverted by FAMA after two doses (Gershon et al., 1990). Using the gp ELISA assay, which was developed at Merck & Co. and which is not commercially available, seroconversion rates approaching 100% have been observed for both children and adults who were immunized (Krause & Klinman, 1995; White, 1997). A problem with this assay, however, is that the antibody titer that is associated with protection is not established; data indicating protection in exposed vaccinees as discussed above for FAMA are not available. It seems likely that this extremely sensitive assay detects antibody levels below those reflective of protection against varicella and thus do not provide clinically useful results. In contrast, commercially available ELISA assays are less sensitive than FAMA and do not identify all individuals who achieve protection from varicella vaccine. For these reasons as well as those of expense and practicality, measurement of VZV antibody titers after immunization is not routinely recommended.

One of the practical problems particularly associated with immunizing adults, is the lack of a rapid, sensitive, commercially available assay to determine VZV antibody titers. A recent comparison of sera from 31 health care workers 6 weeks after vaccination indicated that 87% had antibodies detectable by FAMA but only 61% had titers that were considered positive by a commercial ELISA test (Gershon et al., unpublished). A latex agglutination (LA) assay has shown promise in yielding similar results to FAMA with regard to sensitivity and specificity, but it has been tested extensively in only one laboratory (Steinberg & Gershon, 1991; Gershon et al., 1994). One particular setting in which immunization of adults is of great practical importance is in varicella-susceptible health care workers. Because most susceptible adults respond immunologically to two doses of vaccine, vaccination is useful not only for their own protection but also for protection of their patients and control of nosocomial infections. The availability of a simple and reliable test for VZV antibodies would be especially useful in hospitals and clinics, and is predicted to be highly cost-effective for outbreak control.

Cellular immunity

Cell-mediated immunity to VZV has been measured by a variety of assays including skin testing and lymphocyte stimulation against VZV antigen. These assays usually parallel FAMA titers rather closely. Long-term studies on healthy child vaccinees have demonstrated persistence of cell-mediated responses for up to 5 years after immunization (Watson et al., 1994, 1995; Zerboni et al., 1998).

Immune responses

Primary infection with VZV elicits humoral and cell mediated immune responses that are thought to protect the patient against subsequent varicella and zoster (reviewed by Arvin, 1992, 1996). Neutralizing antibodies appear to block replication of the initial virus inoculum whereas cell mediated immunity probably affects replication at other sites involved in the pathogenesis of VZV infection including the lymph nodes, viscera, skin, and dorsal root ganglia. Thus cellular immunity is critical to control the virus during acute infection and reactivation. Varicella vaccine elicits both humoral and cell mediated immune responses (Arbeter et al., 1984, 1986; Asano et al., 1984, 1985a; Johnson et al., 1989; White et al., 1991; Nader et al., 1995; Arvin, 1996; Varis and Vesikari, 1996; Haumont et al., 1997; Zerboni et al., 1998). In a study comparing antibodies to VZV glycoproteins in healthy children who were vaccinated and children with past varicella, GMTs were lower by a factor of 10 in the vaccinees (LaRussa et al., 1990). However, anti-glycoprotein titers and neutralizing antibody titers in vaccinated children were reported to be comparable to those of adults with previous chickenpox (Haumont et al., 1997).

Immunity conferred by the vaccine appears to be long lasting. There is no indication that the frequency or severity of breakthrough varicella is related to the time elapsed since vaccination (White et al., 1992; Watson et al., 1993; Asano et al., 1994; Johnson et al., 1997). Follow-up studies of small numbers of vaccinees over time have indicated that a high proportion have persistent VZV antibodies. Thirty-six children vaccinated in 1984 and 1987 in the US had detectable VZV antibody titers measured by indirect immunofluorescence (FAMA) 6–10 years following vaccination and did not exhibit a decrease in titer during the later years (Johnson et al., 1997). Approximately 5%, however, lost detectable antibodies by FAMA over time. All 25 of 25 patients studied who were vaccinated in Japan 17–20 years previously and did not have varicella or zoster had positive FAMA titers and also had positive skin test reactions to VZV (Asano et al., 1994). Measured by gp ELISA, persistence of VZV antibodies has been demonstrated for up to 5 years in over 500 American children (Kuter et al., 1991; Watson et al., 1994, 1995; Clements et al., 1995; Arvin & Gershon, 1996; Johnson et al., 1997). In healthy individuals immunized as children it is not uncommon for titers measured by gp ELISA and/or FAMA, to increase

over time, presumably due to exposure to the wild-type VZV with subsequent boosting of immunity.

The explanation of the observed breakthrough cases of varicella that may occur annually in 1–2% of vaccinees is not entirely clear. Undoubtedly some cases represent vaccine failures as has been mentioned; this would also explain the occasional case of severe varicella occurring in a vaccinated child. Because vaccinees who have become seronegative (seroreverters) have developed varicella, a few cases may be due to waning immunity, as has been noted for measles vaccine (Anders et al., 1996). The majority of cases may represent a suboptimal primary response to the vaccine. This explanation is consistent with the observed lack of an increase in severity and/or incidence of breakthrough illness with time (Gershon, 1995; Johnson et al., 1997). It is not known if a suboptimal response to vaccination might have a genetic basis, although this is certainly possible. A further explanation may be that the immunizing dose of virus may be close to the threshold of stimulation of protective immunity. Both of these possibilities are amenable to study.

Safety

Numerous clinical trials have shown that varicella vaccine has a very favorable safety profile. The most common side effects from vaccination are transient pain and redness at the injection site (5–13%) and fever (about 10%) (White, 1996). Perhaps the most important reported adverse event is a rash caused by the vaccine type virus that may follow immunization by 1–6 weeks. Rash after varicella immunization is reported to occur in about 5% of healthy children who are vaccinated (White, 1996). Vaccine-related rash usually occurs about 1 month after vaccination and may be localized to the injection site or generalized. In virologically confirmed cases, rashes occurring sooner than 14 days post-vaccination typically have been due to wild-type VZV whereas those occurring 14–21 days after vaccination have been vaccine strain (White et al., 1991; Sharrar et al., 2000).

The importance of vaccine-associated rash is two-fold. First, its appearance suggests that the virus has multiplied and reached a target organ (the skin) and that the vaccinee is likely to have been successfully immunized. Second, vaccine-associated rash is a risk factor for possible transmission of the vaccine strain to susceptible contacts. Transmission of vaccine-type VZV to others is a well-recognized consequence of vaccination of leukemic children (Tsolia et al., 1990; Hughes et al., 1994). Transmission is much less frequent from healthy vaccinated persons; it has been reported only very rarely (LaRussa et al., 1997; Salzman et al., 1997). In one documented instance representing a circumstance of great concern, vaccine virus was transmitted from a 12-month-old child with 30 skin lesions to his pregnant mother (Salzman et al., 1997). The mother's illness consisted of approximately 100 skin lesions containing vaccine strain virus. She underwent elective abortion and

VZV DNA was not identified in the products of conception by polymerase chain reaction (PCR). Overall the risk that a healthy child will transmit vaccine virus is considered to be very small. At the time of the event described by Salzman and colleagues, several million doses of Merck varicella vaccine had been distributed in the United States with only this one report of spread from one healthy person to another. The risk of transmission of vaccine virus is predicted to be much less than the risk of transmission of wild-type virus via a susceptible child, based on the experience in household contacts of leukemic vaccinees who developed vaccine-related rashes. Therefore withholding vaccination from children living with individuals at high risk of developing complications from VZV is usually not recommended (Committee on Infectious Diseases, 1995; Centers for Disease Control, 1996; Long, 1997). Thus far no instance of transmission to a fetus or transmission in a health care environment has been reported. Whether the vaccine type VZV can cause the congenital varicella syndrome in not known. Vaccine and wild-type viruses are distinguishable by PCR in countries other than Japan (LaRussa et al., 1992) and ongoing studies of rashes that develop in vaccinees are continuing (Sharrar et al., 2000).

Zoster

Cases of clinically diagnosed zoster following varicella vaccination have been evaluated by PCR and other assays, and in some instances the vaccine-type virus has caused zoster in healthy immunized children (Liang et al., 1998; Sharrar et al., 2000). As discussed below, studies in immunocompromised vaccinees have shown that zoster is less frequent after immunization than after natural infection. It seems likely that zoster will also be less frequent in healthy children who have been immunized (White, 1992), but this will take many years of follow-up to prove.

General comments regarding vaccination

There are three general contraindications to routine vaccination: known allergy to the vaccine or one of its components, use in patients who are immunocompromised, and pregnancy. As is the case for most childhood vaccines, varicella vaccine has been demonstrated to be cost effective in several studies (Preblud et al., 1985; Huse et al., 1994; Lieu et al., 1994a,b, 1995; Beutels et al., 1996) (Table 23.1).

Adults

Varicella vaccine has been shown to be effective in adults, but less so than in children. Possibly this is due to a primary cell-mediated immune response that is less vigorous in adults than in children (Gershon, 1995; Nader et al., 1995). In various clinical trials young adults have required two doses of vaccine to reach a seroconversion rate of greater than 90% (Gershon et al., 1986, 1988, 1990; Hardy & Gershon,

Table 23.1 Comparison of responses of children and adults to immunization with live attenuated varicella vaccine, based on numerous clinical trials

	Healthy children (<13 years)	Healthy adults
Doses of vaccine for ≥95% seroconversion	One	Two, 4–8 weeks apart
Vaccine-associated rash about 1 month post-immunization	5%	10%
Rate of breakthrough varicella (usually modified) over time	10%	25%
Estimated loss of detectable VZV antibodies measured by FAMA with time	5%	20%
Increasing GMT with time	Yes	No
Serologic testing after vaccination	Unnecessary	Useful for health care workers

1990). Open label efficacy studies based on household exposure to varicella have shown the vaccine to be about 75% protective against varicella in adults, with most breakthrough infections being modified cases (Gershon et al., 1986, 1988, 1990; Hardy & Gershon, 1990; Kuter et al., 1995; White, 1997). It is recommended that even adolescents above the age of 13 years be given two doses of vaccine (Kuter et al., 1995; White, 1997). Side effects of vaccination are similar to those observed in children, with the vaccine proving to be extremely safe, even after multiple doses (Gershon et al., 1986, 1988, 1990; Hardy & Gershon, 1990; Kuter et al., 1995).

In contrast to children, who often have an increase in VZV antibody titer with time, adult vaccinees may experience loss of VZV antibodies detectable by FAMA months to years following seroconversion. Of 40 healthy adult vaccinees tested 7–13 years after immunization, 18% were found to have become seronegative by FAMA (Gershon, 1995). In contrast, only about 5% of vaccinated children have been reported to lose FAMA antibodies after a similar interval (Johnson et al., 1997). In addition, unlike children, geometric mean titers (GMTs) in adults have not been reported to increase over time, presumably due at least in part to the lack of boosting from exposure to wild-type virus (Asano et al., 1985b; Gershon et al., 1994; Johnson et al., 1997). Adults who have lost detectable antibodies are at risk of developing breakthrough varicella, but usually breakthrough infections, as in children, are modified (Gershon et al., 1990). Thus the significance of the loss of detectable antibody is not entirely clear. Silent re-seroconversions in adults after exposure have also been observed (Gershon et al., 1990). After 8–13 years of

observation, neither the incidence nor severity of varicella in immunized adults increased with time (Gershon, 1995). Thus, there may be some waning immunity in vaccinated adults in that antibodies decline below the limits of detection; however protection against severe varicella is usually observed. As many as 5% of immunized adults may not seroconvert by FAMA after 2–3 doses of varicella vaccine and these adults are not protected against varicella. This risk of primary vaccine failure is another reason why it is preferable to emphasize immunization of children rather than adults (Table 23.1).

Only one case of zoster has been reported in an immunized adult. This case occurred in a physician who seroconverted after immunization but lost detectable VZV FAMA antibodies. Following exposure to a patient with varicella, she re-seroconverted without developing symptoms. A year later, she developed zoster that was shown to be due to wild-type VZV (Hammerschlag et al., 1989).

Varicella vaccine in immunocompromised populations

In the United States, clinical trials of varicella vaccine in children with leukemia were carried out prior to studies in healthy children. Use of varicella vaccine was initially highly controversial in the United States. There was concern about possible long-term adverse effects of vaccination against a disease that is not usually severe in the general population. It was recognized, however, that protection against varicella would be a great advantage for immunocompromised children and at that time they had the most appropriate risk–benefit ratio for initial studies. Because the vaccine was shown to be safe in vaccine trials in Japan (Gershon et al., 1999), it seemed likely that it would also prove safe for American children. Between 1980 and 1990, over 600 children with underlying acute lymphoblastic leukemia in remission were immunized. There were no deaths from varicella or zoster in these children; had they not been immunized a number of deaths due to severe varicella would have been expected. Most were protected against varicella following exposure, and all were protected from severe varicella (Brunell et al., 1982; Gershon et al., 1986, 1989; Arbeter et al., 1990; Gershon, 1995). Although varicella vaccine is not licensed for use in children with leukemia in the United States, a "compassionate use" program for their immunization remains in place (Committee on Infectious Diseases, 1997). The major adverse effect of vaccination of leukemic children is development of a vaccine-associated rash in about 50%, with the need to administer acyclovir to control the rash in about 40% of those with rash. Once immunized, about 85% of these children are completely protected from varicella (Gershon, 1995). Children with solid tumors have also been immunized, but there is no information on vaccine efficacy. Children about to undergo renal transplantation who are immunized have also been protected against varicella. In a French

study of 212 children who were vaccinated prior to transplantation, the incidence of varicella over several years of follow up was 12%, with no severe cases. In contrast, of 49 such children who were not vaccinated, 45% developed varicella during the same interval and there were several deaths from varicella (Broyer et al., 1997).

Vaccine studies and disease pathogenesis

Varicella vaccine trials in immunocompromised patients not only benefited at-risk patients, but also added to our understanding of the natural history of VZV infections. Transmission of VZV from skin lesions was demonstrated in siblings of leukemic children who were studied clinically and serologically for infection with vaccine-type VZV. If the leukemic child vaccinees had no rash, no transmission of VZV was documented when over 100 siblings were followed for seroconversion by FAMA for up to several months after the leukemic children were immunized. If the leukemic vaccinees developed a vaccine-associated rash, however, 21/93 (23%) of their siblings seroconverted to VZV during a similar interval. Five (24%) of these siblings did not develop rash; those with rash had an average of only 38 skin lesions. The mildness of the contact illness in these siblings and the high rate of subclinical infection indicate that the Oka strain of VZV is attenuated, even when transmitted by the natural route of infection (Tsolia et al., 1990).

Vaccine studies provided insight into the pathogenesis and prevention of zoster. Until the 1980s it remained unproven that zoster is due to reactivation of latent VZV. When a few immunocompromised children vaccinated against varicella developed zoster with the Oka strain despite the absence of circulating vaccine-type VZV in the United States, involvement of reactivated latent virus in zoster was proven (Williams et al., 1985; Hardy et al., 1991). Prospective studies of antibody titers and cell-mediated immune reactions in vaccinated leukemic children indicated that children who developed zoster had high antibody titers but low cell-mediated immunity, confirming an earlier finding that low cell-mediated immunity is a major precipitating factor in zoster (Arvin et al., 1978; Hardy et al., 1991). Moreover it was found that there was an association between protection from developing zoster in leukemic children who had more than one dose of vaccine and who had household exposures to varicella (Gershon et al., 1996b). These observations suggest that boosting of cellular immunity by vaccine or exposure to the natural virus may offer protection against development of zoster.

Studies of vaccine in leukemic children also indicated that the incidence of zoster is lower after vaccination than after natural infection (Kamiya et al., 1984; Brunell et al., 1986; Hardy et al., 1991). In a controlled study in the United States, over a period of several years, the incidence of zoster in leukemic vaccinees was 2% and 15% in leukemic children with a past history of varicella (Hardy et al., 1991). More

recent studies in children over a 10 year interval who underwent renal transplantation also indicated that the incidence of zoster was lower in 212 vaccinees (7%) than in 415 patients with past natural varicella (13%) (Broyer et al., 1997).

Immunization of bone marrow transplant patients with an inactivated varicella vaccine indicated that while the incidence of zoster was no lower in vaccinees than in controls, zoster was a significantly milder illness in those transplant patients who had been immunized (Redman et al., 1997).

Thus by immunization with live VZV, protection against zoster may occur in two ways: achieving latency with an attenuated rather than a fully virulent VZV, and boosting of cellular immunity to VZV to protect against zoster immunologically. These results in immunocompromised children offer the hope that healthy children immunized against varicella today will have fewer cases of zoster when they become elderly than did their parents, who were not immunized as children.

The saga of varicella vaccine in immunocompromised patients is by no means over. Hopefully at some point in the future most children will have received varicella vaccine prior to becoming immunocompromised, as is the case for measles vaccine today. However, there will continue to be a need to immunize those immunocompromised children who remain susceptible to varicella, not only to protect them against severe varicella but also against zoster. In children infected with human immunodeficiency virus (HIV), for example, those who develop varicella when their CD4 lymphocyte levels are less than 15% have an 80% chance of developing zoster within 1–2 years after varicella (Gershon et al., 1997; Derryck et al., 1998). Preliminary studies of vaccination of HIV-infected children with CD4 levels that are normal or close to normal indicate that the vaccine is safe and immunogenic. It is hoped that these children will have a lower incidence of zoster since it was possible to provide an attenuated primary infection with VZV while their CD4 cells were still intact (Levin et al., 1999).

