INFLAMMATION, LIFESTYLE, AND CHRONIC DISEASE

THE SILENT LINK

OXIDATIVE STRESS AND DISEASE

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EDITED BY

BHARAT B. AGGARWAL • SUNIL KRISHNAN • SUSHOVAN GUHA

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Preface

Inflammation has been described for thousands of years by many names: in Indian Ayurvedic medicine it is *Sooj*; in traditional Chinese medicine it is *Qi*. It was, however, Cornelius Celsus from first-century Rome who first described inflammation as consisting of heat, pain, redness, and swelling (i.e., calor, dolor, rubor, and tumor). The link between inflammation and various chronic diseases was first suggested by the German physician Rudolf Virchow in the nineteenth century.

The word *inflammation* is derived from the word for *flame* or *fire*. Just as controlled fires can be harnessed for societal benefit in multiple ways, inflammation is an evolutionarily conserved defense mechanism that is essential for diverse human bodily functions.

However, when these flames flare out of control, they can trigger a plethora of unwanted phenomena that eventually culminate in chronic disease. Whereas acute inflammation generated by the immune system serves a therapeutic role, chronic low-level inflammation that may persist "silently" for decades is responsible for chronic diseases. Dysregulated or excessive inflammation, induced by lifestyle factors such as psychological stress, grilled meat, radiation, tobacco, infections, and environmental pollution, is emerging as a fundamental initiator of most chronic human diseases, including cancer, diabetes, obesity, Alzheimer's disease, arthritis, and cardiovascular diseases. Since most of these are diseases of old age, inflammation appears to be linked to the aging process as well.

The current monograph is an attempt to describe the essential role of dysregulated inflammation in various chronic diseases. In many instances, these chronic diseases are preventable, provided major lifestyle changes are made. However, once these diseases manifest themselves, their treatment with steroids and nonsteroidal anti-inflammatory drugs (NSAIDs), the traditional treatments for acute inflammatory diseases, is fraught with devastating side effects that preclude their long-term use. Because chronic diseases caused by chronic inflammation require chronic treatment, many of the chapters in this monograph also address the role of dietary agents, such as fruits, vegetables, legumes, pulses, nuts, and spices, as ideal anti-inflammatory agents that can be consumed chronically. This supports the aphorism by Hippocrates recorded almost 25 centuries ago: "Let food be thy medicine and medicine be thy food."

We first thank all the authors for their exciting contributions to this book. We also thank Dr. Chitra Sundaram for her help in assembling the whole manuscript. We hope that our readers find this book informative and useful.

Bharat Bhushan Aggarwal, PhD

Sunil Krishnan, MD

Sushovan Guha, MD, PhD

Editors

Dr. Bharat Bhushan Aggarwal is the Ransom Home, Jr., Distinguished Professor of Cancer Research in the University of Texas MD Anderson Cancer Center Division of Cancer Medicine's Department of Experimental Therapeutics, and chief of the Cytokine Research Laboratory, in Houston, Texas. He earned a PhD in biochemistry at the University of California in Berkeley, then underwent postdoctoral training at the University of California Medical Center in San Francisco. Afterwards, he worked for 10 years at Genentech, Inc., where he isolated and determined the structure of TNF-α and TNF-β, before returning to a university-based academic position.

The primary focus of Dr. Aggarwal's research has been the role of inflammatory pathways in tumorigenesis, and especially the impact of the transcription factor nuclear factor κB and STAT3 pathways in cancer. His group has also been interested in the use of natural products such as anti-inflammatory nutraceuticals from diet, spices, and traditional medicine, to modulate both inflammatory pathways linked to survival, proliferation, invasion, angiogenesis, and metastasis of tumors, and cancer-induced bone loss. Several of these efforts have led to clinical trials targeting cancer patients using agents that are safe and affordable.

He has published more than 600 papers, has been granted more than a dozen patents, and has been invited to more than 300 national and international conferences to deliver lectures. Dr. Aggarwal's research is currently funded by the National Institutes of Health and by various private organizations. Dr. Aggarwal also cofounded the International Society for Translational Cancer Research. His work has garnered many awards, including the Ranbaxy Award, an Outstanding Scientist Award from the American Association of Indian Scientists in Cancer Research, a McCormick Science Institute Research Award from the American Society of Nutrition, the Kosuna Distinguished Lecture Award from the University of California, Davis, and World Congress on Oxygen Club of California.

Dr. Sunil Krishnan is director of Gastrointestinal Translational Research and associate professor of radiation oncology at the University of Texas MD Anderson Cancer Center in Houston. He received his MD degree at the Christian Medical College in Vellore, India, and then completed an internal medicine residency at Penn State Geisinger Medical Center in Danville, Pennsylvania, and a radiation oncology residency at Mayo Clinic College of Medicine in Rochester, Minnesota, before joining the University of Texas MD Anderson Cancer Center.

The overarching goal of Dr. Krishnan's research is to develop novel strategies to improve radiation treatment outcomes for gastrointestinal malignancies. In the case of incurable malignancies such as locally advanced pancreatic, hepatic, and biliary tract neoplasms, an improvement in efficacy and reduction in toxicity of radiation therapy are likely to translate to an improvement in survival rates and quality of life. In case of resectable malignancies such as rectal, gastric, and gastroesophageal cancers, this approach could potentially result in adoption of organ-sparing alternatives to radical surgery in select subsets of patients. His laboratory's primary focus has been the role of inducible resistance to radiation therapy mediated by inflammatory signaling pathways, especially the impact of the transcription factor nuclear factor kappa B pathway. Although the quest for radiosensitization strategies started with the use of highly targeted pharmaceutical agents, more recently these inquiries have focused on the use of broad-spectrum natural products that simultaneously and seamlessly modulate multiple inflammatory pathways linked to survival, proliferation, DNA repair, invasion, angiogenesis, and metastasis of tumors. Some of these efforts have led to clinical trials in cancer patients.

Dr. Krishnan has published more than 80 papers in peer-reviewed journals, presented numerous seminars and lectures at national and international academic centers and conferences, and is currently funded by the National Institutes of Health and various nonprofit organizations.

Dr. Sushovan Guha is the site director of the Gastroenterology Fellowship Program and assistant professor of gastroenterology, hepatology, and nutrition at the University of Texas MD Anderson Cancer Center in Houston. He earned his MD degree from Jawaharlal Institute of Post-Graduate Medical Education and Research (JIPMER), Pondicherry, India, and subsequently graduated with an MA/MPhil in microbiology and immunology from Columbia University, New York. Next, he completed his internship and residency in internal medicine at the Albert Einstein College of Medicine in Bronx, New York. Dr. Guha then joined the prestigious Specialty Training and Advanced Research (STAR) Fellowship in Gastroenterology and Hepatology at the David Geffen School of Medicine at UCLA, Los Angeles, where he also received his PhD from the Molecular Biology Institute (MBI) under the astute tutelage of Professor Enrique Rozengurt.