Studies of immunization of transplant patients, especially those undergoing renal, liver, and heart transplantation, are sorely in need of being done. While there are some data from France in renal transplant patients, no such studies have been performed in the United States. Moreover, there is no published experience at all in liver and heart transplant patients. Thus there is much still to be done with varicella vaccine as an exploration of potential benefits to be derived from this vaccine in these high-risk patients.

The future

It is expected that use of varicella vaccine in healthy children will increase with time, particularly as state governments require varicella immunization prior to entry into day care or school. It also seems likely that more and more varicella

vaccine will be used throughout the world, given its impressive record of safety and protection. At a recent Infectious Disease Conference, Dr. Stanford Schulman of Children's Memorial Hospital in Chicago cited varicella vaccine as the best vaccine we have against *Streptococcus pyogenes*. A major future milestone will be the successful combination of varicella vaccine with measles–mumps–rubella (MMR) vaccine (White et al., 1997). Thus far, a combination of all four viruses has not resulted in a product that is sufficiently immunogenic against VZV. However, it is expected that an MMRV will eventually become available, and when it does, it will be logical to administer two doses of this vaccine, at 15 months of age and in early childhood. It is crucial to immunize at least 90% of the population in order to assure that the population of adults who were not immunized as children and are susceptible to varicella is vanishingly small (Wharton, 1996). The days when varicella and zoster will become unusual infections in the United States are, hopefully, not too far away.

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24

Prevention of nosocomial transmission

Lisa Saiman and David J. Weber

Introduction

Varicella-zoster virus (VZV) is an important cause of morbidity and mortality in health care settings for both patients and health care workers especially when infection occurs in neonates, pregnant women, otherwise healthy adults, or immunocompromised persons. The Centers for Disease Control and Prevention (Williams, 1983; ACIP, 1996; Garner, 1996), the American Academy of Pediatrics (CID, 1997), the American Medical Association (AMA, 1998), and infectious disease experts (Brunell, 1982; Brawley & Wenzel, 1984; Weitekamp et al., 1985; Sayre & Lucid, 1987; Weber et al., 1988, 1996; Burns et al., 1998; Saiman et al, 1998; Stover & Bratcher, 1998; Saiman & Gershon, 1999), have published recommendations regarding the isolation of patients with VZV infection, the management of patients and health care workers exposed to VZV and immunization of susceptible health care workers with VZV vaccine in efforts to prevent nosocomial outbreaks. This chapter will focus on lessons learned from nosocomial outbreaks of VZV and provide recommendations for the pre- and post-exposure management of health care workers based on the experience at the University of North Carolina (UNC) Hospitals and the New York Presbyterian Medical Center, Columbia campus. The material contained in this chapter is adapted from recent reviews of the management of VZV in health care facilities (Weber et al., 1996; Burns et al., 1998; Saiman et al., 1998).

Patients at high risk for complications of VZV

Control of varicella is critical in health care facilities because varicella and zoster are highly contagious. Many high-risk patients develop VZV infections and require hospitalization or care in outpatient settings. Chickenpox or zoster may also develop during hospitalization for other medical conditions. Thus prompt recognition of VZV infections and proper isolation of patients are critical as will be described below. The risk of complications due to chickenpox and zoster is higher

in neonates (Choo et al., 1995; Wharton, 1996), adults (Miller et al., 1993; Choo et al., 1995) and immunocompromised persons (Feldman et al., 1975; Meyers et al., 1979; Feldhoff et al., 1981; Whitley et al., 1982; Morgan & Smalley, 1983; Locksley et al., 1985; Feldman & Lott, 1987). Infection in otherwise healthy adults frequently results in complications including hospitalization and death. Choo reported that approximately 1.25% of adults with varicella infection required hospitalization, 0.62% developed skin superinfection, 0.78% developed pneumonia, and 0.62% developed other complications (Choo et al., 1995). Similar estimates of hospitalization have been provided by Wharton (1996). Among patients developing chickenpox while undergoing chemotherapy for malignancy or immunosuppressive therapy following organ or bone marrow transplantation, visceral dissemination or severe disease was reported in 30–50% and death in 7–17% (Feldman et al., 1975; Meyers et al., 1979; Feldhoff et al., 1981; Whitley et al., 1982; Morgan & Smalley, 1983; Locksley et al., 1985; Feldman & Lott, 1987; McGregor et al., 1989). However, current rates of morbidity and mortality are likely to be significantly lower due to prophylactic use of varicella-zoster immune globulin (VZIG) and availability of antivirals such as acyclovir.

Neonatal varicella may result if the mother has the onset of clinical varicella within 2 weeks of delivery (Hanngren et al., 1985; Sterner et al., 1990). The onset of varicella in pregnant women within 5 days of birth to 2 days postpartum has been reported to result in severe varicella infection in 17 to 30% of their newborn infants (see Chapter 16). Prophylaxis of infants born to infected mothers with VZIG has decreased mortality associated with neonatal infection but does not fully prevent infection (Hanngren et al., 1985). Thus, such infants require isolation if they remain hospitalized during their incubation period. Finally, chickenpox in pregnant women, including nonimmune health care workers, may lead to the congenital varicella syndrome if infection occurs in the first two trimesters (see Chapter 16).

Transmission of VZV

In most cases, VZV appears to be transmitted from person to person by the droplet route, following aerosolization of virus due to scratching the vesicles and/ or from respiratory secretions. Transmission occurs most efficiently with close contact, but airborne transmission of VZV may also occur. However, VZV is extremely labile and unlikely to be transmitted by inanimate objects. The incubation period of varicella ranges from 8 to 21 days, but most patients develop disease between days 14 and 16. Patients with varicella may become infectious 24 to 48 hours prior to the onset of rash. Normal hosts remain infectious for approximately 5 days after the

onset of the rash. Immunocompromised hosts may remain infectious for a more prolonged period.

The secondary attack rate of varicella among susceptible persons in the household setting has ranged from 61 to 87% (Simpson, 1952; Ross, 1962), but the secondary attack rate among susceptible health care workers may be lower as will be discussed below. Herpes zoster is also infectious and can cause chickenpox. Virus from patients with zoster can be transmitted by the same routes as chickenpox, although it is likely that the risk of transmission from zoster is lower than the risk of transmission from chickenpox.

Nosocomial transmission of VZV

Nosocomial transmission of VZV has been well documented in the literature (Evans, 1940; McKendrick et al., 1976; Asano et al., 1980; Leclair et al., 1980; Morens et al., 1980; Scheifele & Bonner, 1980; Faizallah et al., 1982; Gustafson et al., 1982, 1984; Myers et al., 1982; Tsujino et al., 1984; Hyams et al., 1984; Anderson et al., 1985; Krasinki et al., 1986; Josephson & Gombert, 1988; Stover et al., 1988; Weber et al., 1988; Josephson et al., 1990; Morgan-Capner et al., 1990; Friedman et al., 1994; Faogali & Darcy, 1995; Kavaliotis et al., 1998). Varicella may be introduced into the hospital by infected patients, staff, or visitors. Two years of recent experience investigating varicella exposures at UNC have demonstrated that patients and visitors are more likely to be the source of exposures to VZV than health care workers (Table 24.1). Several investigators have noted that the initial source case for an outbreak was in the incubating phase of varicella prior to the onset (or perhaps recognition) of rash (Krasinski et al., 1986; Weber et al., 1988; Miller et al., 1993).

Nosocomial varicella has occurred among staff and patients who had no direct contact with the index case, supporting airborne transmission (McKendrick et al., 1976; Josephson & Gombert, 1988). Epidemiologic and tracer studies have confirmed relative airflow from the rooms occupied by the index case as a major risk factor for the acquisition of infection among susceptible hosts in the hospital (Josephson & Gombert, 1988). VZV DNA has been detected by the polymerase chain reaction (PCR) 1.2 to 5.5 m from the beds of patients with varicella and from the room air of immunocompromised patients with zoster (Sawyer et al., 1994). However, it is not known if this VZV DNA is infectious.

Continued propagation of epidemics has occurred due to acquisition of varicella in exposed staff (Gustafson et al., 1984; Faogali & Darcy, 1995). Exposure to dermatomal (Morens et al., 1980; Faizallah et al., 1982; Weber et al., 1988) or disseminated (Hyams et al., 1984; Krasinski et al., 1986) zoster in immunocompromised patients has led to transmission of varicella in the hospital setting. Dermatomal

Table 24.1 VZV exposure evaluations, UNC hospitals, 1997–1998

Year	Number of reported episodes of possible exposures to VZV	Source (*n*)	Persons exposed (*n*)	Exposure location (*n*)**
Varicella				
1998	16 episodes	Staff (2)	Staff only (4)	Inpatient (10)
	(7 no exposures* including	Patient (11)	Patient only (0)	Outpatient (7)
	5 in which all staff immune)	Visitors (3)	Staff & patients (6)	Emergency Room (0)
1997	17 episodes	Staff (2)	Staff only (1)	Inpatient (13)
	(12 no exposures including	Patient (12)	Patient only (0)	Outpatient (4)
	9 in which all staff immune)	Visitors (3)	Staff & patients (4)	Emergency Room (2)
Herpes zoster				
1998	20 episodes	Staff (4)	Staff only (2)	Inpatient (10)
	(14 no exposures* including	Patient (15)	Patient only (0)	Outpatient (8)
	12 in which all staff immune)	Visitors (1)	Staff & patients (4)	Emergency Room (3)
1997	13 episodes	Staff (2)	Staff only (1)	Inpatient (5)
	(10 no exposures* including	Patient (11)	Patient only (0)	Outpatient (5)
	4 in which all staff immune)	Visitors (0)	Staff & patients (2)	Emergency Room (3)

Notes:

* No exposures implies possible source case not found to have varicella or that all exposed individuals found to be immune to varicella.

** More than one exposure location per episode may have occurred.

zoster in immunocompetent hosts has led to transmission of VZV to susceptible health care workers via the airborne route or droplet route (Josephson & Gombert, 1988; Weber et al., 1988).

Costs associated with VZV control

The costs associated with VZV outbreaks or overall VZV control have been provided by several investigators. These costs include performing serologic testing; furloughing exposed nonimmune staff during their incubation period; administering varicella-zoster immune globulin (VZIG) to exposed, immunocompromised, nonimmune patients and staff; providing single rooms for exposed patients during the incubation period; time and effort expended by Infection Control personnel and the Occupational Health Service during the assessment of VZV exposures; and the use of antiviral agents, if indicated. Containment of single outbreaks of VZV infection within a hospital setting in the US has been reported to cost from $9100 to $19 000 (Faizallah et al., 1982; Hyams et al., 1984; Stover et al., 1988). The costs of

one year of VZV control in a hospital have been reported to be $41 500 in 1986 by Krasinski and colleagues (Krasinski et al., 1986) and $55 934 in 1988 by Weber and coworkers. Decision analysis demonstrated that immunization of health care workers susceptible to varicella is cost-effective for health care facilities (Hamilton, 1996; Gray et al., 1997; Nettleman & Schmid, 1997).

Control of nosocomial exposures

The Centers for Disease Control and Prevention (Williams, 1983; ACIP, 1996; Garner, 1996), the American Academy of Pediatrics (CID, 1997), and infectious disease clinicians (Brunell, 1982; Brawley & Wenzel, 1984; Weitekamp et al., 1985; Sayre & Lucid, 1987; Weber et al., 1988, 1996; Saiman et al., 1998; Stover & Bratcher, 1998) have published guidelines or algorithms designed to aid clinicians in the control of nosocomial exposures of VZV. A suggested guideline is shown in Figure 24.1 and Table 24.2. There are several areas of controversy among these various guidelines. First, the CDC suggests that exposed sero-susceptible patients be placed on isolation and exposed sero-susceptible employees be removed from work from days 10 to 21 post-exposure while the American Academy of Pediatrics (CID, 1997) suggests isolation from days 8 to 21 post-exposure. This is because patients may be infectious by the respiratory route 1 to 2 days before the onset of rash. Second, the CDC continues to recommend that nonimmunocompromised patients with dermatomal zoster be placed on only contact isolation as opposed to airborne and contact isolation (Weitekamp et al., 1985). It is our practice to place patients with dermatomal zoster on airborne and contact isolation because of the difficulty of defining "immunocompromised patients" and reports of airborne or droplet transmission of varicella from nonimmunocompromised patients with dermatomal zoster (Josephson & Gombert, 1988; Weber et al., 1996). Third, CDC has recently recommended that all patients with varicella or disseminated zoster and immunocompromised patients with dermatomal zoster be placed in rooms meeting engineering requirements suitable to house patients with tuberculosis (i.e., private room, negative pressure of room with respect to corridor, at least 6 air exchanges per hour, and air exhausted directly to the outside). We are unaware of any nosocomial outbreaks in which transmission was linked to recirculated air without air exchanges of infected patients placed in private rooms with negative pressure. Further, negative pressure rooms have been reported to be adequate in preventing transmission of varicella from hospitalized patients (Anderson et al., 1985). Given the current incidence of varicella and tuberculosis, it is unlikely that all facilities will have adequate rooms for both tuberculosis and VZV infections.

VZV outbreaks in Neonatal Intensive Care Units (NICU) are relatively rare (Gustafson et al., 1984; Stover et al., 1988). Most infants are kept in isolettes, which

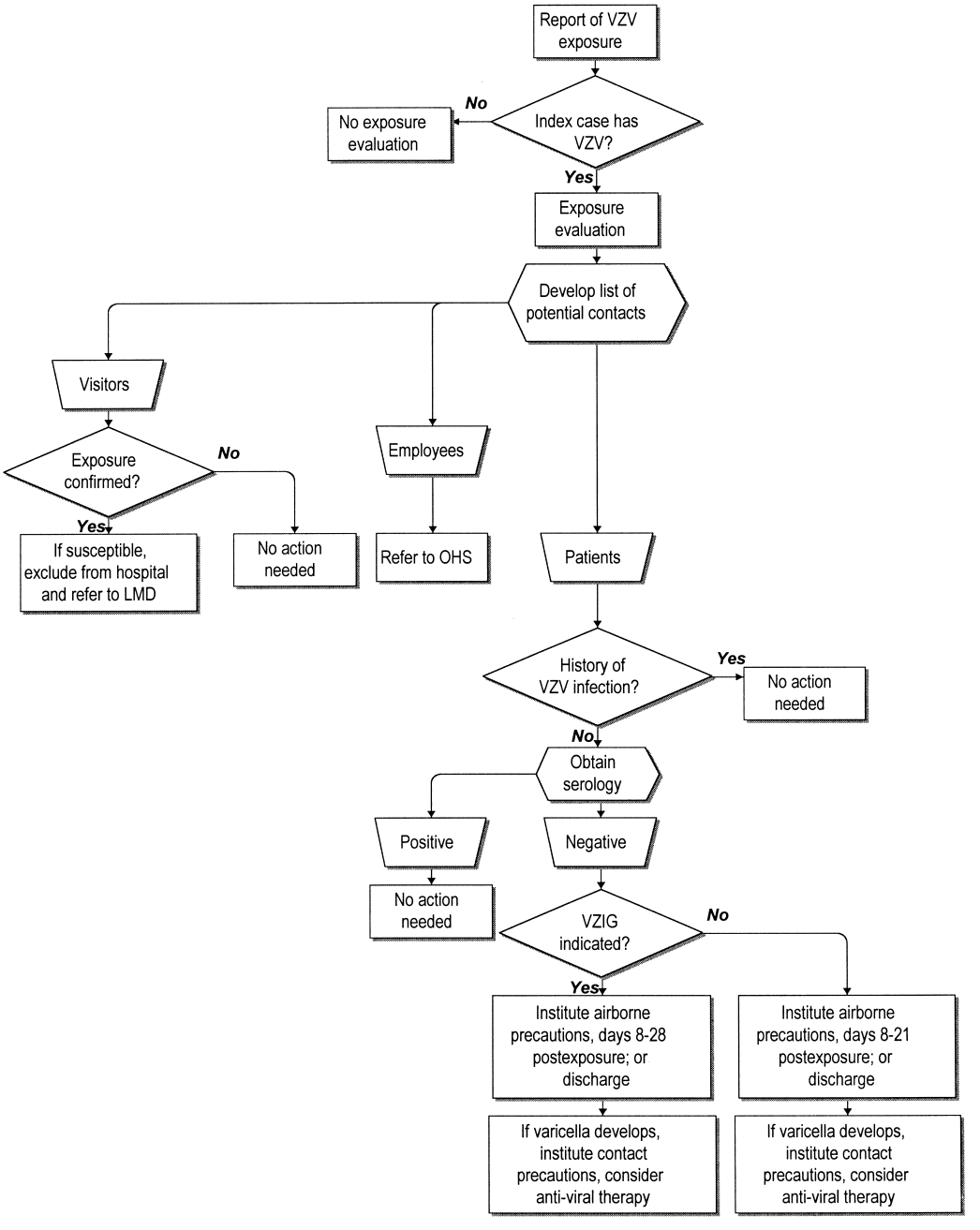

Figure 24.1 Management of VZV exposures in health care settings. A suggested algorithm for management of VZV exposures to visitors, employees and patients in health care settings is shown. VZIG, varicella-zoster immune globulin

Table 24.2 Infection control guidelines for the prevention and management of nosocomial VZV infection in patients and visitors

A. *Screening pediatric patients and visitors*

1. Both inpatients and outpatients on the Pediatric Service should be screened for infectious disease exposures and/or communicable infectious diseases, including VZV. Patients with a potentially communicable disease should be evaluated by the admitting physician for the necessity of isolation including those children who have had VZV immunization.
2. All hospital visitors under 13 years of age should be screened for the presence of actual or incubating infectious diseases.
3. The immunization status of all children should be reviewed during outpatient and inpatient hospital visits. Varicella vaccine should be offered to children as per recommendations of the Advisory Committee on Immunization Practices and American Academy of Pediatrics.

B. *Management of patients exposed to VZV*

1. Confirm that the source case has VZV infection. Typical varicella-zoster may be diagnosed by history and physical exam. In uncertain cases, diagnosis may be aided by Tzank prep, direct immunofluorescent stain, culture and/or PCR.
2. If VZV infection is diagnosed in the source case, a determination should be made of whether exposure has occurred. Exposure is defined as being in an enclosed airspace with the source case (e.g., same room) or in intimate face-to-face contact with the source in an open area during a potentially contagious stage of illness. Varicella is considered contagious beginning 48 hours prior to the onset of rash and until all lesions are dried and crusted, which is generally 5 days in immunocompetent hosts or longer in immunocompromised hosts. Zoster is considered contagious only from the onset of rash.
3. Susceptible patients are placed on airborne precautions if available. If unavailable, they should be placed in private negative pressure rooms if available. If feasible, only varicella immune personnel should care for patients during the incubation period. If nonimmune personnel are used, they should don a N-95 respirator prior to room entry. Isolation should be maintained from days 8 to 21 post-exposure, unless the patient received VZIG, in which case isolation is continued from days 8 to 28 following exposure. If varicella occurs, the patient is also placed on contact isolation.
4. All exposed patients should be considered for VZIG prophylaxis using standard criteria.
5. Attempts should be made to discharge all susceptible exposed patients during the incubation period.

C. *Management of patients with chickenpox or zoster*

1. All patients with chickenpox or zoster are placed on airborne, if available (private negative pressure room, air exhausted directly to the outside, $\geq$6 air exchanges per hour) and contact isolation. Gloves are worn when entering the room; gowns are worn when contact with the patient or environmental surfaces is likely. Immune health care workers may enter the room without a mask.

Table 24.2 (*cont.*)

2. Susceptible employees should refrain from the care of patients with VZV infection when possible. When not possible, they should wear an N-95 respirator.
3. Patients are maintained on isolation until all lesions are dry and crusted.

D. *Management of staff with varicella or zoster infection*

1. Staff with varicella or zoster on exposed areas are excused from work until all lesions are dry and crusted. Staff with zoster on unexposed areas may work if lesions are covered.
2. Staff with varicella or zoster should be offered antiviral therapy.

minimize the opportunity for transmission. However, it is generally impossible to determine precisely when infants are removed from isolettes for procedures, feeding, and stimulation. Thus, when exposure to VZV occurs in a NICU, it is recommended that VZIG be administered liberally to preterm infants less than 28 weeks gestation or infants older than 28 weeks gestation whose mothers do not have a history of varicella or are seronegative.

Previously immunized children exposed to chickenpox and hospitalized during their incubation period may develop chickenpox while hospitalized. While recent studies have suggested that modified, mild chickenpox may occur in up to 10% of immunized children (see Chapter 23), such children may be infectious. To prevent possible nosocomial exposure, VZV serology may be obtained in such children upon admission. If antibodies are detected, the child is considered immune and does not require isolation.

VZV immunity among health care workers

Varicella is primarily a disease of childhood in countries with temperate climates with the peak incidence of infection occurring in younger children 1 to 4 years of age (Wharton, 1996). However, it has been estimated that 4 to 8% of persons older than age 20 are susceptible to varicella (Gray et al., 1990; Miller et al., 1993). There is also a suggestion that a larger proportion of adults in the US and the UK may be susceptible due to immigration from countries with tropical climates (Sinna, 1976; Ooi et al., 1992).

Studies have noted that 60 to 86% (median 78%) of health care workers in the US report a history of varicella (Myers et al., 1982; Steele et al., 1982; Hyams et al., 1984; Alter et al, 1986; Krasinski et al., 1986; Haiduven-Griffiths & Fecko, 1987; Stover et al., 1988; McKinney et al., 1989). A history of prior varicella by a health care worker is highly correlated with immunity as measured by serology. Only 0 to 1.6% (median 0%) of health care workers with a positive history were serosuscept-

ible (Myers et al., 1982; Steele et al., 1982; Shehab & Brunell, 1984; McKinney et al., 1989). However, a history of prior household exposure to VZV is not a reliable indicator of immunity in the absence of clinical illness (Myers et al., 1982). Thus, a history of varicella is supported by statements such as "I had chickenpox when I was five years old" or "My mother said that I had chickenpox when I was two." rather than statements such as "All my brothers and sisters had chickenpox, so I must have also had chickenpox."

Among hospital personnel with a negative or uncertain history of VZV infection, reported serosusceptibility has varied from 4 to 47% (median 15%) (Myers et al., 1982; Steele et al., 1982; Shehab & Brunell, 1984; Alter et al., 1986; Krasinski et al., 1986; Haiduven-Griffiths & Fecko, 1987; Stover et al., 1988; Weber et al., 1988; McKinney et al., 1989). Overall, the susceptibility of health care workers to varicella has ranged from 1 to 7% (median 3%) (Steele et al., 1982; Gustafson et al., 1984; Shehab & Brunell, 1984; Alter et al., 1986; Krasinski et al., 1986; Stover et al., 1988; McKinney et al., 1989; Haiduven et al., 1994). Following nosocomial exposure to VZV infection, 2 to 16% of susceptible staff have developed clinical varicella (Krasinski et al., 1986; Weber et al., 1988; Miller et al., 1993). However, five of five pediatric residents with a negative history of varicella (two of whom had positive serologies by indirect fluorescent antibody) developed chickenpox 3 to 23 months (median 8 months) after starting their residency (Oshiro et al., 1996). Notably, only one resident had a known nosocomial exposure.

Serologic testing for VZV

All health care workers, including volunteers and per diem workers, should be screened for VZV immunity at the time of their initial-employment evaluation. Alternatively, current employees may be screened at the time of their annual tuberculosis and immunization evaluation or via a special program. Employees with a history of VZV infection may be considered immune. Employees without a definitive history of VZV infection should undergo serologic testing, and if negative, be immunized as will be discussed below. A suggested algorithm for evaluating health care workers immunity to VZV is shown in Figure 24.2.

At present, none of the currently available serologic assays are ideal to detect immune individuals or to detect seroconversion after immunization with VZV vaccine (LaRussa et al., 1987; Provost et al., 1991). Serologic tests used to detect VZV antibodies have included complement fixation (CF), indirect fluorescent antibody (IFA), fluorescent antibody to membrane antigen (FAMA), indirect hemagglutination (IHA), immune adherence hemagglutination (IAHA), radioimmunoassays (RIA), enzyme linked immune-absorbent assay (ELISA), gp ELISA, and latex agglutination (LA). The complement fixation (CF) test is not sufficiently

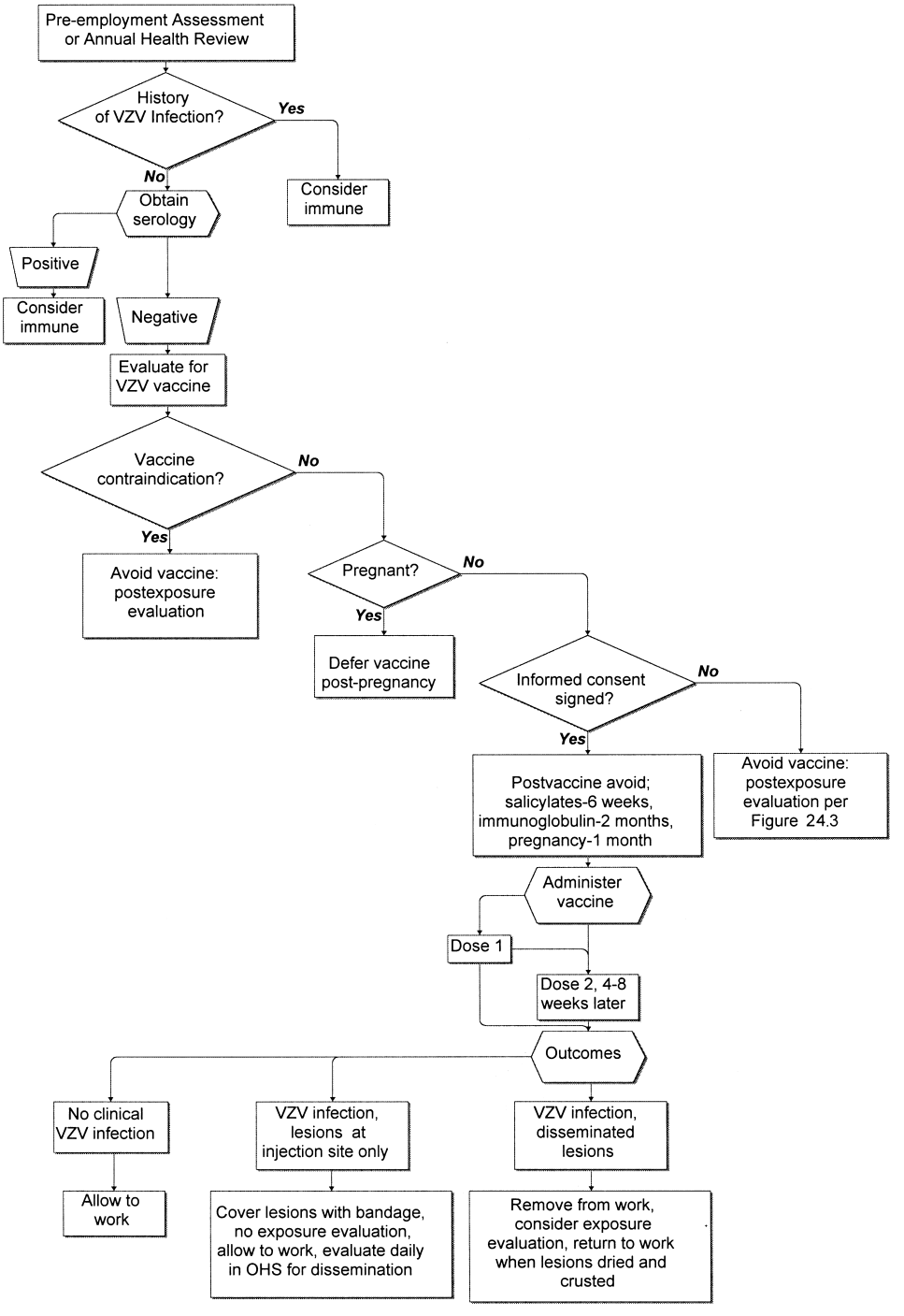

Figure 24.2 Assessment of health care workers for VZV immunity. A suggested algorithm for assessing health care workers for VZV immunity including assessment for VZV vaccine is shown.

sensitive for screening purposes. The IFA, FAMA, and RIA are sensitive, but are not available for general clinical use. ELISA is a useful test to identify individuals who require immunization, but these assays may miss persons with low antibody levels. However, it is safe to immunize someone who is already immune to VZV. In addition, ELISA may not detect seroconversion after immunization. The LA assay is more useful to identify seroconversion after immunization, but may be difficult to interpret for those inexperienced with this assay and it is not automated. Moreover, there is a prozone effect wherein persons with high antibody titers may have a negative result by LA unless the serum is serially diluted. Health care workers without a history of chickenpox have developed chickenpox despite being seropositive by IFA, ELISA or LA assay (Oshiro et al., 1996, L. Saiman and D. Weber, personal communication) and secondary attacks have been documented (Junker et al., 1991). The gpELISA developed by Merck utilizes glycoproteins of VZV as the antigen and is thought to lack specificity, detecting higher levels of conversion after immunization than other assays and detecting antibodies in persons who develop chickenpox. The gpELISA is also not commercially available. Thus, more accurate serologic testing is sorely needed.

Immunization of susceptible health care workers

Immunization of nonimmune health care workers with the varicella vaccine has been recommended by the Advisory Committee on Immunization Practice (ACIP), the Hospital Infection Control Practices Advisory Committee (HICPAC), the American Medical Association (AMA), the authors and other infectious disease experts (ACIP, 1996; Weber et al., 1996; CDCP, 1997; AMA, 1998; Bolyard et al., 1998; Burns et al., 1998; Saiman et al., 1998; Stover & Bratcher, 1998; Saiman & Gershon, 1999). There are several compelling reasons why health care workers should be immune. First, health care workers with incubating or clinical varicella have transmitted VZV infection to hospitalized patients. Such infections may lead to substantial morbidity in high risk patients. Second, health care workers are at risk of acquiring VZV from patients with chickenpox or zoster, and as described previously, even healthy adults have a substantial risk of varicella complications. Further, secondary VZV infections may occur among their hospital contacts including patients, coworkers, and visitors. Finally, the presence of susceptible hospital staff results in significant costs for health care organizations as described above. We believe that mandatory vaccination programs are preferable to voluntary programs. However, legal or contractual restraints may preclude such mandatory immunization programs.

Standard guidelines should be practiced for varicella immunization including proper vaccine storage, administration, assessment of contraindications and

precautions, medical record documentation, and informed consent (see Chapter 23). The following information should be recorded in the medical record: employee name, date, vaccine given, lot number, site of immunization, and informed consent. In the US, significant adverse reactions should be reported to the Food and Drug Administration (FDA) via the vaccine adverse events reporting system (VAERS). Susceptible female workers planning a pregnancy should be counseled about congenital varicella and neonatal varicella. In consultation with their obstetricians, it is recommended that attempting pregnancy be deferred until 1–3 months after immunization (ACIP, 1996, Chapter 16). Susceptible male health care workers planning a family can be immunized.

Adolescents and adults require two doses of vaccine administered 4 to 8 weeks apart. If a worker is not immunized within 8 weeks, the second dose can be given at longer intervals; there is no need to restart the vaccine series. Additionally, the second dose should be given even if a vaccine-associated rash developed after the first dose. Studies have reported a seroconversion rate of approximately 75% four weeks after the first dose and 90% four weeks after the second dose (Gershon et al., 1992; Gershon, 1995; Arvin, 1996). Antibody levels have been shown to persist at least one year in 97% of adolescents and adults given two vaccine doses. In adults, a 56–70% reduction of varicella following household exposure has been demonstrated (Gershon et al., 1988; Arvin & Gershon, 1996). The risks of transmission of varicella to close susceptible contacts of immunized immunocompetent persons appears to be very low (less than 1% of healthy household contacts) and does not occur without a vaccine-associated rash (Tsolia et al., 1990). Diaz and colleagues did not demonstrate any transmission of varicella from 37 healthy vaccinated children to their 30 susceptible immunocompromised siblings (Diaz et al., 1991). However, none of the healthy children had developed a vaccine-associated rash.

The peak occurrence of side effects from varicella vaccine that have been reported in adults following the first and second dose respectively are as follows: fever $>100°$F, 10.2% and 9.5% (0–42 days); injection site complaints at 0–2 days, 24.4% and 32.5%; varicella-like rash at injection site 3% (6–20 days) and 1% (0–6 days); and generalized varicella-like rash 5.5% (7–21 days) and 0.9% (0–23 days). Rash is more common after the first dose than the second dose and the median number of lesions is five. Recent studies using PCR to distinguish between wild type VZV and the vaccine strain have indicated that most persons presenting within two weeks of vaccination with a vesicular rash have wild type virus infection indicating an exposure prior to immunization (LaRussa et al., 1992). Such workers should be questioned about possible exposures to chickenpox or zoster in the workplace as well as at home.

Workers should be educated about the possible development of a vaccine-associated rash. If any rash develops in a health care worker after immunization,

the worker should be evaluated by the Occupational Health Service to determine if the rash is consistent with varicella. It should be remembered that the rash may be papular and not vesicular and that low concentrations of virus are present. PCR has been performed on scrapings from lesions to distinguish wild type from vaccine strains (LaRussa et al., 1992). If the vaccine-associated rash cannot be covered with a bandage and/ or clothing, the worker should be furloughed for the duration of the rash. If the rash can be covered, some centers allow the worker to have patient contact and some centers limit this to contact with nonimmunocompromised patients. Covering varicella lesions is consistent with recent recommendations, which advised that health care workers with dermatomal zoster that can be covered may work (Bolyard et al., 1998). However, some centers choose to exclude workers with dermatomal zoster until all lesions are dry and crusted.

Performing a post-immunization serology is controversial and may not be cost effective.. As described above, commercial tests may lack the sensitivity to detect the lower antibody levels associated with immunization compared with natural infection. Some centers do not perform serologic testing because the rate of seroconversion among adults is over 90% (Weber et al., 1996). However, some centers perform post-immunization serologic testing and do not assign seronegative workers to patients with VZV infection. Still other centers give a third dose of VZV vaccine to workers who have not seroconverted, although there are no data supporting this practice. Health care workers frequently have questions about varicella vaccine. Prepared questions and answers in the form of an information packet often facilitate the immunization process. Suggestions for such questions and answers are provided in Table 24.3.