Dr. Guha is a board-certified clinical gastroenterologist, internist, and physician-scientist at the University of Texas MD Anderson Cancer Center. The research focus in Dr. Guha's laboratory consists of unraveling signal transduction pathways in pancreatic cancer (PaCa), a devastating disease quite incalcitrant to conventional therapeutic regimens. Thus, there is an urgent need to develop novel therapeutic regimens, which will arise from a better understanding of the genetic and epigenetic changes leading to mitogenic signal transduction pathways. His current focus is to dissect G-protein-coupled receptor (GPCR)-mediated protein kinase D (PKD)-induced mitogenic and angiogenic signaling pathways in PaCa. His group showed that PKC-PKD signaling pathways downstream of GPCRs contribute to both mitogenesis and angiogenesis in PaCa. His laboratory uses various molecular and cellular biological techniques to unravel PKD-dependent critical signaling pathways. His group has developed an orally available specific small-molecule inhibitor of PKD with strong therapeutic potency and is performing preclinical studies in multiple animal models of PaCa. His group is also developing genetically engineered models in mice to study the role of PKD in initiation and progression of PaCa. Finally, his laboratory is characterizing novel downstream targets (substrates) of PKD that modulate key cellular processes, including oncogenic *Ras*-dependent mitogenesis, migration, drug resistance, and epithelial-to-mesenchymal transition in PaCa. He has published more than 75 papers in peer-reviewed journals, presented numerous seminars and talks at national and international academic centers and conferences, and is currently funded by the National Institutes of Health and nonprofit organizations.

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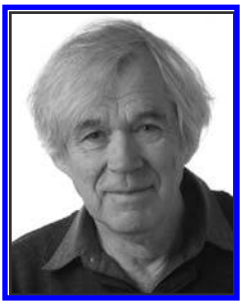

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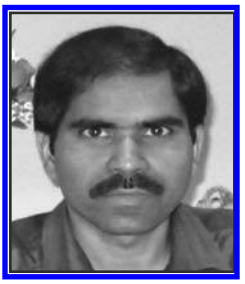

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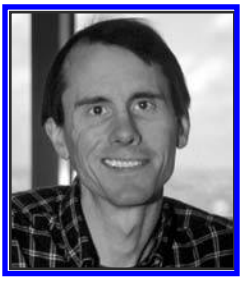

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1 Roles of Innate Immunity and Inflammation in the Aging Brain

Eitan Okun, XinZhi Chen, Milan Basta, and Mark P. Mattson

CONTENTS

1.1 INTRODUCTION

Aging is associated with increased incidence of several neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), as well as stroke (Joseph et al. 2009). All these disorders involve chronic inflammatory changes in the affected regions of the central nervous system (CNS) that contribute to the pathologic process (McGeer et al. 2004). There is evidence that in each of these disorders both the intrinsic (innate) and peripheral (humoral) immune systems are involved, perhaps initially as an adaptive response that subsequently becomes deleterious. While both the innate and peripheral immune processes are interconnected in various ways, we will mostly focus in this chapter on the roles of the innate immune response in the brain during aging and in age-related neurodegenerative conditions. For more information on the involvement of the peripheral immune system in the aging brain, see Kin and Sanders (2006).

Innate immune cells and innate immune receptors are expressed in both non-immune cells (neurons and astrocytes, for example) and classical immune cells, such as microglia. Microglial cells arise from bone marrow/mesenchymal cell-derived monocytes that enter the brain during development and differentiate intraparenchymally; microglia may reside in either a resting (surveying) phenotype with a small soma and highly branched processes, or an activated amoeboid form (Aloisi 2001). There is some evidence that new microglia may also be generated from bone marrow cells during adult life, particularly in response to injury (Guo et al. 2004). Microglia are similar, if not identical, to macrophages; they are considered innate immune cells because of their ability to respond directly to a pathogen without the need to communicate with the humoral immune system. Innate immune receptors, also referred to as pattern recognition receptors (PRRs), bind directly to pathogen-associated molecular patterns (PAMPs) in molecules produced by microbial pathogens. Danger-associated molecular patterns (DAMPs) are altered intrinsic molecules generated in damaged or severely stressed cells; these molecules act as ligands that can activate various innate immune receptors, including Toll-like receptors (TLRs) and RIG1-like receptors (RLRs).

1.2 INNATE IMMUNE EFFECTORS IN THE CENTRAL NERVOUS SYSTEM

The two most studied innate immune protein families in the context of CNS physiology and pathology are the TLRs and the complement system. In the following sections we will briefly describe these two protein families and expand on how they and related innate immune components are involved in molecular, cellular, and functional changes that occur in the brain during aging.

1.2.1 Toll-Like Receptors

TLRs are major PRRs that have a central role in the initiation of innate immunity against invading microbial pathogens. These integral membrane proteins have a single membrane-spanning domain, a leucine-rich extracellular domain, through

which they recognize PAMPs, and a cytoplasmic Toll/IL-1 receptor (TIR) domain similar to that of the interleukin-1 receptor (IL-1R), which initiates downstream signaling (Kawai and Akira 2007). Each TLR by itself or in combination with other TLRs recognizes distinct PAMPs that include lipids, lipoproteins, nucleic acids, and proteins. TLRs are ubiquitous, present in both immune and nonimmune cells, and their expression is rapidly altered in response to pathogens, cytokines, and environmental stressors (Akira et al. 2006).

Thus far, 11 human and 13 mouse TLRs have been identified. TLRs rely on receptor dimerization to achieve specificity in agonist recognition. TLRs may be segregated into groups based on the specific PAMPs they recognize. For example, TLRs 1, 2, 4, and 6 recognize lipids, while TLR4 predominantly recognizes lipopolysaccharides (LPSs) from Gram-negative bacteria. TLR2 dimerizes with TLR1 to recognize triacylated lipopeptides from bacteria, such as Pam3Csk4, or with TLR6 to respond to a variety of PAMPs, including peptidoglycan, diacylated lipopeptides such as Pam2Csk4, lipopolysaccharides of Gram-positive bacteria, fungal zymosan, and mucoplasma lipopeptides. TLR10, which is expressed only in humans, can heterodimerize with TLR2 and TLR1 (Akira et al. 2006). The second class of TLRs includes TLR5 and TLR11, which are activated in response to protein ligation. TLR5 is mainly expressed in the intestine, where it senses bacterial flagellin protein (Uematsu et al. 2008). TLR11 recognizes an unknown ligand of uropathogenic bacteria and a profilin-like molecule of the protozoan parasite *Toxoplasma gondii* (Akira et al. 2006). TLRs 3, 7, 8, and 9 comprise the third group in the TLR family, and are localized intracellularly, where they are ideally positioned for activation by nucleic acids of bacterial and viral origin. TLR3 is activated in response to double-stranded RNA (dsRNA) of viral origin. Human TLR8 and its murine orthologue, TLR7, recognize imidazoquinoline and viral ssRNA. TLR9 recognizes unmethylated CpG dinucleotides found in bacteria as well as viral genomes. An illustration of the different TLRs and their respective cellular localizations is shown in Figure 1.1.