At present, there are inadequate data regarding the long-term risk to immunized health care workers who are caring for patients with chickenpox or zoster. In view of the protection conferred by the vaccine, we assign immunized workers to such patients. Workers may benefit from exposures as this may "boost" their immunity. Should VZV disease develop, it will most likely be mild with fewer lesions and therefore potentially less infectious.

Management of health care workers exposed to VZV

All potential exposures to varicella need the coordinated assessment of Infection Control and the Occupational Health Service. Employees potentially exposed to varicella or zoster should be evaluated to determine their immune status as soon as feasible and ideally within 24 to 48 hours of exposure so that VZIG can be given if needed. All efforts to confirm VZV infection in the source case should be made. Generally, history and physical exam alone are sufficient to diagnose varicella or zoster. A Tzank preparation will not distinguish between VZV infection and herpes

Table 24.3 Frequently asked questions regarding varicella vaccine and suggested responses

Question	Suggested response
1. Is varicella a serious disease in the adult?	Yes. Between 1 and 2% of adults will require hospitalization.
2. Who recommends varicella vaccine for health care workers?	The Advisory Committee on Immunization Practices (CDC), the American Academy of Pediatrics, and many infectious disease experts.
3. Do all employees need varicella vaccine?	No. Employees with a history of varicella (chickenpox) or zoster (shingles) should be considered immume. Employees with a negative or uncertain history of varicella should have a blood test and be tested for immunity via serology. Employees with negative serology should be considered vaccine candidates.
4. How is the vaccine administered?	Adults should receive two vaccine doses about 4–8 weeks apart.
5. Is serological testing after vaccine recommended to assure immunity?	The currently available serologic tests may not be sufficiently sensitive to reliably detect the antibody level induced by the vaccine.
6. What is the frequency of local side effects?	About 25–30% of vaccinees experience injection site complaints.
7. What is the frequency of varicella-like rash?	About 1–5% of vaccinees develop a local rash and/or systemic rash.
8. What is the frequency of serious side effects?	Very low and much lower than seen with chickenpox in adults.
9. Can the varicella vaccine be administered simultaneously with the MMR?	Yes. A separate syringe and injection site should be used.
10. Can the vaccine be given to a health care worker who lives with a pregnant woman an immunocompromised host, or a woman trying to get pregnant?	Yes. The best way to protect high-risk individuals is "herd" immunity, i.e. ideally, persons in closest contact with the high risk person are immune to varicella. In addition, the risk of possible transmission of vaccine virus from vaccine associated rash is much less than the risk of transmission from wild-type infection.

Table 24.3 (*cont.*)

Question	Suggested response
11. Will a booster be required?	Currently there is no recommendation for a booster. Immunity induced by the vaccine appears to persist for years. However, it is possible that a booster will be recommended at some time in the future.
12. Can I get shingles after having the vaccine?	Yes. However, the likelihood of shingles appears to be substantially reduced compared to having been infected with the wild virus.

simplex virus infection. Direct immunofluorescent staining of vesicular fluid is a more specific test, but is not universally available.

In all potential exposures, the potential for VZV acquisition should be assessed. We define VZV exposure as being within a confined airspace (e.g. same room) or face-to-face contact with an infectious patient (Weber et al., 1996). As previously stated, employees with a history of VZV infection may be considered immune. Employees without a previous history or an uncertain history should be serologically tested for immunity if they have not already had such testing. Employees with a positive test can be considered immune. All susceptible employees who did not wear a respirator should be considered for post-exposure prophylaxis with VZIG according to standardized guidelines (see Chapter 21). Such workers are removed from duty from days 8 through 28 post-exposure if VZIG is given and from days 8 through 21 if VZIG is *not* given. Employees who develop varicella may return to work when clinically well and after all lesions are dried and crusted (usually about 5 days).

There are several options for management of immunized health care workers who are exposed to VZV. One proposed option would be to test serologically such employees immediately post-exposure regardless of immune status after immunization. Serosusceptible employees could be retested for an amnestic response 5 to 7 days post-exposure although this strategy is costly and serologic testing may not be accurate in vaccine recipients. Employees testing nonimmune would be relieved from duty from days 8 to 21 post-exposure. A second proposed option would be to remove immunized employees only if they develop clinical varicella. Some centers have advocated daily visits to Occupational Health Service for evaluation and examination for prodromal or disease symptoms and rash. However, if hospitals choose the latter option and an immunized employee develops varicella, this employee could be a potential source case for patients or other

staff. Exposed susceptible health care workers have been reassigned to areas lacking susceptible persons, assigned to perform projects at home, or allowed to continue to work wearing masks as long as they are disease free. Such an alternative management strategy is perhaps more practical and cost-effective in some institutions, but may be problematic in other settings. A suggested algorithm for management of vaccinated health care workers after exposure to varicella is shown in Figure 24.3.

In the collaborative varicella vaccine study conducted in the United States between 1980 and 1993, adult recipients of varicella vaccine were closely followed after a household exposure to varicella, usually to one or more of their children. Of 57 vacinees who had household exposures to varicella, 42 (74%) were completely protected against the disease (Gershon et al., 1988; Arvin & Gershon, 1996). Thus it is estimated that as many as 30% of vaccinated adults may develop mild chickenpox. Those with a breakthrough illness generally had mild infections with a mean number of 43 skin lesions, which is less than 10% of the number of lesions expected with wild type chickenpox (Gershon et al., 1988). It can be predicted therefore that most vaccinated health care workers are likely to be protected and that those who are not will have only a few cutaneous lesions (Hardy & Gershon, 1990). As the spread of varicella is directly related to the presence of a skin rash and to the severity or extent of the skin rash (Tsolia et al., 1990), it is predicted that immunization of health care workers will have the effect of decreasing nosocomial spread of VZV.

Post-exposure prophylaxis may also be provided by administering varicella immune globulin (VZIG), acyclovir, or immunizing nonimmune health care workers. Prophylactic acyclovir (10–20 mg/kg every 6 hours) given for 7 days, beginning one week after exposure to chickenpox, has been used successfully to prevent disease (Asano et al., 1993; Suga et al., 1993; Abe & Bradley, 1995). However, the prophylactic use of acyclovir was associated with a decreased rate of seroconversion; approximately 50% of the children remained serosusceptible.

Several studies have investigated the ability of the Oka strain varicella vaccine to attenuate or prevent clinical varicella in healthy children when administered as post-exposure prophylaxis (Asano et al., 1977, 1982, 1993; Udeda et al., 1977; Katsushima et al., 1982, 1984; Naganuma et al., 1984; Sugino et al., 1984; Tsujino et al., 1984; Arbeter et al., 1986; Boda et al., 1986). The vaccine was most effective when administered within three days of exposure and is generally ineffective when administered more than five days after exposure. In a double-blind, placebo-controlled study in children, lots of varicella vaccine prepared for research were reported to be 67% effective in completely preventing illness and 100% effective in modifying varicella to a milder illness when administered within five days of exposure (Arbeter et al., 1986). The efficacy of post-exposure prophylaxis in adults is unknown.

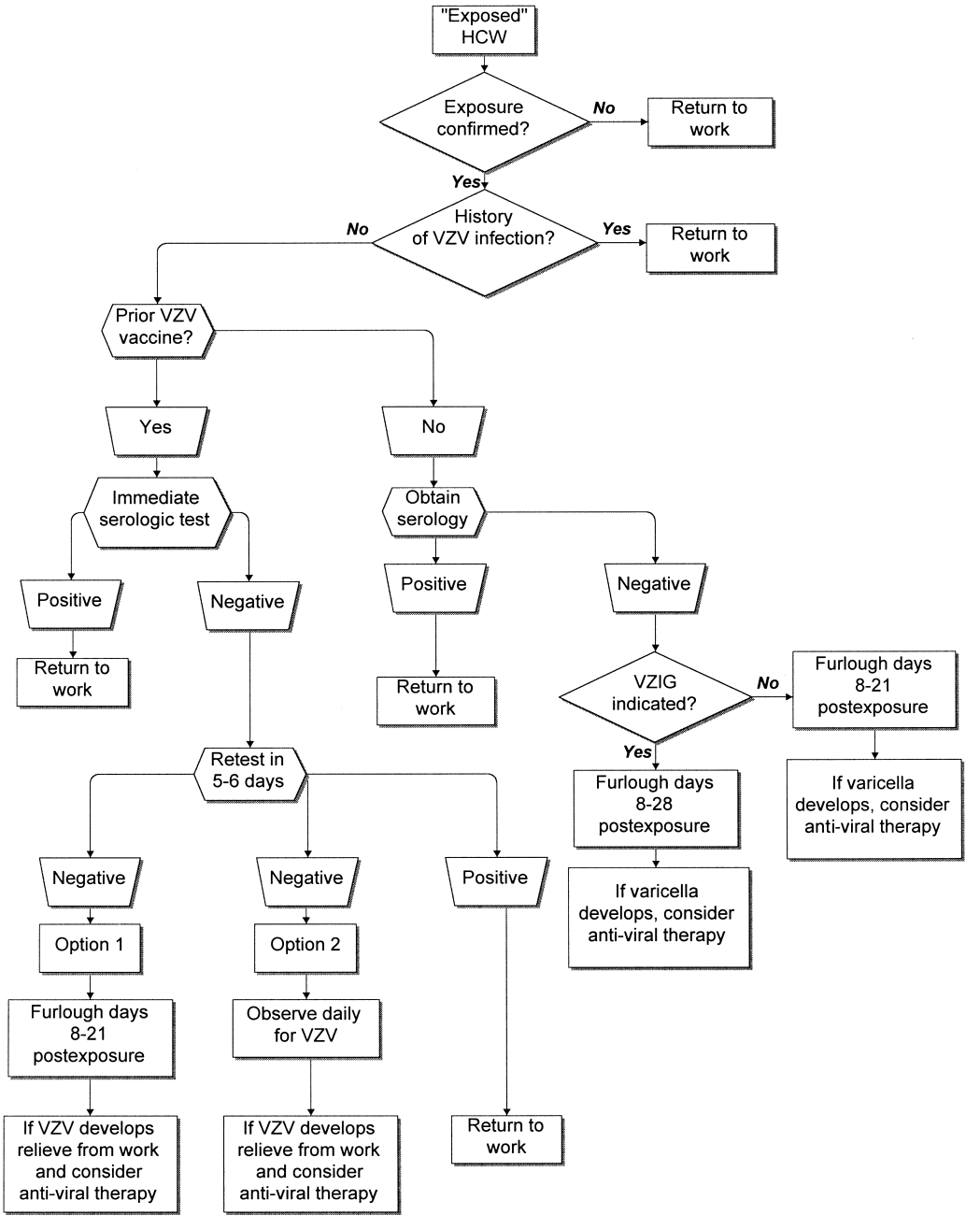

Figure 24.3 Management of health care workers exposed to VZV. A suggested algorithm for managing health care workers exposed to VZV including immunized workers is shown. HCW, health care worker; VZIG, varicella-zoster immune globulin.

Management of the VZV infected health care worker

All health care workers with VZV infection should be evaluated by the Occupational Health Service. Following confirmation of infection, staff and patients exposed to the HCW should be appropriately managed (Figure 24.2 and 24.3 and Table 24.2). All health care workers should be considered for recommended antiviral therapy (see Chapter 21).

Conclusions

All health care workers should be immune to varicella. Employees who do not have a history of VZV infection and are serologically negative should be immunized with VZV vaccine. A VZV immunization program will reduce the risk of VZV infection and its complications for the serosusceptible employees, and decrease the likelihood of nosocomial transmission of VZV to patients. In addition, a program of VZV immunization will probably be a cost-effective strategy for health care facilities. Future research is needed to better define the risks, if any, of transmission of vaccine strain VZV to patients following employee immunization. In addition, future research should better define the post-exposure management and outcome of the immunized health care worker exposed to VZV.

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25

Immunization against herpes zoster

Myron Levin

Introduction

Reactivation resulting in infectious VZV in ganglia may occur sporadically throughout life, but the clinical importance of this phenomenon may be limited by VZV-specific immune responses. This might be accomplished either by a direct mechanism to inhibit reactivation or by rapid local clearing of reactivated virus, such that no signs or symptoms occur (subclinical reactivation). Infectious VZV has not been recovered from ganglia at autopsy, except in the special circumstance where the deceased had HZ at the time of death. There are no data on reactivation frequency as a function of age, but the concept of subclinical reactivation is supported by fluctuations in VZV-specific antibody and asymptomatic viremia observed during longitudinal studies of certain populations (Gershon et al., 1982; Ljungman et al., 1986; Wilson et al., 1992), and by case reports of neuropathic syndromes without cutaneous manifestations (zoster sine herpete) (Barrett et al., 1993; Gilden et al., 1994). Subclinical reactivation might be an important factor in maintaining VZV-specific immunity long after primary infection with varicella.

Role of VZV-specific immunity

There is very strong evidence that deficient VZV-specific immunity is closely correlated with the occurrence of clinically apparent reactivation. This has been observed in three clinical settings: (i) HZ occurs with an increased frequency at an early age in children who were exposed to VZV in utero (maternal infection) or in early infancy, probably because in these situations the normal immune response does not develop (Baba et al., 1986; Vachvanichsanong, 1991; Terada et al., 1994); (ii) the age-specific incidence and severity of HZ is much greater in both pediatric and adult patients with immunosuppressive illnesses (e.g., HIV infection) and in those receiving immunosuppressive therapies (Feldman et al., 1973; Dolin et al., 1978; Ljungman et al., 1986; Colebunders et al., 1988; Wilson et al., 1992; Kawasaki et al., 1996; Derryck et al., 1998); (iii) the incidence and severity of HZ increases

with increasing age, which in turn correlates directly with a decline in VZV-specific immunity (Hope-Simpson, 1965; Miller, 1980; Berger et al., 1981; Burke et al., 1982; Ragozzino et al., 1982; Donahue et al., 1995).

Importance of cell-mediated immunity (CMI)

The critical immune responses are probably T cell-mediated. This presumption is based on several observations: (i) Th1 and cytotoxic T cell activity are essential for recovery from herpesvirus infections (Arvin et al., 1986; Manickan et al., 1995); (ii) varicella and HZ occur with normal frequency and severity in children with congenital immune deficiencies limited to agammaglobulinemia and in adults with common variable immune deficiency; (iii) immunocompromised patients who lack only antibody (e.g. multiple myeloma patients before chemotherapy) have no excess of HZ; (iv) bone marrow transplant recipients who receive supplemental intravenous γ-globulin nevertheless suffer an increase in frequency and severity of HZ (Ljungman et al., 1986; Wilson et al., 1992; Redman et al., 1997).

VZV-specific immunity and aging

VZV-specific antibody measured by several different methods in 55–70-year-old individuals is at levels which are 80–90% of those of young adults (Berger et al., 1981; Gershon & Steinberg, 1981; Hayward et al., 1996). Conversely, starting in young adulthood, there is a progressive, linear decline in VZV-specific CMI, as measured by delayed hypersensitivity using VZV skin test antigen or by in vitro lymphocyte proliferation assay (LPA) in response to VZV antigen (Miller, 1980; Berger et al., 1981; Burke et al., 1982). This is also demonstrated by a cross-sectional analysis of responder cell frequency (RCF) as a function of age. RCF is a limiting dilution assay that enumerates peripheral blood mononuclear cells (PBMC) that respond to VZV antigen in vitro (Hayward et al., 1996). The readout is the number of PBMC required to detect a single VZV-responding lymphocyte. Thus, a falling RCF indicates an increase in the prevalence of specific memory cells. The RCF assay measures primarily CD4+ memory T cells that express the CD45RO phenotype (Hayward et al., 1994). The responding population includes cells with MHC class II-restricted cytotoxicity for targets expressing VZV antigens (Hayward et al., 1986) and cells making either γ-interferon or IL-4 (Zhang et al., 1994). The RCF assay has good intra- and inter-assay reproducibility, and is more suitable than LPA for the multi-year longitudinal studies that will be described below. The frequency of responding cells increases from undetectable ($<$1:100 000) in varicella-naïve children, to approximately 1:40–50 000 in adolescence in children with prior varicella infection. This peaks in young adulthood at 1:20 000 (possibly because of environmental exposure), and then declines progressively thereafter with increasing age to 1:60–80 000 (Figure 25.1).

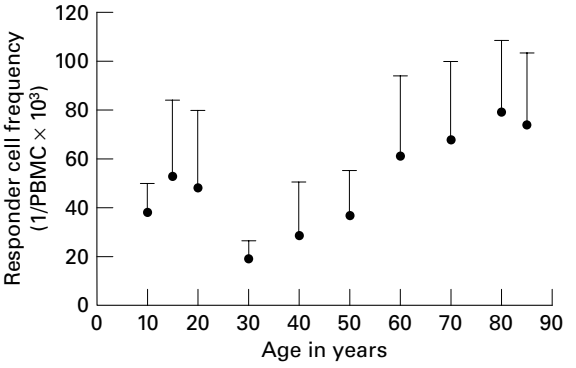

Figure 25.1 Mean (± SD) of VZV responder cell frequency in PBMC as a function of age. Number of observations are: 0–9 years, 33; 10–14 years, 26; 15–19 years, 16; 20–29 years, 14; 30–39 years, 13; 40–49 years, 16; 50–59 years, 74; 60–69 years, 156; 70–79 years, 66; 80 years, 10.