In addition to the numerous exogenous ligands that activate the different TLRs, endogenous TLR ligands (or DAMPs, as defined above) have been identified in recent years. Endogenous TLR ligands include an array of extracellular matrix (ECM) proteins, such as low molecular weight hyaluronic acid (HA), fibrinogen, fibronectin, heparin sulfate proteoglycans, and immune-related proteins such as β-defensins (Pandey and Agrawal 2006). During tissue injury or proteolysis, ECM components undergo cleavage, with one or more of their cleavage products gaining the ability to act as TLR ligands. For example, high molecular weight HA is cleaved to low molecular weight HA, which subsequently binds TLRs 2 and 4 and activates signaling cascades downstream of these TLRs. Heat shock proteins, released from stressed cells, may also activate TLR4 (Lehnardt et al. 2008). In this way innate immune inflammatory responses may be activated without the presence of invading pathogens (Shimada et al. 2008).

Functional TLR signal transduction is complex and relies on receptor dimerization as well as the presence of accessory proteins and coreceptors, which regulate the signaling pathways initiated by each receptor. After recognition of PAMPs, TLRs activate the signaling components that mediate immune responses required for host defense. The cytoplasmic region of TLRs contains a TIR domain, which mediates

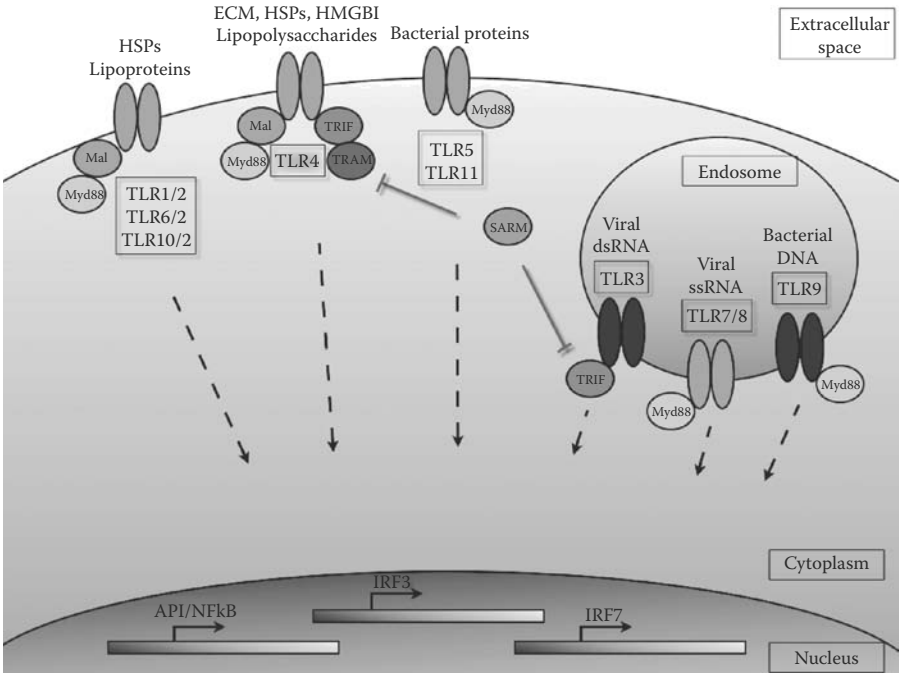

FIGURE 1.1 Illustration of the cellular localizations of the different TLRs. Plasma membrane TLRs: TLR2 can heterodimerize with either TLR1, TLR6, or TLR10. TLR4 forms mostly homodimers that can recognize lipopolysacharides from Gram-negative bacteria or various DAMPs, such as HSPs, high mobility group B1 (HMGB1) proteins, or different extracellular matrix components, such as low molecular weight hyaluronic acid and fibronectin. TLR5 and TLR11 form mostly homodimers as well, and are thought to recognize bacterial proteins. Endosomal TLRs: TLR3 detects viral dsRNA, but can also detect endogenous RNA from ruptured cells. TLR7 and TLR8 detect ssRNA, whereas TLR9 detects CpG-rich bacterial DNA. Elliptical circles represent the different TIR domain-containing adaptor proteins (Mal, Myd88, TRIF, TRAM, and SARM) used by various TLRs, with SARM the only inhibitor adaptor protein capable of inhibiting TRIF-mediated signals from TLR3 and TLR4. Signaling from all TLRs culminates in activating members of the interferon regulatory factors, AP1, or NF-κB families of transcription factors.

homo- and heterophilic interactions between TLRs and TIR-containing adapters. TLRs recruit a set of adaptor proteins with TIR domains by homophilic interaction of their TIR domains. The signaling pathways activated by TLRs are broadly classified into myeloid differentiation factor 88 (MyD88)-dependent and -independent pathways; MyD88 is the universal adapter protein recruited by all TLRs except TLR3 (Kawai and Akira 2007).

Upon receptor activation and interaction with MyD88, one or more TIR-containing adapter proteins, TIRAP/Mal (TIR-domain-containing adapter/MyD88 adaptor-like), TICAM1/TRIF (TIR-domain-containing adaptor molecule 1/TIR-domain-containing adaptor-inducing interferon-po, and TRAM (TRIF-related adaptor molecule), are recruited along with IL-1R-associated kinases (IRAKs)-1, 2, 3, 4, or the inhibitory

IRAK-M. Once phosphorylated, IRAKs dissociate from MyD88 and interact with TNF receptor-associated factor 6 (TRAF6). TRAF6 forms a complex with Ubc13 and Uev1A to promote the synthesis of lysine 63-linked polyubiquitin chains, which in turn activate transforming growth factor β-activated kinase 1 (TAK1), a mitogen-activated protein kinase kinase kinase (MAPKKK) (Wang et al. 2001). TAK1, in combination with an activator subunit TAB1, TAB2, or TAB3, activates two downstream pathways involving the IKK complex and the MAPK family (ERK, JNK, or p38). The IKK complex, composed of the catalytic subunits IKKα and IKKβ and a regulatory subunit IKKγ, catalyzes the phosphorylation of IκB proteins (Kawai and Akira 2007). This phosphorylation leads to the degradation of IκBs and the subsequent nuclear translocation of the transcription factor nuclear factor κB (NF-κB). Members of the MAPK family phosphorylate and activate the transcription factor activator protein 1 (AP-1). Activation of the transcription factors NF-κB and AP-1 results in expression of pro-inflammatory cytokines such as interleukin (IL)-6, IL-1 and tumor necrosis factor (TNF)-α.

Most of the TLRs seem to be absolutely dependent on the expression of MyD88 for all of their functions, whereas TLR3 and TLR4 are capable of signaling through a MyD88-independent pathway. SARM, the fifth known TIR domain-containing adaptor protein, is the only inhibitory adaptor protein; SARM inhibits TLR3, as well as the MyD88-independent responses to TLR4 activation (Kenny and O'Neill 2008). Both TLR3 and TLR4 differ from other TLRs by their ability to activate interferon regulatory factor 3 (IRF3). Following TLR4 activation, a MyD88-independent pathway can be activated when TRIF is recruited in concert with TRAM. This culminates in MAPK signaling and activation of the transcription factors NF-κB and IRF-3. TRIF-dependent signaling following TLR3 activation acts through recruitment of the IKKs, TBK1, and IKK, which activate IRF3 (Arancibia et al. 2007). Alternatively, TLR3 may activate IRF2 through TRIF-dependent activation of phosphatidylinositol 3-kinase and Akt (Sarkar et al. 2004). Exceptionally, MyD88-dependent signaling of TLR7, TLR8, and TLR9 can also induce type I IFN production (Kawai and Akira 2007).

1.2.2 The Complement System

The complement system has at least 35 circulating and membrane-bound components, factors, regulatory proteins, and inhibitors. The complement system can be viewed as a link between innate immunity and humoral immunity, because its activation results in opsonic, chemotactic, and cytolytic activities to clear invading pathogens as an important component of the immune system. Excessive or unregulated complement activation may exacerbate host tissue injury associated with a variety of pathologic states, including aging and aging-related disease in the CNS (Gasque et al. 2002). The complement system consists of three activation pathways: (1) the classical pathway, (2) the lectin pathway, and (3) the alternative pathway (Figure 1.2). All three pathways eventually converge to a single terminal pathway involving regulatory proteins and complement receptors (Kinoshita 1991).

The classical pathway is commonly activated by antibodies binding to an antigen, but it also can be activated by aggregated amyloid proteins, petraxins, C-reactive

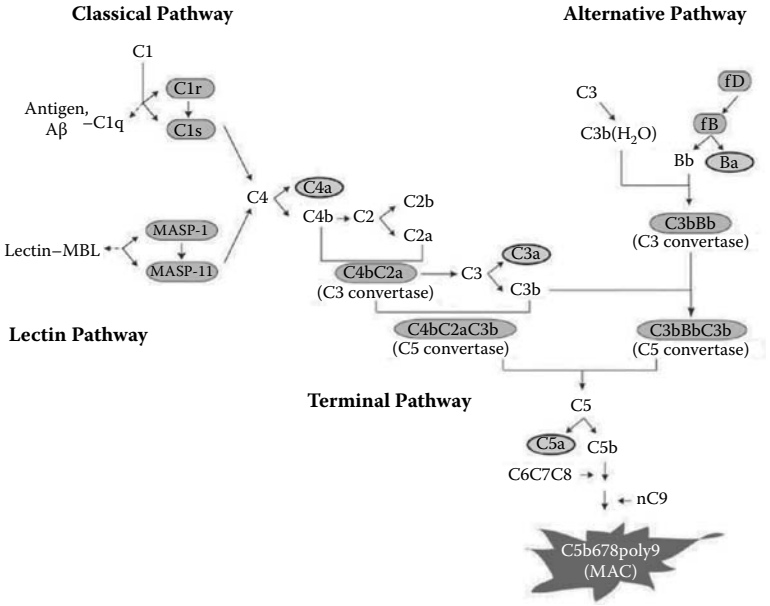

FIGURE 1.2 Schematic diagram of complement activation. According to the initial stimuli, complement activation can be induced by the classical, alternative, and lectin pathways. All three pathways can converge into the terminal pathway inducing target cell disruption if the membrane attack complex (MAC) is formed once the complement system is fully activated. Abbreviations: Aβ, amyloid β peptide; C1q, complement component 1 subcomponent q; MBL, mannose-binding lectin, MASP, mannose-activating surface protein; fB, factor B; fD, factor D. Gray tone circles indicate proteases with enzymatic activities. Oval shapes with dark outlines indicate generated anaphylatoxins during a series of events of complement activation.

protein, and necrotic or apoptotic cells without antigen–antibody interactions (Rogers et al. 1992; McGeer and McGeer 2004). The classical pathway is initiated by the attachment of C1q to a target that causes autoactivation of serine proteases C1r and C1s. Active C1s cleaves C4 into C4a and C4b, and then forms C3 convertase (C4bC2b) following C4b binding to C2 and release of C2a. The C3 convertase cleaves C3 into C3a and C3b, which in turn incorporates into C5 convertase (C4bC2bC3b) to cleave C5 into C5a and C5b. The attached complement fragments then become ligands for complement receptors on phagocytes, such as the microglia of the brain (Pinckard et al. 1975). The small fragments, such as C3a and C5a anaphylatoxins, have multiple pro-inflammatory effects (Hugli 1990).

The lectin pathway is initiated by binding of serum mannose-binding lectin (MBL) to simple carbohydrates (such as mannose and fucose) expressed on bacteria and viruses in a pathogen-specific manner (Matsushita 1996). The mannose-binding lectin-associated serine proteases MASP1 and 2 are structurally and functionally similar to the C1r and C1s in the classical pathway by their ability to cleave C4 and then C2 to generate C3 and C5 convertases (Thiel et al. 1997).

The alternative pathway is initiated by spontaneous activation of C3 by water molecules, without engaging early components of the classical pathway. The

spontaneously hydrolyzed C3 molecule, called C3 (H_2O), is able to form complexes with factor B. In the context of this complex, factor B is subject to proteolytic attack by factor D, resulting in a smaller fragment Ba (that gets released into the fluid phase), while the larger Bb fragment remains bound to the hydrolyzed C3 molecule. Under normal circumstances factors H and I mediate dissociation of the above complex that is capable of proteolytic cleavage of C3 into C3a and C3b. Amplification of the alternative pathway is initiated when cellular surfaces of pathogenic microorganisms (yeast cell wall component zymosan or LPS in the bacterial cell wall) provide a site where spontaneously activated C3b molecules are protected from the regulatory functions of factors H and I. Following stabilization of C3bBb molecule by properdin (that reduces the rate of decay of the complex), the amplification C3 convertase is formed. This convertase creates more C3bBb via an amplification loop that involves cleavage of more native C3 in the fluid phase. After several rounds of amplification, multimers of C3bBb molecules then reach a critical mass, sufficient to form the alternative pathway C5 convertase, and the pathway then proceeds through the common membrane attack complex (MAC) formation stage (McGeer and McGeer 2002).

Although each of the three complement pathways is initiated differently, all three have a common terminal portion of the pathway that begins with cleavage of C5 into C5a and C5b. The formation of C5b initiates a sequence of conformational and hydrophobic changes of complement components C6 through C9, resulting in the formation of the lytic C5b678poly9 complex or membrane attack complex (D'Ambrosio et al. 2001). Pores 9–12 nm in diameter form within the target membrane, depending upon the number of C9 proteins assembling. The MAC is usually inserted into foreign bacteria and viruses and can also damage host cells if cells are inadequately protected.

To counteract deleterious complement activation, cells are equipped with a series of endogenous membrane-bound complement inhibitors, such as binding proteins, receptors, and cofactor proteins. This is to ensure that the small complement fragments that stimulate inflammation do not harm host cells from uncontrolled complement-mediated damage (McGeer and McGeer 2002). Moreover, depending on the type of stimulus, although the complement system is meant to confer protection in the short term, it can also cause damage to the brain due to its strong cytotoxic capabilities by amplifying neuroinflammation unless tightly regulated (Rogers et al. 1992; McGeer et al. 1993). Many of the adverse effects of complement proteins can be counteracted by immunoglobulins, and data suggest that intravenous immunoglobulin can reduce injury and improve functional outcome in experimental models of stroke (Arumugam et al. 2007).