Prophylactic immunization of the intact host

Proposed model

The information described in the previous section can be encompassed by a model in which random reactivation of VZV occurring sporadically in latently infected ganglia is limited by VZV-specific T cell-mediated immunity. If, or when, these immune responses fall below a critical threshold, then reactivated VZV will propagate within a ganglion (ganglionitis is the observed pathology), resulting in prodromal, acute, and, in some cases, post-herpetic neuralgia. Antegrade spread of VZV to the skin from the ganglionitis produces the dermatomal eruption. Consistent with this model is the observation of Tanaka et al. that the skin test response to VZV antigen was negative in 1 of 8 patients within the first two weeks after onset of ophthalmic herpes zoster, whereas 25 of 27 matched controls had skin test reactivity (Tanaka et al., 1984).

Central hypothesis for immunization

The central hypothesis for the prevention of HZ follows from this model; namely, that any means of *enhancing critical immune responses in at-risk individuals will result in prevention or attenuation of HZ.* Clinical evidence in support of this presumption is the paucity of second cases of HZ in normal elderly individuals, in whom an attack of HZ presumably serves as an immunizing event that is sufficient to protect them for the remainder of their lives (Hope-Simpson, 1965). A corollary of the model is that any phenomenon which: (i) alters the quantity or genotype of latent VZV in ganglia (such as vaccination of naïve individuals); or (ii) alters the immune response to VZV (such as antiviral therapy of varicella or presence of per-

Table 25.1 VZV vaccination of older individuals

Reference	*n*	Age (yr)	Vaccine (PFU × 10^3)	Vaccine Antigen (U/ml)	Immune test	Duration (yr)
Berger et al. (1984)	33	55–65	2.7[c]	n.a.	LPA	<0.16
Berger et al. (1985)	29	50–65	6–12[c,d]	n.a.	LPA, Skin test	<0.10
Starr et al. (1987)	25	50–74	4.4[e]	6.0	LPA	<0.33
Sperber et al. (1992)	97	18–49	0.28–2.8–2.8[e]	0.08–8.4	Antibody	1
Takahashi[a]	37	>50	3.0[d]	4.0	Skin test	1
Berger et al. (1998)	200	55–88	3.2–8.5–41.7[f]	0	LPA/RCF	1
Levin et al. (1998)	202	60–82	3–6–12[e]	4.0–12.0	RCF/CK/Antibody	6–8
Hayward et al. (1994b)[b]	167	55–79	4.0 vs. nil[e]	5.2	RCF/CK/Antibody	3

Notes:

[a] Personal communication; [b] Also Levin and Hayward, unpublished; [c] Manufacturer, RIT; [d] Received VZV skin test prior to vaccination; [e] Manufacturer, Merck Research Laboratories; [f] Manufacturer, Pasteur Merieux Connaught; n.a., not available; LPA, lymphocyte proliferation assay; RCF, responder cell frequency assay; CK, induced cytokine array.

sistent maternal antibody at the time of infection); or (iii) decreases the natural boosting from environmental exposure (e.g., as a consequence of universal childhood vaccination to prevent varicella) might influence HZ disease.

Potential problems with active immunization

At least eight studies, spanning a 15-year period, have utilized active immunization to enhance VZV-specific immunity in previously infected individuals (Table 25.1). One goal of these experiments was to establish the safety of administering live attenuated vaccine-strain varicella to older individuals. Although aging is associated with declining VZV-specific CMI, vaccination was felt to pose a minimal risk, given the clinical observations that HZ ultimately heals in elderly patients and that they are protected when exposed to varicella. In all studies to date vaccine-related morbidity has not been a problem.

A second goal was to determine the immunogenicity of this live vaccine, since this might be adversely affected by the residual (albeit lower) VZV-specific CMI present in many older individuals and by their ability to maintain high levels of anti-VZV antibody. Once again clinical observations, the most important of which is the rarity of second attacks of HZ, suggested that a robust recall immune response is intact in aging individuals. The immunologic laboratory correlate of this is demonstrated by three studies. In 1980, before the first vaccination attempt,

Hata et al. showed that skin testing with VZV antigen was negative in 14 of 25 HZ patients within 4 days of onset, but repeat testing after 11 days was uniformly positive (Hata, 1980). Berger et al. studied LPA responses in 20 older patients within 4 days of onset of HZ, at which time only 15% of patients had a strong response, 30% had a weak response and 55% had no response. Six weeks later this was reversed: 55% had a strong response, 15% had no response, and 30% were still weak (Berger et al., 1985). We subsequently did a similar experiment in which eight matched control individuals older than 60 years had an RCF of 1:82 000 ($\pm$ 16 000), whereas ten patients studied up to 6 years after HZ had an RCF increased to 1:35 500 ($\pm$ 24 000) (Hayward et al., 1991). These three experiments indicate that older individuals can significantly increase VZV-specific CMI responses to endogenous VZV antigen, and maintain them for many years.

Immunogenicity of varicella vaccine in elderly vaccinees

In 1984 Berger et al. immunized 33 volunteers, 55–65 years old, who were VZV-seropositive, but had a negative VZV-specific LPA, with 2700 plaque-forming units (PFU) of the Oka strain of varicella (Berger et al., 1984). Eight weeks later 17 vaccinees had a strong CMI response, 11 had a weak response, and 5 (15%) failed to respond. Antibody was increased in more than 50% of vaccinees. A follow-up study was done on 50–65-year-old volunteers who had a negative skin test response to VZV antigen before vaccination (Berger et al., 1985). After administration of 6000 ($n = 12$) or 12 000 ($n = 17$) PFU of varicella vaccine approximately 60% of vaccinees had a positive LPA at 2–3 weeks, whereas only 1 of 9 placebo recipients did so. Almost all placebo and vaccinated volunteers converted their skin test and had a rise in antibody, indicating that inactivated varicella antigen used for skin testing might be a useful immunogen, and that skin testing might be more sensitive than the LPA then in use.

In 1987 Starr et al. immunized 25 volunteers 50–75 years of age with a varicella vaccine similar in process and dose (3500 PFU) to that currently used for childhood immunization (Starr et al., 1987). The LPA response 4 months post-vaccination was age-related. Eighty-five percent of 50–59-year-old vaccinees had an increase in LPA, whereas only 20% of those older than 70 years developed an increase, thus raising questions about the influence of age on the response to active immunization and possibly explaining the correlation between the severity of HZ and age.

During numerous clinical trials of primary immunization with the varicella vaccine in this country some seropositive adolescents and adults received vaccine (Arbeter et al., 1986). This occurred without noteworthy reactions and approximately one-half had a 4-fold rise in antibody. These studies are omitted from Table 25.1 because the details are not available and the vaccinees were not elderly, although the small number of vaccinees (mostly young adults) receiving the

booster had a significant increase in LPA response measured at 6–8 weeks.

In 1992 Sperber et al. immunized 102 young adults (18–49 years; mean age of 32 years) divided into six groups receiving 28 000, 2800, or 280 PFU of live vaccine, or heat inactivated vaccine made from the same three live doses (Sperber et al., 1982). In this study only antibody response was measured. The frequency of mild local reactions was similar with all preparations and there were no significant systemic reactions, thereby indicating the safety of large doses of live VZV vaccine. At 42 days after vaccination there was a 3-fold increase in mean antibody titer after 28 000 PFU and a 2-fold increase after the 2800 PFU dose. It was especially noteworthy that the antibody response was similar for the live and the equivalent amount of inactivated vaccine. Antibody response returned to baseline by 11 months. This experiment confirmed and complemented the observation that VZV antigen alone was immunogenic, and indicated that its role as a component of live vaccine preparations deserved additional study. Bergen et al. had previously demonstrated that a one-third reduction of VZV antigen in the presence of a constant quantity of PFU resulted in a decrease in antibody and CMI response at one year after primary vaccination of children (Bergen et al., 1990).

In 1997 Takahashi et al. vaccinated 37 people $\geq$50 years old with commercially available live vaccine (M. Takahashi and K. Yamanishi, personal communication). Seven of eight vaccinees who had a negative skin test with VZV antigen prior to vaccination converted their skin test 5–7 weeks after vaccination, and 9 of 15 with weak or intermediate skin test reactions had an increase in reactivity after vaccination. The skin test converters maintained a positive skin test at one year post-vaccination. The remainder had strong reactions throughout the experiment. Thirty-three of 37 vaccinees showed an increase in anti-VZV antibody.

Berger et al. have published their experience with immunization of 200 volunteers age 55–88 years (mean age of 65 years) (Berger et al., 1998). Groups of 50 vaccinees received 3200, 8500, or 41 650 PFU of a live VZV vaccine, and a control group received pneumococcal vaccine. This study confirmed the safety of large doses of live VZV vaccine in this age range, and demonstrated that local reactions, which occurred in 26–41% of vaccinees, were minor in nature and less severe than those occurring after administration of pneumococcal vaccine. There were no systemic symptoms. A single recipient had a vesicular rash at the injection site. The vaccinees were tested prior to immunization and 6 weeks later for VZV-specific antibody, LPA, and RCF. The CMI assays utilized either crude VZV antigen, purified VZV glycoproteins, or a single VZV gene product (IE62). With all assays the controls had no booster response at 6 weeks compared to baseline, whereas the VZV vaccine recipients had an approximate 2-fold increase in CMI in the LPA assay and a 2–3-fold increase in the RCF assay. Booster responses were most apparent when measured with crude VZV antigen. Antibody concentration increased by 30–40%

after vaccination, but the magnitude of the antibody boost did not correlate with the CMI boost. There was no dose effect for boost in either CMI or antibody.

The deficiencies in the aforementioned studies include (in various combinations): (i) small number of vaccinees; (ii) vaccinees not within the high-risk age group for HZ; (iii) absent or inadequate measures of CMI; (iv) use of skin test antigen (which itself is immunogenic) to define a study group; (v) no information on the persistence of any booster effect.

Many of these problems have been addressed by a study of Levin et al. that describes at least 6 years of follow-up on 202 volunteers who received vaccine in 1988–89 (Levin et al., 1998). The vaccinees were 60–87 years of age, and randomly chosen to receive either 3000, 6000, or 12 000 PFU, or 3000 PFU followed by another 3000 PFU three months later. Less than 25% of vaccinees had local reactions, including those that had two doses of vaccine. These reactions were mild and similar to those described in the large study of Berger et al. Six vaccinees reported a small number (<10) of new skin lesions, usually at the vaccination site, within 21 days of immunization. Only one of these could be shown to contain VZV by virus isolation. There were no systemic symptoms. Immunologic measurements were made prior to immunization, at 3 months (6 months if two doses were received), and annually. Antibody was measured by ELISA. VZV-specific CMI was determined by measuring γ-interferon production by VZV-stimulated PBMC, and by RCF assay.

Antibody at baseline, as previously reported in these older vaccinees, was at levels approximating 85% of levels of young adults. There was a significant antibody boost following vaccination that lasted for only one year. γ-Interferon responses were also boosted, but this effect was lost by one year post-vaccination (Figure 25.2). RCF was enhanced by immunization (Figure 25.3; Curve C), then gradually diminished, but was still apparent, 6 years post-vaccination. The half-life of the RCF enhancement was 56 months. The booster effect was not influenced by the age of the vaccinee, gender, or RCF at time of vaccination. Similar to the observations of Berger et al., there was no discernable dose effect on the magnitude of the RCF during the first two years of follow-up. However, a dose effect became apparent after two years by comparing the highest and lowest doses. Thus, dose was a determinant of the persistence of the response at 4 years (Figure 25.4), but the dose effect was lost at 6 years. It is important to note that the immunologic results in the first year of the studies of Levin et al. and Berger et al. were very similar.

There are four important additional comments pertinent to this experiment. The first is that the group mean RCF was initially boosted to the level observed in people 50 years of age (see Figure 25.1). Secondly, it is likely that the booster effect is underdetected by the RCF assay. This is because the format of the RCF assay used

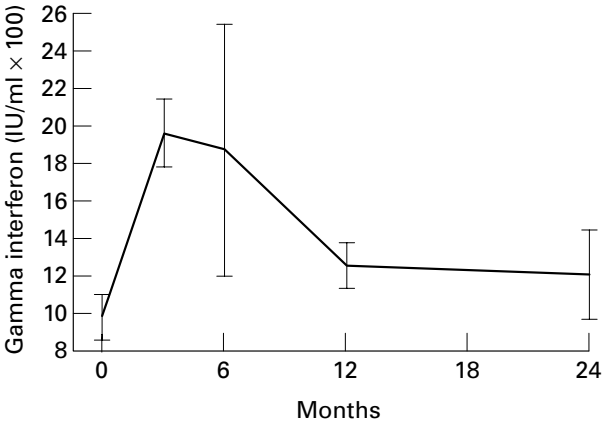

Figure 25.2 Mean (± SD) γIFN synthesis in vitro by PBMC from elderly recipients of live attenuated varicella vaccine. γIFN was measured by ELISA on supernatants from PBMC stimulated with VZV antigen. γIFN level at 3 months was significantly higher than prevaccination level ($P < 0.001$). Number of observations: time 0, 193; 3 months, 196; 6 months, 39; 12 months, 149; 24 months, 53.

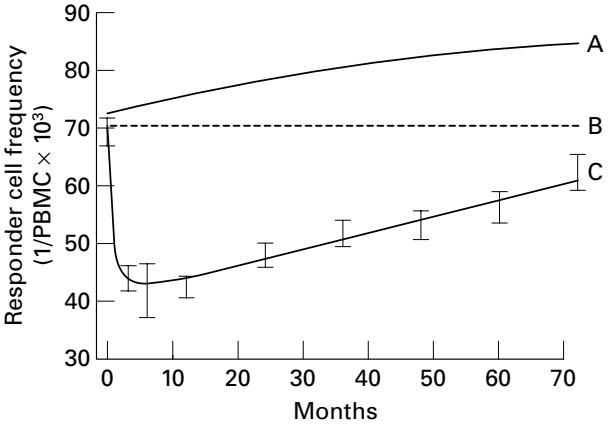

Figure 25.3 Mean (± SD) of VZV responder cell frequency (RCF) in PBMC from elderly recipients of live attenuated varicella vaccine compared with the expected RCF of a cohort of the same age who had not been vaccinated. Curve A represents expected RCF if the subjects had not been vaccinated. This was calculated from the distribution of RCF values of vaccinees at baseline, using the formula: RCF = − 311.38 + 9.80(age) − 0.06(age). Curve B represents the mean baseline RCF of all vaccinees. Curve C represents the RCF of vaccinees at times after vaccination. Frequency at time 0 was significantly less ($P < 0.05$) than at all other times. Number of observations: 0 months, 188; 3 months, 190; 12 months, 186; 24 months, 177; 36 months, 166; 48 months, 160; 60 months, 153; 72 months, 124.

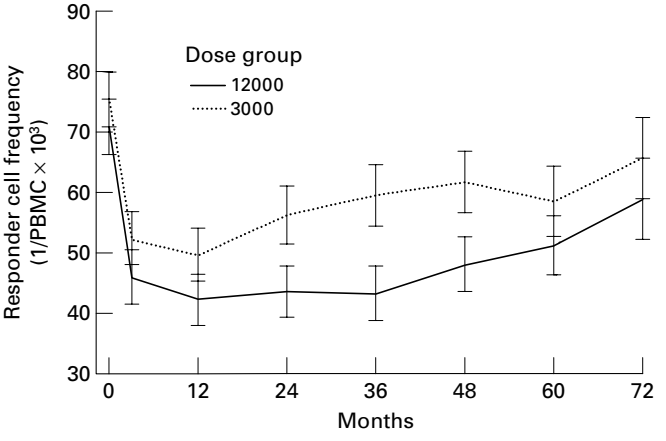

Figure 25.4 Mean (± SD) of VZV responder cell frequency (RCF) in PBMC from elderly recipients of two different doses of live attenuated varicella vaccine. A longitudinal analysis of their time response curves indicated that RCF at the 12 000 PFU dose is significantly greater ($P < 0.05$) through 48 months than at each corresponding point at the 3000 PFU dose. Number of observations: 3000 PFU/12 000 PFU = 0 months, 52/48; 3 months, 52/49; 12 months, 52/46; 24 months, 46/46; 36 months, 41/40; 48 months, 40/38; 60 months, 41/38; 72 months, 28/32.

in this study does not detect responding cells at a frequency of less than 1/100 000 PBMC and did not detect a VZV-specific responder frequency greater 1/12 500 PBMC. Thirdly, judging the booster effect by comparing the response at 6 years to the baseline level at the time of vaccination (Figure 25.3; Curve B) ignores the ongoing and predictable natural decline of VZV-specific immunity which these vaccinees would otherwise have experienced. This baseline decline was calculated from the RCF of vaccinees at baseline regressed on their age at baseline, and is depicted as Curve A in Figure 25.3. This consideration indicates that there was continuing enhancement of RCF in vaccinees (difference between curves A and C in Figure 25.3) at 6 years after vaccination. Fourthly, it is necessary to consider the potentially unique fraction of individuals who have no detectable responding cells after vaccination. At the beginning of this experiment almost 40% of vaccinees had no detectable VZV-responding cells prior to vaccination (Figure 25.5). This percentage of "nonresponder" vaccinees fell to 10–12% during the three years following vaccination; rose to 21% at year 4; and was 38% by year 6. Age was not a predictor of nonresponse in the vaccinated cohort. As mentioned above, the sensitivity of the assay may be an issue here. Moreover, both Takahashi & Yamanishi (personal communication), and Berger et al. (1998), also found that 5–10% of their subjects failed to respond to vaccination. This raises the possibility that the initial nonresponder cohort (regardless of the assay characteristics), and/or those who

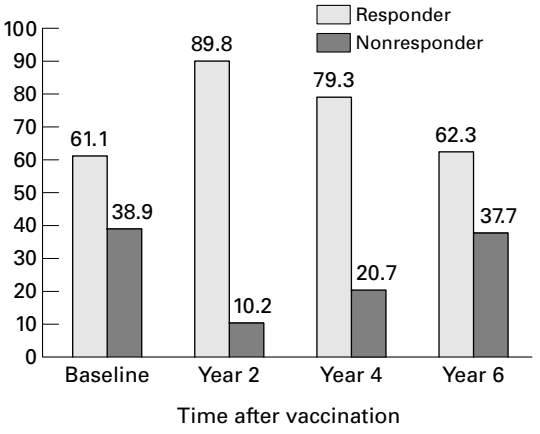

Figure 25.5 Presence of at least one VZV responder cell per 100 000 PBMC (responder) in elderly recipients of live attenuated varicella vaccine at different times after vaccination. Number of observations at each time point is as indicated in Figure 25.3.

fail to boost after vaccination, may consist of individuals who are especially prone to develop HZ, in which case the current vaccination strategy would fail. Furthermore, the loss of detectable responding cells by a large number of vaccinees may define the duration of a protective effect (as does the 56 month half-life of the booster effect), suggesting that additional booster vaccinations will be required at yet to be established intervals.