1.3 CELLULAR AND MOLECULAR CHANGES THAT OCCUR IN THE BRAIN DURING AGING

Several major alterations that occur in the brain during the aging process set the stage for hyperactivation of innate and humoral immune pathways. Similar to other tissues, these changes include oxidative stress, increased production of pro-inflammatory

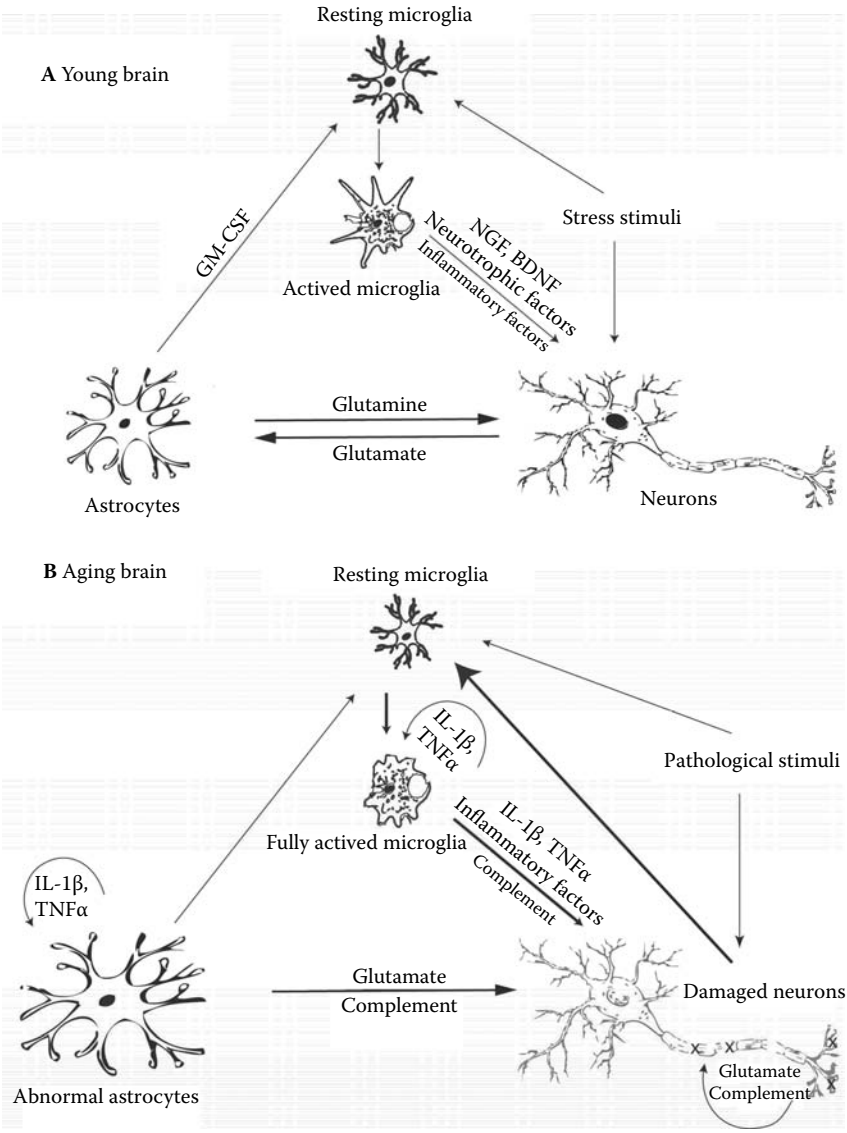

FIGURE 1.3 A simplified schematic representation of interactions of key cells in the young and aged brain.

cytokines, cellular damage and death, and the recruitment and activation of innate immune effector cells (microglia/macrophages) and circulating lymphocytes. These inflammatory processes may be relatively subtle in some individuals or more pronounced and closely associated with the disease process in those who suffer from AD, PD, and other age-related neurodegenerative conditions. A simplified illustration of the key cells that are affected in the brain during aging is shown in Figure 1.3. In this section we describe immunity-related changes that occur during normal aging

in the major cell types in the brain (astrocytes, microglia, and neurons). As this topic has been reviewed in considerable detail previously (Bishop et al. 2010; Mrak et al. 1997; Lucin and Wyss-Coray 2009), we will pay particular attention to the impact of age-related immune alterations on neural stem cells (NSCs) because of their potential to replace neurons and glial cells damaged in aging, injury, and disease.

1.3.1 Changes in Glial Cells during Aging

The numbers of activated astrocytes and microglia are increased during normal aging (Morgan et al. 2007). Data suggest that total numbers of astrocytes and pericytes may increase by ~20% in the aged cortex and other brain regions, whereas the number of oligodendrocytes and microglia does not change (Pilegaard and Ladefoged 1996; Peinado et al. 1998; Rozovsky et al. 1998). Astrocytes with an activated phenotype increase during aging in multiple brain regions (Cotrina and Nedergaard 2002). The increase in activated glial cells with age is sex-dependent and region-specific (Mouton et al. 2002), and in some brain regions such as the hippocampus, the numbers of astrocytes and microglia may not increase with age (Long et al. 1998).

1.3.1.1 Inflammation and Gliosis

Upon activation by tissue damage, severe cellular stress, or infection, microglia proliferate and undergo a morphological transformation from a ramified to an amoeboid appearance. Depending upon the nature of the activation stimulus, microglia respond so as to perform a specific task that is usually beneficial (e.g., removal of apoptotic cells or abnormal proteins). If the disturbance is relatively minor, microglia may secrete anti-inflammatory cytokines and supportive growth factors. This type of activation is also regarded as alternative activation, or M2 (Colton and Wilcock 2010). If the disturbance poses a more serious threat, such as a pathogen invasion, microglia can release toxic factors to kill the pathogen and recruit help by releasing pro-inflammatory cytokines. This type of activation is also referred to as classical activation, or M1 (Mantovani et al. 2004). M2 microglia are typically considered less inflammatory than M1 cells and are characterized by reduced nitric oxide production and increased anti-inflammatory cytokine production. Accordingly, there is heterogeneity of microglial activation states depending upon the nature, intensity, and duration of a pathological condition (Colton et al. 2006; Maier et al. 2008). The microglial phenotype could mean the difference between a beneficial outcome and a detrimental outcome if the response is either too aggressive or too passive (Lucin and Wyss-Coray 2009). This is especially important in the CNS, in which superfluous inflammation could result in excessive collateral damage to neurons, with dire functional and cognitive implications.

In response to injury and acute infection, microglia may release a combination of factors that function to limit the extent of the injury or infection. These include factors known to promote the survival and plasticity of neurons, including TNF-α and neurotrophic factors like brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) (Morgan et al. 2004; Cullheim and Thams 2007; Trapp et al. 2007), and protect neurons against glutamate-mediated excitotoxicity by, for example, increasing the expression of the glutamate transport

protein GLT-1 (Persson et al. 2005; Shaked et al. 2005) and Ca^{2+}-binding proteins (Cheng et al. 1994). On the other hand, parenchymal microglia derived from old mice exhibit an altered inflammatory profile (Streit 2006; Sierra et al. 2007), which may play a role in the previously characterized decrease in neurogenesis with aging (Kuhn et al. 1996). A lower turnover rate of parenchymal microglia during aging may result in a preponderance of microglia with the M1 phenotype that appears to dominate the scene in age-related neurodegenerative disorders.

1.3.2 Changes in Neurons during Aging

During normal aging, in the absence of disease, neuron numbers are believed to remain relatively stable in some brain regions, with modest neuronal loss occurring in other brain regions. For example, a stereological analysis of the cerebellum from human subjects aged 19 to 94 years of age revealed no evidence for loss of either granule neurons or Purkinje cells; however, the volumes of white matter and Purkinje cell somata were reduced, suggesting a reduction in the size of individual neurons (Andersen et al. 2003). Even dopaminergic neurons in the substantia nigra, which are considered to be particularly prone to degeneration and exhibit extensive loss in PD, are maintained at constant levels during aging in some individuals (Alladi et al. 2009). As described in Section 1.3.2.1, however, there is considerable evidence that the functionality of neuronal circuits declines during aging in many regions of the nervous system. While most neurons in the adult mammalian brain are believed not to be replaced, at least two populations of neurons are replaced: granule neurons in the dentate gyrus of the hippocampus and interneurons in the olfactory bulb (Conover and Notti 2008). The impact of aging on this process of neurogenesis is described in Section 1.3.2.2.

1.3.2.1 Neuronal Dysfunction and Degeneration during Aging

The efficiency and accuracy of most behaviors declines during aging. Decrements in vision, hearing, smell, and taste contribute to worsening functions in activities of daily living (Corso 1971). However, there are also deficits in the central processing of information, as reflected in poorer performance in tests of learning and memory (Lister and Barnes 2009) and motor coordination (Seidler et al. 2010), for example. Because neuronal loss is minimal during normal aging, it is likely that age-related deficits in neuronal circuit function are the result of more subtle structural and functional alterations. In this regard, changes in numbers of synapses or functional plasticity of synapses occur in at least some brain regions. Perhaps the most thorough analysis of normative brain aging has been performed by Peters et al., who examined area 46 in the prefrontal cortex of rhesus monkeys (Luebke et al. 2010). Their findings reveal loss of white matter, regression of dendritic arbors, and loss of dendritic spines and synapses. In a study of CBA mice of five ages (4, 8, 12, 18, and 24 months) there was an impairment of cerebellum-dependent delay eyeblink conditioning in the 24-month-old mice (Woodruff-Pak et al. 2010). Stereological analysis of their cerebella indicated significant loss of Purkinje neurons in the 18- and 24-month-old mice, and electrophysiological analysis demonstrated a significant deficit in long-term depression, whereas hippocampal long-term depression was normal in the old mice.

Neurons are maintained well beyond the age of 65 in both lamina III and V of the frontal cortex in human subjects (Scheff et al. 2001). The lack of synaptic decline in the frontal cortex in neurologically normal individuals older than 65 years lends support to the idea that many stereotypic views of age-related changes in the CNS do not apply to all brain regions. Therefore, neuron loss may contribute to age-related dysfunction in some brain regions, whereas in other regions the dysfunction results from loss or dysfunction of synapses.

Changes in neurons that occur during normal aging may be antecedents of common age-related neurodegenerative disorders, including AD and PD. Synapse loss occurs in the frontal cortex of AD patients, as demonstrated by electron microscope-based analysis of biopsy tissue samples (DeKosky and Scheff 1990). Analysis of the dentate gyrus of the hippocampus demonstrated a reduction in synapse number and an associated decline in the width of the molecular layer; interestingly, there was an increase in the size of remaining synapses (Scheff et al. 1996). Individuals with mild AD had fewer synapses in region CA1 of the hippocampus than age-matched individuals who were neurologically normal or were experiencing mild cognitive impairment (MCI); those with MCI had significantly fewer synapses than controls (Scheff et al. 2007). There was a positive correlation between performance on cognitive tests and total CA1 synapses; however, the total number of synapses showed no relationship to numbers of plaques and neurofibrillary tangles. Interestingly, despite the abundance of neurofibrillary pathology, there was no change in synaptic density in the entorhinal cortex between control and Alzheimer subjects (Scheff et al. 1993). Thus, the entorhinal cortex differs from other cortical areas that show a significant decline in synaptic numbers with AD.

1.3.2.2 Neurogenesis in the Aging Brain

Adult neurogenesis is a complex process involving pools of self-renewing progenitor cells that, upon receiving certain signals in their immediate environmental niche, can stop dividing and differentiate into neurons (Lathia et al. 2007). The newly generated neurons can grow axons and dendrites, and form functional synapses with preexisting neurons (Klempin and Kempermann 2007). While neurogenesis dramatically decreases in late embryonic and early postnatal periods, it does take place during adulthood and is thought to play a physiological role in learning and memory (Garthe et al. 2009).

In the adult mammalian brain, neural progenitor cells (NPCs) are located in the hippocampal dentate gyrus subgranular zone (SGZ) and the subventricular zone (SVZ) lining the lateral ventricles (Suh et al. 2009). The production of new neurons from NPCs continues throughout life in rodents, nonhuman primates, and humans. In the olfactory bulb two types of interneurons are generated from a dividing precursor cell population in the SVZ (Altman 1969; Corotto et al. 1993; Luskin 1993; Winner et al. 2002). The continuous addition of interneurons, which modulate spatial and temporal coding of olfactory information, might provide a substrate for adapting to environmental changes (Cecchi et al. 2001; Doetsch and Hen 2005). In the dentate gyrus of the hippocampus, new granule cells are continuously generated from precursor cells in the subgranular zone (Altman and Das 1965; Kaplan and Hinds 1977; Cameron et al. 1993; Kuhn et al. 1996). The formation of new granule cells in the

SGZ is modulated by a large number of physiological stimuli, including exercise (van Praag 2009), dietary energy restriction (Lee et al. 2002b), and environmental enrichment (Rossi et al. 2006). Increasing evidence suggests a role for hippocampal neurogenesis in learning and memory and mood regulation, and a role for olfactory bulb neurogenesis in olfactory discrimination and memory (Abrous et al. 2005).

Across the life span, a progressive reduction of adult hippocampal neurogenesis occurs. With advancing age, there is a decline in precursor cell proliferation and net neurogenesis (Kuhn et al. 1996). This reduction takes place in the context of other structural changes (Rosenzweig and Barnes 2003; Driscoll and Sutherland 2005). Recent findings have shown there are at least two subpopulations of NPCs in the hippocampus, only one of which is adversely affected by aging (Lugert et al. 2010). Regular exercise can counteract the adverse effect of aging on hippocampal neurogenesis in mice (van Praag et al. 2005). Similar to the hippocampus, neurogenesis is impaired in the olfactory bulb of old, compared to young, animals (Brown et al. 2003; Enwere et al. 2004; Luo et al. 2006). Aged mice show olfactory discrimination deficits, attributed to a decline in olfactory neurogenesis (Enwere et al. 2004).

Aging has sometimes been designated as a strong negative regulator of adult hippocampal neurogenesis. Although neurogenesis decreases with advancing age and in old age lingers at a few percent of the value in early adulthood, whether age regulates neurogenesis is problematic. The decline need not be "regulated," but instead may be secondary to general age-related changes, including oxidative stress and elevated glucocorticoid levels. Indeed, experimental clamping of corticosterone at its level in young animals resulted in maintenance of neurogenesis as the animals aged (Cameron and McKay 1999). While most studies have demonstrated a reduction of neurogenesis during aging, counting numbers of proliferating cells alone might be deceiving. For example, even if the baseline level of adult neurogenesis is very low in old age, the relative regulation that is possible from this baseline might be much larger than early in life (Kempermann et al. 1998).