Immunization of older patients with inactivated vaccine

It had previously been reported that subcutaneous vaccination with a heat-inactivated vaccine or skin testing with inactivated VZV antigen resulted in boosting of antibody titer or skin test response to VZV antigen. The advantage of an inactivated vaccine is that it would pose little threat of serious adverse events in the occasional older vaccinee who had an undetected immune deficiency (e.g., lymphoma) or was unexpectedly varicella-naive. A trial comparing live attenuated and inactivated vaccine was undertaken in 167 individuals, mean age 65 ± 1 years (minimum age 55 years), who were randomized to receive 4000 PFU of Merck Oka strain vaccine or an equal volume of vaccine which had been heat-inactivated at 56 °C for 7 days (Hayward et al., 1994; Levin & Hayward, unpublished). This vaccine contained <2 PFU of VZV. Protein immunoelectrophoresis indicated that the vaccines were identical. There were no differences in the immune responses to the two vaccines measured at 3, 12, 24, and 36 months after vaccination. As in the previous study, significant antibody and γ-interferon boosts were detected at 3 months, but returned to baseline at one year (Figure 25.6). The RCF after both vaccines was improved from 1:65–70 000 at baseline to 1:39 000 at three months post-vaccina-

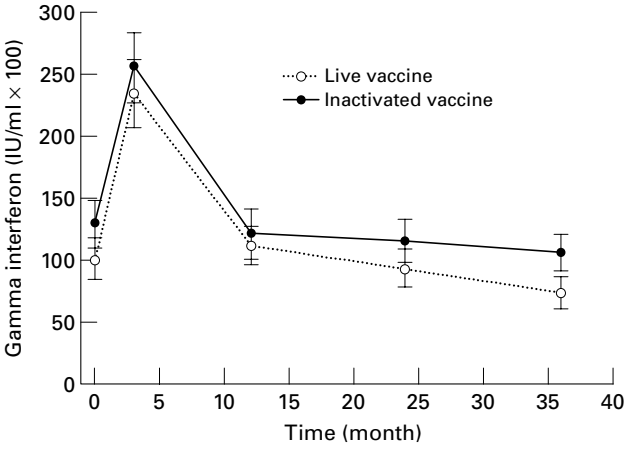

Figure 25.6 Mean (± SD) γIFN synthesis in vitro by PBMC from elderly recipients of live attenuated or inactivated varicella vaccine. γIFN was measured by ELISA on supernatants from PBMC stimulated with VZV antigen. γIFN level at 3 months was significantly higher than prevaccination level ($P < 0.001$). Number of observations with live or inactivated vaccine: time 0, 84/81; 3 months, 82/81; 12 months, 71/72; 24 months, 67/66; 36 months, 61/60.

tion (Figure 25.7), very similar to that seen in the prior study (Levin et al., 1998). The half-life of the response was shorter (23 months); however, the mean dose of virus was also lower for this study (4000 PFU vs. 6250 PFU) and dose appears to influence persistence (Levin et al., 1998). In this trial approximately 40% of patients had no detectable RCF at baseline, with a residual of 15% still undetectable after either vaccine. In summary, there is strong evidence that inactivated VZV vaccine has the same immunogenic potential as live vaccine over a 3-year period.

In this comparative trial some vaccinees were also immunized with tetanus toxoid 3 months prior to varicella vaccination and their RCF to tetanus toxoid determined before and after this secondary immunization. Assays for immune response to varicella vaccine were performed in these double vaccinees. There was a weak correlation between the specific RCF boost in response to each vaccination. However, half of the VZV nonresponders in this subset developed a tetanus-specific response, suggesting the absence of a common mechanism for failure of secondary immunization in this older age group (Hayward et al., 1994). The pre- and post-immunization RCF did not correlate with the baseline percentage of PBMC with cell surface CD3+, CD4+, CD8+, or CD3CD45R0+ markers, indicating that variation in memory cell phenotype did not explain variations in the response to VZV vaccines.

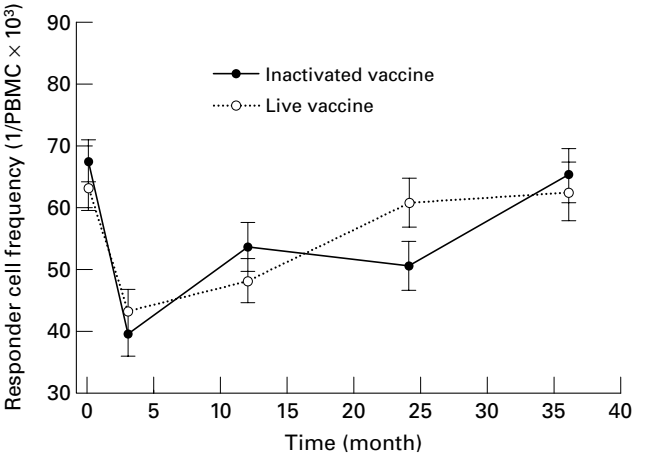

Figure 25.7 (± SD) of VZV responder cell frequency in PBMC from elderly recipients of live attenuated or inactivated varicella vaccine. Frequency at time 0 was significantly less ($P < 0.05$) than at 3, 12, and 24 months. There were no significant differences between the two vaccines. Number of observations were as given in Figure 25.6.

Cytotoxic T lymphocyte response in older individuals after booster vaccination

Among the vaccinees described in the previous section a subset had their PBMC tested for ability to lyse cells bearing VZV antigens, using autologous Epstein-Barr virus lymphoblasts as targets for HLA Class I restricted cytotoxicity (Hayward et al., 1996). Human fibroblasts served as targets for unrestricted (natural killer) cytotoxicity. Both the live and inactivated vaccines stimulated a significant, but similar increase, in natural killer activity at 3 months after vaccination. Both vaccines also increased Class I restricted cytotoxicity, but the enhancement was significantly greater with the live vaccine (Figure 25.8).

Current status of clinical trials

There is ample evidence that a variety of markers of VZV-specific CMI can be boosted in elderly individuals by either a live or an inactivated varicella vaccine. However, there is no data that clearly indicates which (if any) of these represent sensitive markers for prophylaxis against HZ. The several long-term studies of Levin et al. included more than 1100 patient-years of passive follow-up of vaccinees for HZ (Hayward et al., 1994; Levin et al., 1998). There were 10 reports of mild cases of presumed HZ, but with few lesions, a shortened course, and little pain. This suggested that vaccination might not alter the frequency of HZ, but might greatly attenuate its severity (see Table 1 in reference; Levin & Hayward, 1996). This might be analogous to the "breakthrough" disease seen in recipients of primary

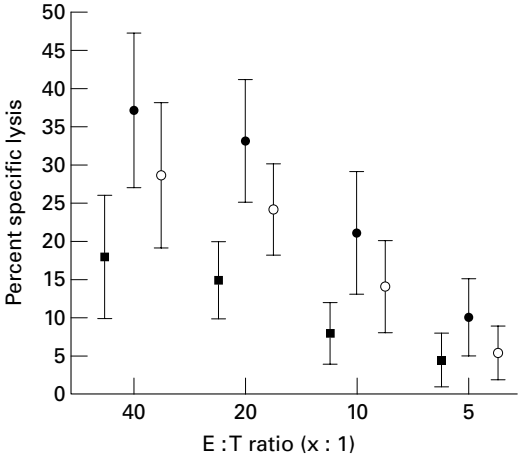

Figure 25.8 Lysis of autologous Epstein-Barr virus (EBV) targets infected with a VZV gp1 vaccinia recombinant either before a booster immunization (■) or after immunization with live (●) or killed (○) VZV vaccine. Results are the mean ± 1 SD for eight subjects in each group.

immunization of varicella vaccine (White et al., 1992), in that the recall response to a VZV challenge may be inadequate to prevent some clinical signs of infection, but rapidly amplifies to limit disease. However, the follow-up studies cited do not prove this concept, since they were not designed as efficacy trials and may have been susceptible to a variety of biases.

In preparation for an appropriately powered efficacy trial, several dose-finding experiments were undertaken in 1997–98 in an additional 515 vaccinees ≥ 60 years of age (143 were age ≥ 76 years) at three separate sites (C. Chan, M. N. Oxman, M. J. Levin et al., unpublished). These trials were designed to determine the formulation of a "shingles" vaccine. Such a vaccine must contain a quantity of live virus which is both safe and at the optimal portion of a dose–response curve. Also, based on the observations that inactivated VZV vaccine is immunogenic, the candidate vaccines for these penultimate trials contained various combinations of live virus and VZV antigen. A shingles vaccine was chosen based on an endpoint of RCF at 3 months post-vaccination. This shingles vaccine, which contains 10–20 times more virus and antigen than was used in the previous long-term studies, is the vaccine to be used in an efficacy trial.

The definitive test of concept began in December, 1998 with a double-blind efficacy trial to encompass 37 200 vaccinees, two-thirds 60–69 years old and the remainder ≥ 70 years. This study (CSP 403; M. Oxman and Study Executive Committee) was designed to utilize 15 sites within the Veterans Administration Collaborative Trials system and six sites supported by the National Institute of

Allergy and Infectious Diseases. Given the possibility that some cases of HZ might be attenuated by booster vaccination, rather than prevented, several types of endpoints have been chosen. These include cases of HZ and of postherpetic neuralgia, and, as the primary endpoint, measures of "burden of illness", which is a parameter combining pain severity and duration over an extended observation period. With a study group of this size it is calculated that it will take 4.5 years of follow-up per vaccinee (total study duration of 5.5 years), assuming >3 cases of HZ per 1000 vaccinees per year. This experiment attempts to develop a surrogate marker for protection against HZ. Such a marker would be very valuable if changes in the vaccine are contemplated in the future. Toward this end CMI (RCF and a variety of induced cytokine assays) is to be measured in a subset of 1200 vaccinees/placebo recipients before and after booster vaccination, and these assays will also be performed on samples from all cases of HZ, early after onset and at later time points. One unlikely possibility is that enough HZ will occur in this subset to validate a marker. A second possibility is that some CMI measurement early after HZ will be significantly lower than values seen at baseline or after immunization in age-matched controls who do not develop HZ. A third possibility is that some measure of CMI during attacks of HZ will correlate with the severity of these attacks (measured as burden of illness or postherpetic neuralgia), and that this might also differ between the study arms. Finally, it will be important to determine if the vaccine-induced responses are of the same magnitude reached by patients who develop HZ. This would indicate that the vaccine mimics natural boosting from HZ, which appears to be sufficient to prevent second attacks in most older individuals.

Prophylactic immunization of the immunocompromised host

Live, attenuated varicella vaccine has not been used to boost VZV immunity in this patient population, because of safety considerations and because the immune response might be limited in many potential target groups. However, since the Oka/Merck vaccine virus is both safe and efficacious for primary immunization of varicella-naive leukemic children (Gershon et al., 1986, 1990), it is likely that secondary immunization to prevent HZ should be successful in selected immunocompromised patients. This hypothesis will be tested in certain groups of HIV-infected children in early 1999 (A. Gershon and M. Levin, personal communication).

Studies of heat-inactivated VZV are underway in bone marrow transplant recipients, a patient population with VZV latent in dorsal ganglia, but initially without VZV-specific immunity (Ljungman et al., 1986; Wilson et al., 1992). Thirty-eight transplant recipients have been immunized with 4.5 units (equivalent to 2900 PFU of live commercially available vaccine) of VZV antigen (Redman et al., 1997). A

single dose given to 14 patients at one month after transplantation significantly increased the VZV-specific LPA at three months post-transplantation compared to a randomly assigned control group. There was no protective effect. However, three doses given monthly, starting a month post-transplantation, was both immunogenic and protective. Although the incidence of HZ was the same as that of the control group, there was strong attenuation in terms of lesion number, initial pain, and postherpetic neuralgia. This practical consequence of immunizing bone marrow transplant recipients supports the preliminary observations made by Levin et al. that HZ might be modified, rather than prevented, by booster doses of varicella vaccine (Levin & Hayward, 1996; Levin et al., 1998). A modified approach is under study in which one dose of inactivated vaccine is given prior to bone marrow transplantation and three doses are given after transplantation (A. Arvin, personal communication).

Other factors which might alter herpes zoster in the community

It is important to consider that there are some potential epidemiological and environmental factors that might alter the frequency and severity of HZ in future generations, and that some of these factors might influence the efficacy of vaccination to prevent HZ. At this time there is no way to assess the importance of these factors.

Universal immunization

Varicella vaccine is recommended for all normal children and adults who have never had varicella. Successful application of these recommendations might limit circulation of wild type VZV, which in turn will remove the environmental boost that probably contributes to the level of VZV-specific immunity in adults (Arvin et al., 1993; Asano et al., 1994; Krause & Klinman 1995). There is already evidence in communities with extensive use of this vaccine that the incidence of varicella has declined, both in vaccinees and in children who have not been vaccinated. This natural boosting may be important for delaying, preventing, or attenuating HZ in seropositive individuals, whether they are immune because of varicella or vaccination. Environmental boosting could also impact the efficacy of a shingles vaccine. Conversely, people who remain susceptible may benefit from a paucity of environmental VZV, since they will be more likely to avoid infection and thereby not develop HZ.

Antiviral therapy of varicella

Approximately 1–3% of children in the USA receive nucleoside analogs (usually acyclovir) for treatment of varicella (Lieu et al., 1994; Yawn et al., 1997). This is

more common in adolescents. Antiviral therapy probably has little effect on viremia, which is minimal by the time treatment is started, but reduces the number of skin lesions in children and adults, and thereby might decrease the amount of latent VZV in ganglia. Conversely, very early therapy of varicella could decrease the level of VZV-specific immunity, and thereby increase the likelihood of HZ or reduce the response to a shingles vaccine. A comparison of VZV-specific RCF in acyclovir-treated and untreated children demonstrated no difference at three years after varicella (Rotbart et al., 1993).

Latency of Oka strain VZV

The vaccine virus (Oka strain) becomes latent in ganglia. However, viremia and skin lesions appear to be minimal following vaccination, suggesting that the quantity of latent genome in ganglia will be less than after varicella. Moreover, Oka strain VZV is temperature sensitive and replicates 7-fold less readily in cultured human neurons than does the parent strain (Hayakawa et al., 1984; Somekh & Levin, 1993). HZ caused by Oka strain has been documented in immunocompromised and normal vaccinees (Hardy et al., 1991; Gershon et al., 1996; Merck Research Laboratories, personal communication). The age-specific incidence of HZ in vaccinees is not known, and there are no experiments underway to determine this. The passive collection system maintained by the manufacturer suggests a lower incidence in vaccinees compared to historical controls, but this conclusion suffers from the probability of underreporting and the relatively short period of latency for vaccinees, compared with naturally infected individuals. An optimistic view of the preventative effect of vaccination could be drawn from the data of Hardy et al. (1991). They compared well-matched groups of leukemic children who had either prior varicella or Oka vaccine. The vaccinated group had an approximately 3-fold lower incidence of HZ, and vaccinees receiving two doses of vaccine had a lower incidence of HZ than those receiving a single dose (Gershon et al., 1996).