1.3.2.3 The Effects of Inflammation on Neurogenesis: A Role for Microglia

An important component of the disease process in many neurological disorders is inflammation; microglia are major players in such states (Kerschensteiner et al. 2009). With their diversity of cell types and activation states, microglial effects on adult neurogenesis may range from detrimental to supportive (Simard and Rivest 2004). The turnover rate of microglia in the healthy adult brain is probably low (Lawson et al. 1992). However, during pathological conditions, both intrinsic proliferation of parenchymal microglia and recruitment of monocytes could be substantial (Flugel et al. 2001; Ladeby et al. 2005; Djukic et al. 2006; Ajami et al. 2007). Microglial processes and arborizations are highly mobile and malleable, which may enable microglia to scan the environment without disturbing neuronal networks (Davalos et al. 2005; Nimmerjahn et al. 2005). Sudden appearance of factors that are not usually detected, damage to neuronal membranes, and loss of inputs can all result in focal and transient changes in the microglial activation profile (van Rossum and Hanisch 2004; Hanisch and Kettenmann 2007; Pocock and Kettenmann 2007).

While microglial activation and the resulting inflammation could be detrimental to adult neurogenesis, this may not always be the case. Evidence indicates that microglia

under certain circumstances can be beneficial and support the different steps in adult neurogenesis. Most studies so far have primarily focused on the microglial reaction after an acute injury, or have used exogenous administration of the bacterial endotoxin lipopolysaccharide (LPS; a TLR4 ligand) (Ekdahl et al. 2003; Monje et al. 2003). LPS mimics the infection by Gram-negative bacteria, which results in a massive TLR4-mediated antimicrobial defense reaction with an acute excessive activation of microglia that can trigger the death of newly formed neurons (Hanisch and Kettenmann 2007). However, as mentioned above, microglial functional phenotype is context-dependent and probably adapts as the microenvironment changes in order to cope with altered homeostasis. Therefore, LPS-stimulated microglia do not reflect all microglial functions, but can only provide proof of principle that this particular functional phenotype (probably the M1 phenotype) is detrimental for survival and differentiation of newly formed neurons in the adult brain (Ekdahl et al. 2003; Monje et al. 2003). Further support for a detrimental effect of the M1 microglial activation state can be inferred from observations in transgenic mice, which exhibit chronic expression of interleukin-6 (IL-6) by LPS-activated microglia and an associated decrease in the production of new neurons (Vallieres et al. 2002). In addition, cell culture studies showed that NSC survival is compromised when NSC are exposed to IL-6 (Monje et al. 2003). Together with other inflammation-induced cytokine products like interferon-γ (IFN-γ), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α), IL-6 may suppress neurogenesis in inflammatory states (Ben-Hur et al. 2003; Monje et al. 2003; Cacci et al. 2005; Iosif et al. 2006; Koo and Duman 2008).

Further evidence for an adverse effect of microglia on neurogenesis comes from a study in which minocycline, a microglia inhibitor, administered for 5 weeks resulted in an increase in the number of newly formed neurons, while the microglia population decreased (Monje et al. 2003). However, other studies have shown that the natural killer cell- and T cell-derived protein IFN-γ can be both neurotoxic and supportive of neurogenesis. The deleterious effect of IFN-γ is well-characterized, but recent observations have indicated that on the contrary, microglia stimulated with low levels of IFN-γ support neurogenesis (Butovsky et al. 2006), and that IFN-γ enhances neuronal differentiation directly when administered to NSCs or neuronal cell lines (Wong et al. 2004; Song et al. 2005). In addition, IFN-γ transgenic mice exhibit increased NSC proliferation and differentiation in the adult dentate gyrus associated with neuroprotection and improved spatial cognitive performance (Baron et al. 2008). The previously reported neurotoxic effect by IFN-γ in this area could be due to its occurrence in high concentrations or to the concomitant presence of inflammatory mediators such as LPS or TNF-α during severe infection (Baron et al. 2008).

When acutely activated microglia change into an anti-inflammatory M2 phenotype following an injury, the cells either maintain their phenotype or divert into a more deleterious activation state. This transition can be demonstrated by changes in their cytokine production profile when cultured microglia are exposed to LPS for a prolonged time period. Newly formed neurons that did not die during the first month of deleterious microglial activation continued to survive for at least 6 months following the insult (Bonde et al. 2006). Interestingly, this long-term survival occurred despite the concomitant chronic microglia response, suggesting an ability of NSC to adapt to an inflammatory environment (Bonde et al. 2006). An instructive,

beneficial role of microglia in adult neurogenesis is supported by studies of NSCs cocultured with microglia or grown in conditioned media from microglia (Aarum et al. 2003; Morgan et al. 2004; Walton et al. 2006; Nakanishi et al. 2007).

Studies exploring the interaction between brain inflammation and neurogenesis have so far mainly focused on the short- and long-term influence of microglia on progenitor proliferation and survival of new neurons. However, it is conceivable that microglia could also influence the functional properties and synaptic connectivity of the new neurons. For example, activated microglia secrete cytokines and growth factors, such as TNF-α and BDNF, which can modulate excitatory (Pickering et al. 2005) and inhibitory (Henneberger et al. 2005) synaptic transmission and alter dendritic spine morphology (Schratt et al. 2006; von Bohlen und Halbach et al. 2006). In fact, recent findings support roles for inflammatory mediators in the development of the functional synaptic connectivity of the new neurons. Thus, new hippocampal neurons born after induction of epileptic seizures received decreased excitatory and increased inhibitory synaptic drive (Jakubs et al. 2006). The latter response may involve TNFα, a cytokine that modulates synaptic plasticity and vulnerability of neurons to seizure activity (Bruce et al. 1996; Albensi and Mattson 2000). Moreover, LPS-induced inflammation without seizure activity and neuronal death, leading to chronic elevation of the numbers of activated microglia, resulted in enhanced inhibitory input to the new hippocampal neurons (Ekdahl et al. 2009).

The risk for brain tumors increases with advancing age (Flowers 2000). The cellular environment surrounding a brain tumor differs considerably from the pro-inflammatory environment in affected brain regions of patients with neurodegenerative disorders. Soluble factors released by the tumor cells change the phenotype of surrounding microglia; TNFα and IL-6 are downregulated, and expression of metalloproteases is upregulated, which stimulates the growth and invasiveness of the tumor cells (Markovic et al. 2005; Sliwa et al. 2007). Conversely, the intrinsic properties of the microglia population seem to be an important factor. A reason that glioblastomas are almost always fatal is that they harbor a small population of cancer stem cells that are resistant to chemotherapy and radiation. Similar to NPCs, self-renewal of glioblastoma stem cells depends upon the tonic repression of neuron-specific genes by a transcriptional repressor called REST and an associated protein called TRF2 that stabilizes REST (Zhang et al. 2009). Both NSCs and cancer stem cells can be induced to stop dividing and establish a neuronal phenotype by experimental treatments that target REST or TRF2. Though not yet established, it may also be possible to suppress neural tumor growth by activating innate immune pathways that induce differentiation of cancer stem cells or reduce their survival. Consistent with the latter possibility, it was recently shown that both TLR3 (Lathia et al. 2008) and TLR2 (Okun et al. 2010) suppress neurogenesis.