Conclusions

Developing an immunization strategy to prevent HZ in aging individuals is hampered by limited information concerning the age-related VZV-specific immune defects that are the basis of this disease. There is ample evidence that several measures of VZV-specific CMI can be boosted in older vaccinees by either a live attenuated or a heat-inactivated varicella vaccine. A carefully designed, appropriately powered efficacy trial is underway to evaluate a "shingles" vaccine. Presumably this trial will also help us address certain lingering issues, such as: (1) the safety of large amounts of live varicella virus in a large cohort of elderly vaccinees; (2) the persistence of VZV-specific immune responses stimulated by this vaccine, and

implications for additional doses if immunity wanes; and (3) the search for a surrogate marker associated with protection against HZ. The study may fail to clarify: (1) the significance, in terms of HZ risk, of those vaccinees who fail to boost after vaccination; (2) the relative importance of the live and inactive components of the vaccine. Furthermore, several community-based epidemiological factors, such as universal varicella vaccination, may influence the final interpretation of this trial.

Prophylactic immunization for immunocompromised patients is also under intense study. It is likely that a variant of the current live varicella vaccine will be useful in immunocompromised patients who are carefully selected on the basis of residual immunity. For others with more profound immune deficits, protection with an inactivated vaccine may be safer. In both cases it will be necessary to undertake long-term follow-up studies in order to determine the persistence of enhanced immunity, and it will be important to undertake efficacy trials designed to correlate protection with immune measurements. In certain groups of immunocompromised patients this may require a schedule consisting of multiple doses of vaccine, revaccination, or some combination of sequential inactive vaccine followed by live vaccine.

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Page numbers in bold indicate illustrations; in italics, tables.

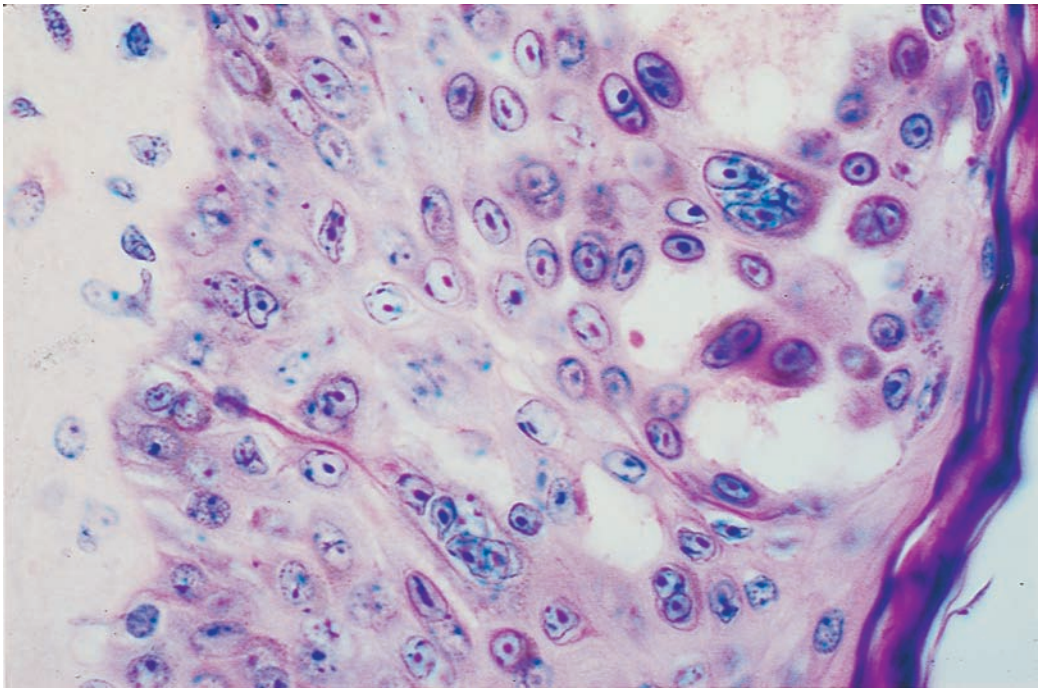
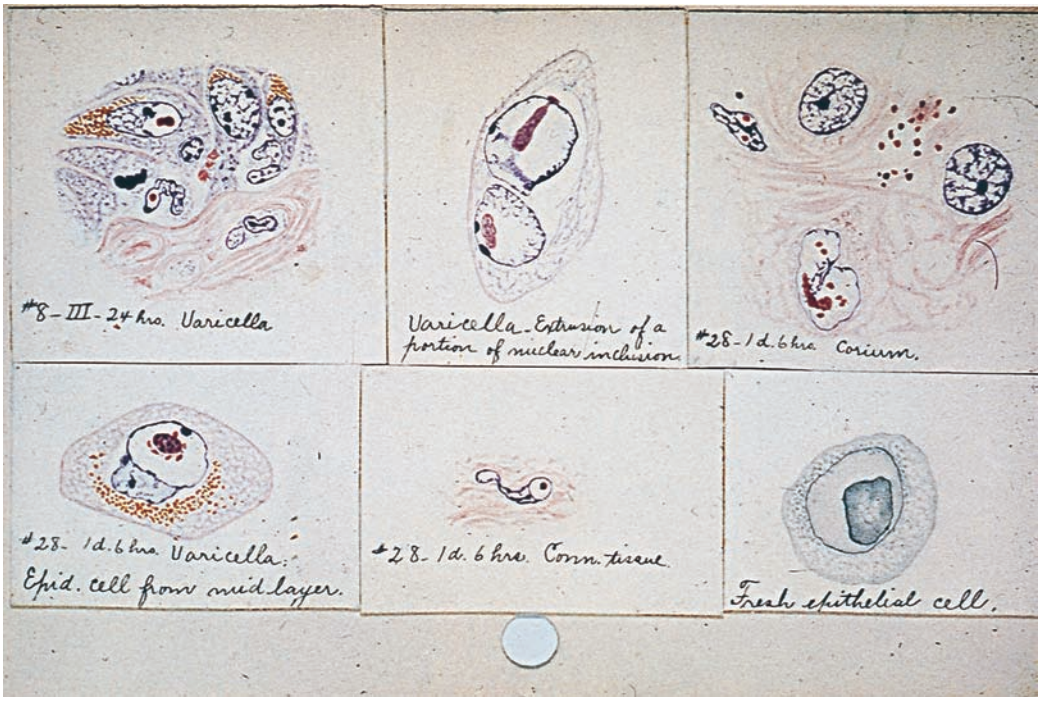


Figure 1.1 Photomicrograph of a day 1 cutaneous varicella lesion prepared by Dr. Tyzzer on June 8, 1904 in the course of his study in the Philippines. Stain: Eosin–methylene blue.

Figure 1.2 Camera lucida drawings made by Dr. Tyzzer of the nuclear changes observed in cells in the cutaneous lesions of varicella.



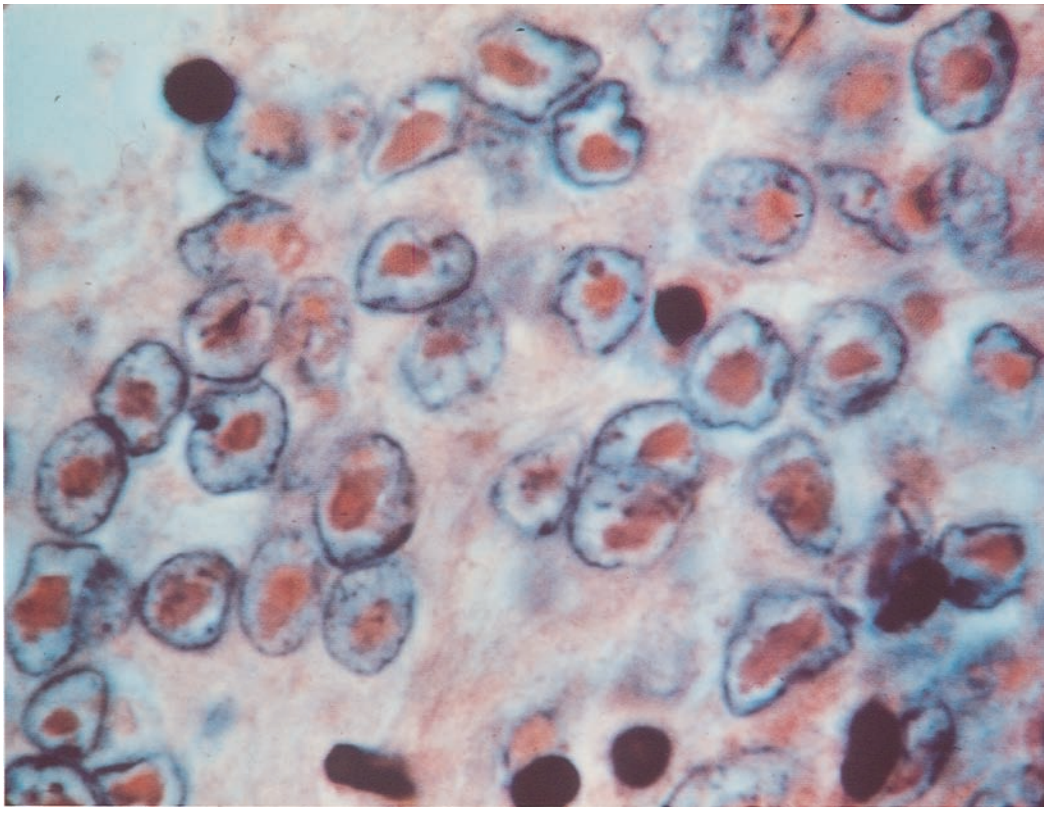
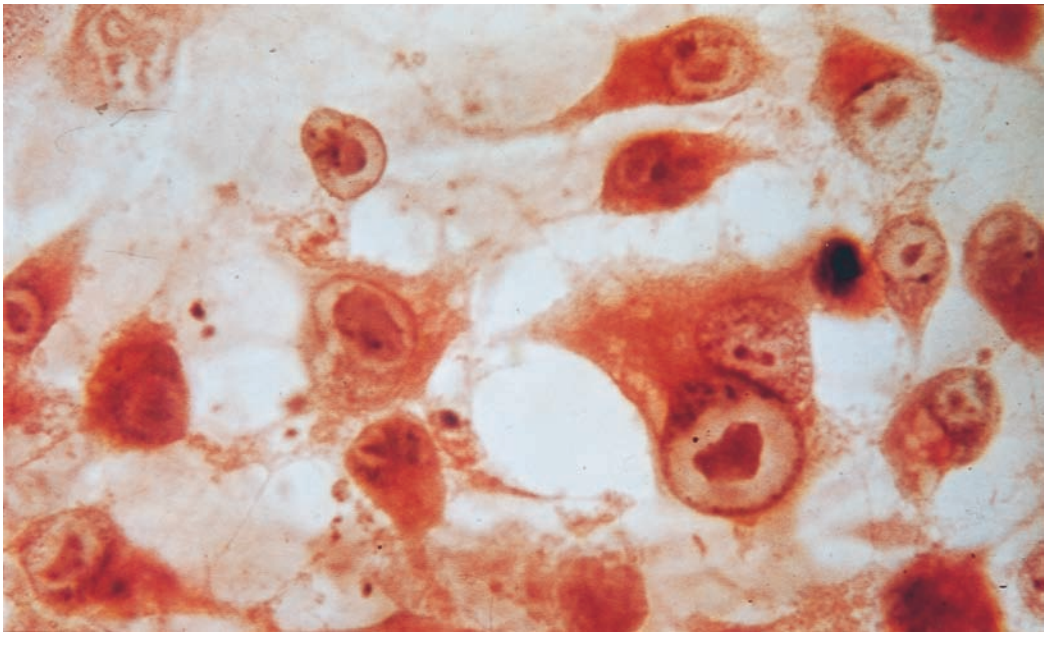


Figure 1.3 Fragment of human embryonic tissue from a Maitland type culture inoculated 14 days earlier with varicella vesicle fluid, showing nuclear inclusion bodies. From the first successful suspended cell culture; prepared March 19, 1949. Stain: hematoxylin–eosin.



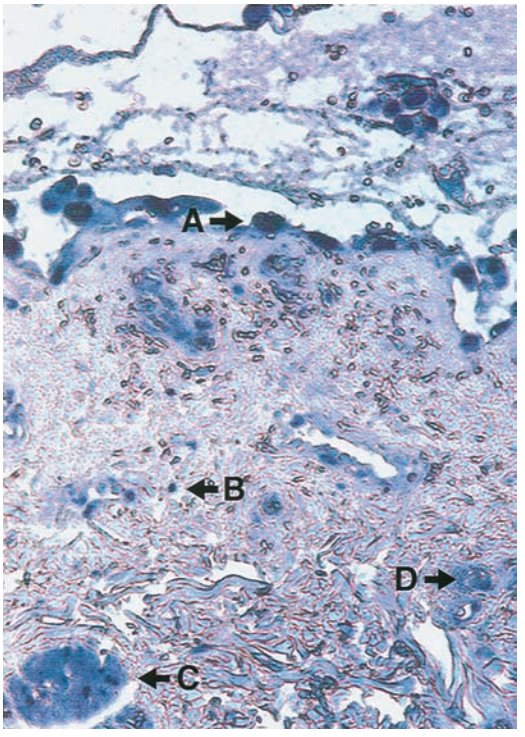


Figure 6.1 Immunohistochemical detection of VZV protein in a skin biopsy. A section of a punch biopsy from a patient with chickenpox was analyzed for the presence of ORF29 by reacting the tissue sample with a polyclonal rabbit antiserum prepared against ORF29p. (A) Indicates positive epithelial cells, (B) points to positive inflammatory cells, (C) indicates positive dermal nerves, and (D) shows positive endothelial cells. The products of ORFs 4, 62, 63, 21, and 29 were detected using purified anti-VZV proteins, rabbit antibodies, and an alkaline phosphatase (AP)-conjugated goat anti-rabbit immunoglobulin secondary antibody. Signal was visualized with AP substrate.

Figure 6.2 In situ detection of VZV ORF29-encoded RNA in a satellite cell adjacent to a large sensory neuron in human trigeminal ganglia.

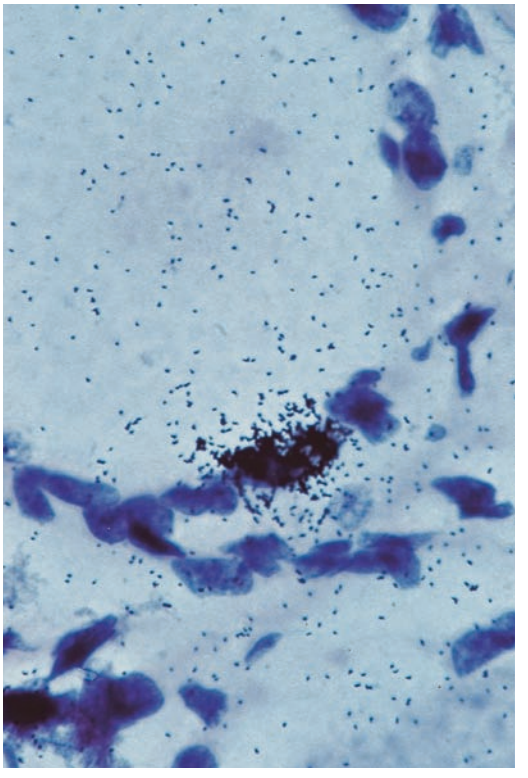


Figure 1.4 (*opposite*) Edge of a focal lesion in the first successful roller-tube experiment. Tissue harvested 13 days after inoculation with varicella vesicle fluid (PWel.strain) showing numerous intranuclear inclusion bodies. Prepared November 19, 1952. Stain: hematoxylin–eosin.

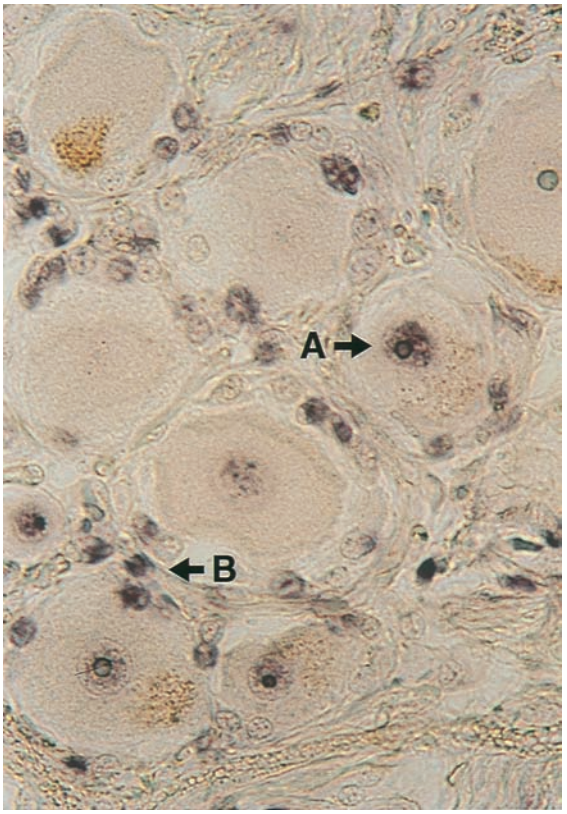
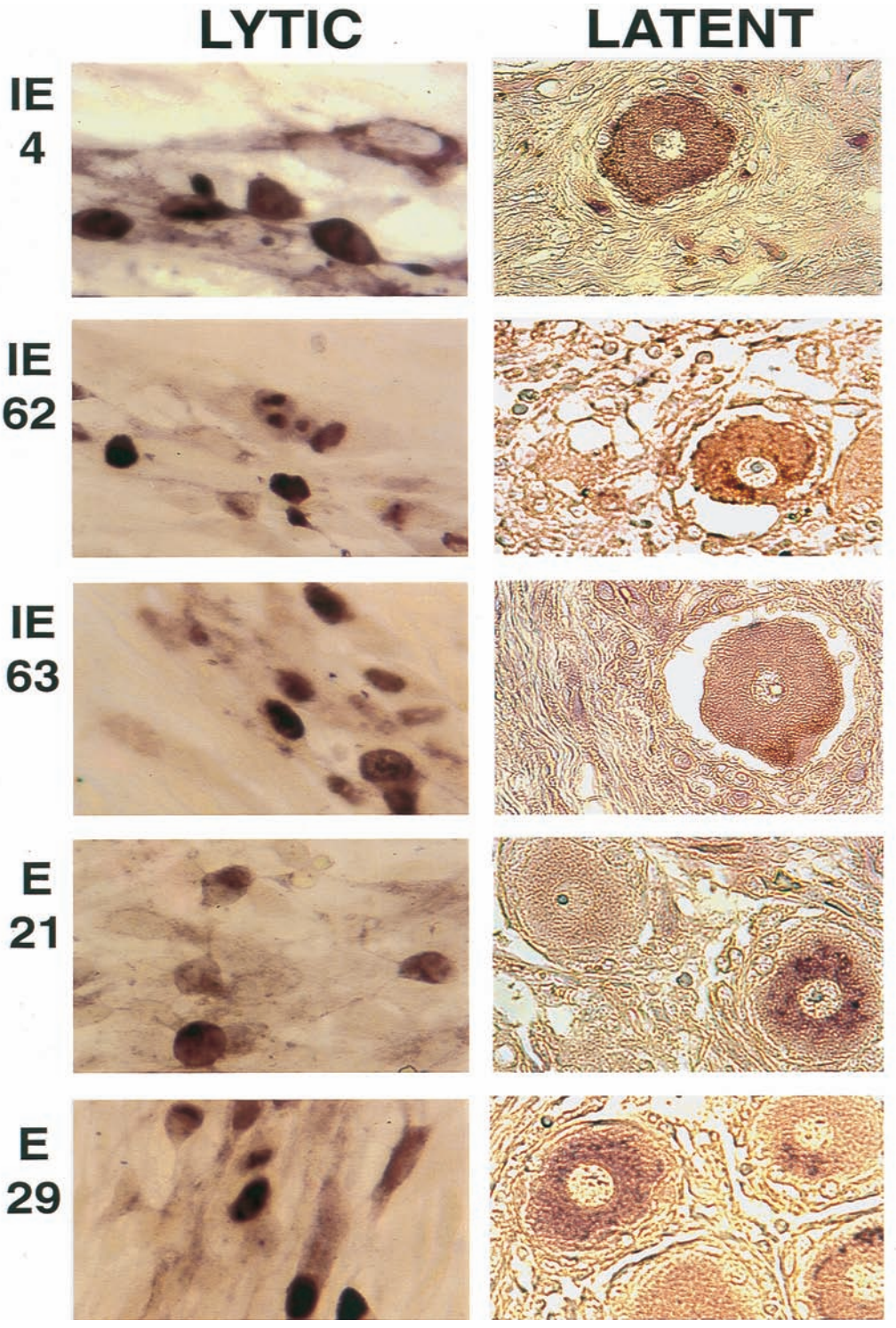


Figure 6.3 In situ detection of VZV DNA in human dorsal root ganglia. VZV DNA was detected using a fluorescein-labeled oligonucleotide probe complementary to 27 bp within ORF54 and an AP-conjugated anti-fluorescein antibody. (A) denotes a positive neuron, and (B) identifies satellite cells harboring VZV DNA.

Figure 6.4 (*opposite*) Detection of VZV proteins in productively infected cells and in human dorsal root ganglia. Immunohistochemical detection of VZV proteins was performed in fibroblasts (HELF) infected with VZV strain Ellen and in dorsal root ganglia harboring latent virus. the products of ORFs 4, 62, 63, 21, and 29 were detected using purified anti-VZV proteins, rabbit antibodies, and AP-conjugated goat anti-rabbit immunoglobulin secondary antibody. Signal was visualized by developing with AP substrate. The specimens used are identified on the top of the figure, and the individual genes whose products were analyzed are shown on the left. The arrows indicate neurons with positive nuclei. The staining seen near the plasma membrane of some neurons is from coloration of lipofuschin.



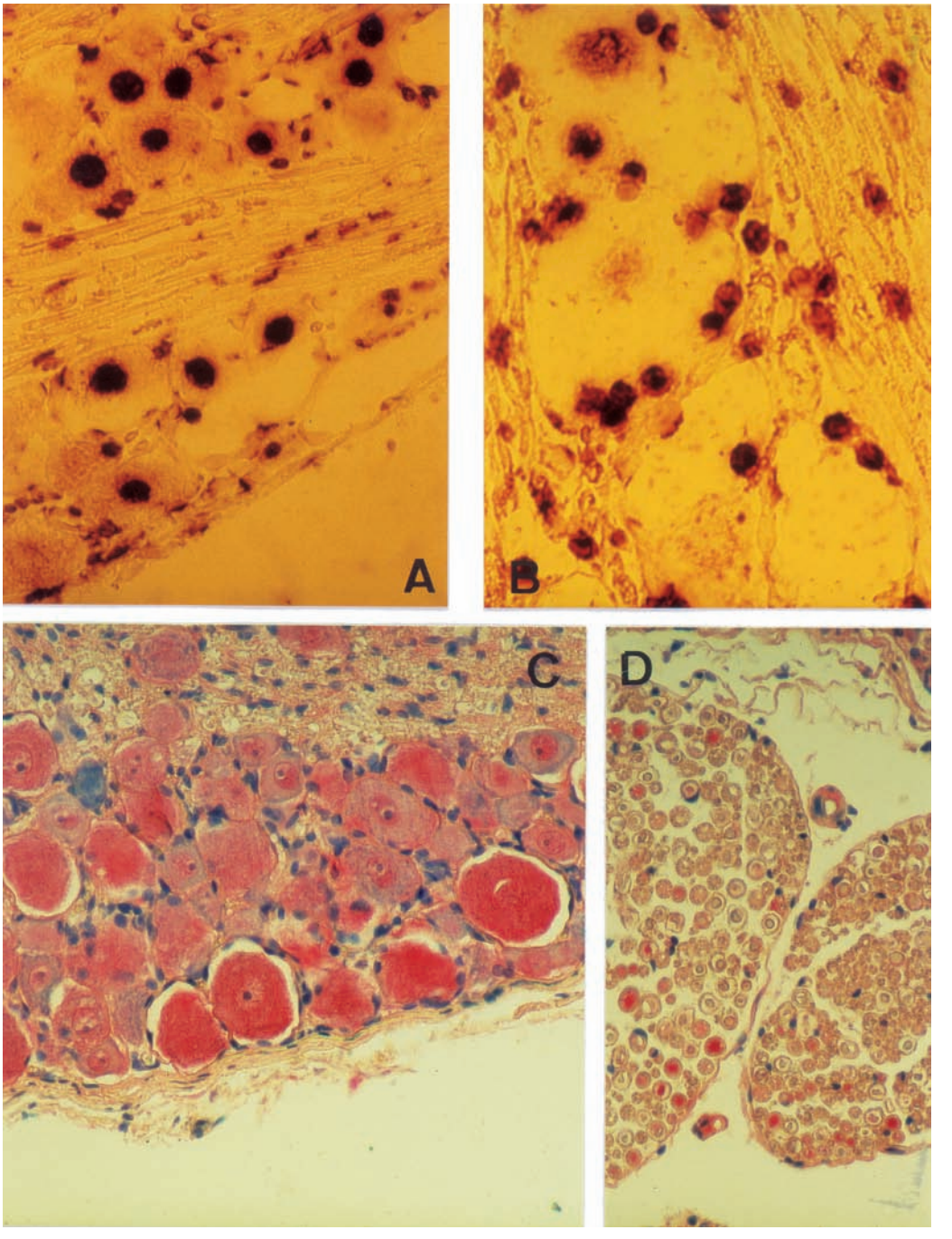


Figure 9.3 (a) and (b) Detection by in situ hybridization of varicella-zoster virus nucleic acids in DRG sections of an infected adult rat, 7 days after infection, using digoxigenin-labeled mapping about 50% of the viral genome. Depending on the animal, viral genome is detected mostly in nuclei of neurons (a) or in both non-neuronal cells and neurons (b). Immunohistochemical detection of VZV IE63 protein in a lumbar ganglion of an infected adult rat, 6 weeks after inoculation (c), and in the corresponding root (d) indicating that the protein can accumulate in axons.

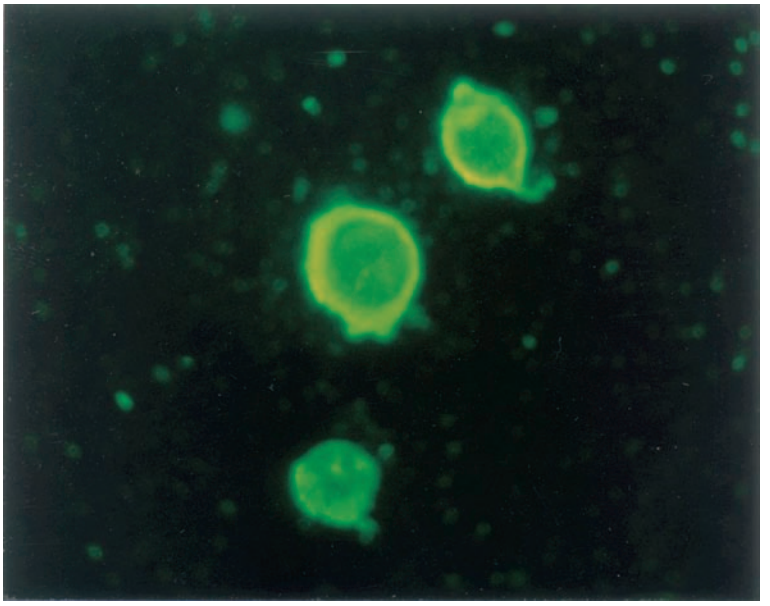
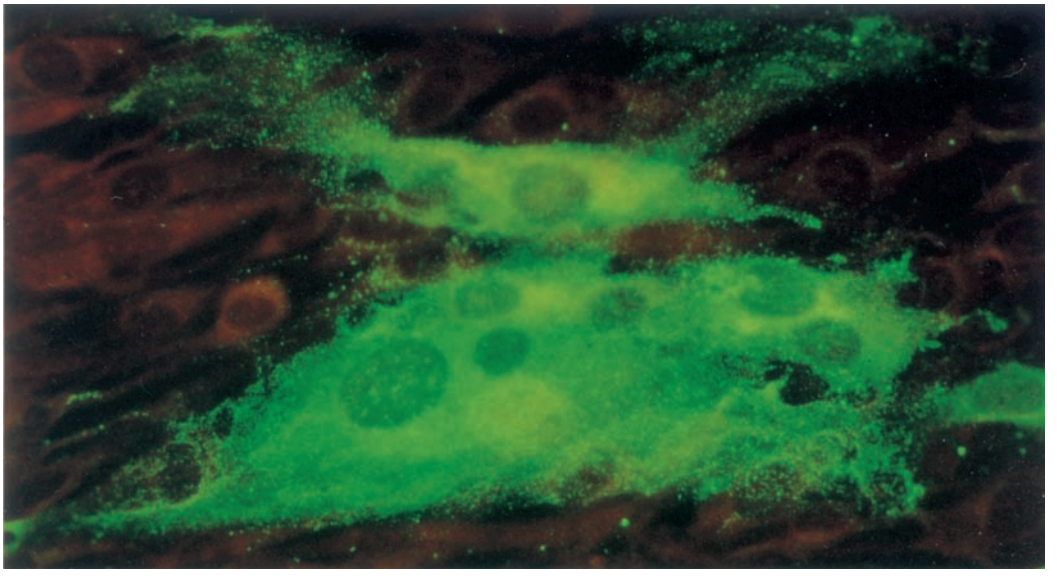


Figure 17.1 Immunofluorescent antibody staining of skin lesion from a child with varicella. Note the bright cytoplasmic and nuclear fluorescence stain of epithelial cells with large nuclei reacted with pooled mAbs against VZV proteins.

Figure 17.3 Indirect immunofluorescent staining of acetone fixed human fetal diploid lung cells 48 hours after inoculation with cell-free varicella-zoster virus; reacted with mAb against VZV gC. Note the Intense specific green fluorescence staining in the cytoplasm, cytoplasmic membrane, and Golgi apparatus of a cell with multiple nuclei. The reddish-brown cells are uninfected cells counter-stained with Evan's blue.



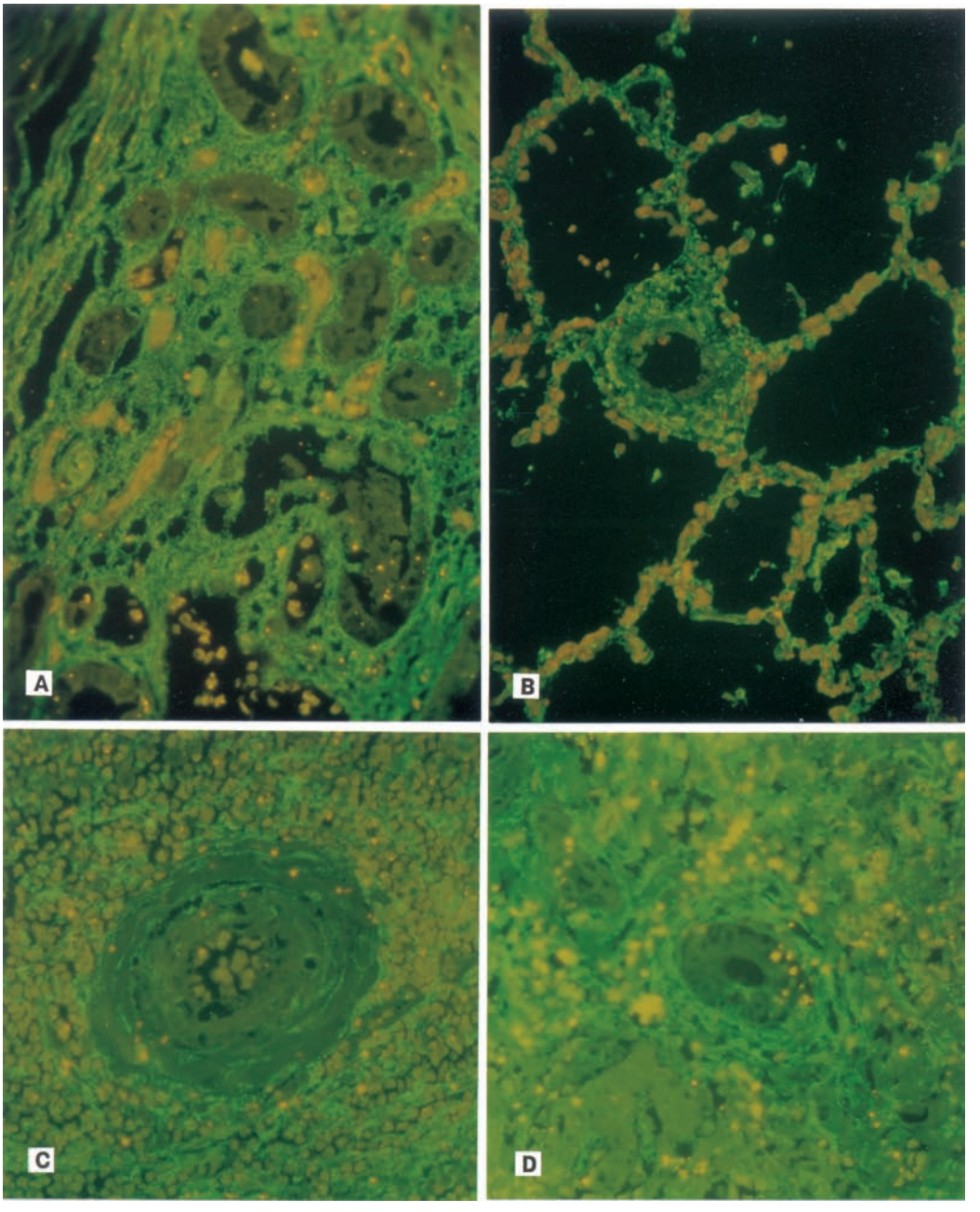


Figure 17.4 Indirect immunofluorescent staining of kidney, liver, lung and spleen of formalin-fixed paraffin-embedded tissue sections from an AIDS patient, reacted with pooled mAbs against several VZV gene products and counter stained with Evan's blue. (A) Strong focal fluorescent staining is seen in the interstitium of kidney tissue sections, while no staining is observed in the tubules. (B) Strong fluorescent staining is seen in the blood vessel within the interstitium and the endothelium of the vessel, and the interstitial compartment of alveolar sacs and the pneumocytes. (C) Positive fluorescent staining is seen in the interstitium of portal triads of the liver with focal staining of the bile duct cells. (D) Positive fluorescence staining is seen in a splenic artery surrounded by white pulp, smooth muscle, endothelium and the surrounding interstitium.