1.3.2.4 Microglia and Acute Brain Injuries

Neurogenesis may be increased in response to an acute brain injury such as severe epileptic seizures and stroke; such injury-induced neurogenesis can occur in the SVZ and the SGZ in the hippocampus (Bengzon et al. 1997; Parent et al. 1997; Arvidsson et al. 2001, 2002). Interestingly, ischemia-induced neurogenesis gives rise to new neurons not only in the SGZ and SVZ, but also in areas that are nonneurogenic in

the intact brain, the striatum, and to a minor extent, the cerebral cortex (Lindvall and Kokaia 2010). Similarly, following hippocampal damage caused by epilepsy, aberrant migration of new neurons is seen toward the necrotic area in the dentate hilus (Parent et al. 1997; Scharfman et al. 2000, 2002). These findings raised the possibility that stimulation of neuronal replacement by neurons produced by endogenous neurogenesis could become of value for restoring function after stroke and other conditions leading to neuronal loss. However, although there are animal studies reporting that increased neurogenesis may be associated with improved recovery after stroke, definite proof for a causal relationship is lacking (Lindvall and Kokaia 2010).

Also after stroke, the microglial population changes over time with respect to morphology, phenotype, and cytokine expression. Consistent with a cytotoxic action of microglia early after the insult, administration of the anti-inflammatory drug indomethacin improved the survival of the stroke-generated neuroblasts in the striatum (Hoehn et al. 2005). Similarly, delivery of minocycline, which reduces microglia activation, during 1 month after MCAO increased the number of new neuroblasts and mature neurons in the dentate gyrus (Liu et al. 2007). Importantly, data suggest that neurogenesis continues for at least 1 year after a stroke (Kokaia et al. 2006; Thored et al. 2006), suggesting the possibility of the formation of new neuronal circuits and restoration of function even long after the stroke occurred.

The inflammatory system may also be involved in the migration of the new neurons toward ischemic areas, acting through the chemokine stromal cell-derived factor-1α (SDF-1α) and its receptor CXCR4 (Thored et al. 2006), the latter being highly expressed by neural progenitors (Ni et al. 2004). SDF-1 is upregulated by the glial population after a stroke and is implicated as an inflammatory stimulus that could enhance both progenitor proliferation and chain migration (Imitola et al. 2004). Moreover, SDF-1 has been reported to promote the differentiation of newly generated neurons into inhibitory GABAergic neurons (Luo et al. 2008), which may provide a mechanism to suppress unrestrained neuronal activity that can occur in brain injury.

1.4 INFLAMMATORY RESPONSES ARE EXAGGERATED IN THE BRAIN DURING LATE LIFE

As in many other organ systems (Libby 2007), inflammatory processes increase during aging as the result of oxidative stress, and cell damage and death. Hypersensitivity to innate immune activation in the brain is evident in several models of aging. Mixed glial and coronal sections from the brains of aged rodents are hyperresponsive to LPS stimulation and produce more inflammatory cytokines (e.g., IL-1β and IL-6) than those of cultures from younger animals (Ye and Johnson 2001; Xie et al. 2003). Further, older mice are more sensitive to septic shock induced by intracerebroventricular (ICV) administration of LPS. Old mice had elevated TNF-α production in the brain and plasma after LPS challenge compared with young adult controls (Kalehua et al. 2000). In another murine model of aging, microarray analysis revealed that peripheral injection of LPS induced a higher expression of IL-1β and TNF-α in the hippocampus of aged mice compared to young mice (Terao et al. 2002). Another microarray analysis

showed increased markers of glial reactivity, including major histocompatibility complex (MHC) class II, CD68, and glial fibrillary acidic protein (GFAP), in the brains of aged mice (Godbout et al. 2005). In this model peripheral stimulation of the innate immune system with LPS caused an exaggerated inflammatory cytokine response in the aged brain with increased production of IL-6 and IL-1β.

Aged mice that experienced an amplified and prolonged neuroinflammatory response to LPS showed a delayed recovery from sickness behavior (Godbout et al. 2005). In a rat model of aging, in which increased reactive glia with MHC class II expression were detected, peripheral injection of *Escherichia coli* promoted higher levels of IL-1β in the hippocampus of old compared to young animals (Barrientos et al. 2006). This increased IL-1β production in the hippocampus of aged rats after *E. coli* challenge was associated with impaired long-term hippocampus-dependent memory (Barrientos et al. 2006). Neither of these studies (Godbout et al. 2005; Barrientos et al. 2006) found peripheral inflammatory cytokines to be a reliable indicator of the exaggerated inflammatory responses in the CNS. We recently measured levels of a panel of cytokines, neurotrophic factors, and stress response proteins in brain tissue samples (cerebral cortex and striatum) of young, middle-age, and old mice that had been subjected (or not) to a stroke. Old mice exhibited higher levels of pro-inflammatory cytokines (TNFα, IL-1β, and IL-6) and lower levels of neuroprotective proteins (BDNF, bFGF, HSP70, GRP78, and HO-1) than young mice (Arumugam et al. 2010). Interestingly, even the side of the brain not directly affected by the stroke exhibited elevated levels of pro-inflammatory cytokines in old compared to young and middle-age mice, suggesting aging reduces the ability of the brain to contain inflammatory processes within the brain region directly affected by the injury. Taken together, these results suggest that the presence of reactive glia in the aged or diseased brain is permissive to an amplified, spreading, and prolonged neuroinflammatory response, which may lead to subsequent behavioral and cognitive complications.

1.5 HORMONAL CHANGES IN THE BRAIN DURING AGING THAT AFFECT IMMUNITY

One well-known aspect of aging is progressive changes in the status of multiple neuroendocrine systems. In women, estrogen levels decline precipitously at menopause, and in men testosterone levels decline steadily with advancing age (Chahal and Drake 2007). There is considerable evidence from experimental cell culture and animal models that estrogen and testosterone can suppress inflammatory processes (e.g., microglial activation and pro-inflammatory cytokine production) in the brain, and can protect neurons against dysfunction and death (Bruce-Keller et al. 2000; Pike et al. 2009). Insulin resistance and consequent diabetes are increasingly common with advancing age and may promote inflammatory processes in the brain as a result of increased oxidative stress caused by this metabolic state (Craft 2007). Mice that are insulin resistant as the result of either overeating a normal diet or consuming a diet high in saturated fats and sugar exhibit deficits in learning and memory and impaired hippocampal synaptic plasticity and neurogenesis (Stranahan et al. 2008a, 2008b). The relative contribution of innate and humoral immune systems to

insulin resistance/diabetes-induced brain dysfunction and degeneration remains to be determined.

Another endocrine system that is altered during aging is the hypothalamic–pituitary–adrenal (HPA) axis, which controls the production of the glucocorticoid cortisol. Basal levels of corticosteroids are generally elevated during aging, probably due to impairment of negative feedback mechanisms that normally suppress the HPA axis after a surge of corticosteroids. Aging is also associated with a blunted activation of the HPA axis by stress or inflammation (Nicolson et al. 1997; Terrazzino et al. 1997; Kudielka et al. 2004). Moreover, the diurnal fluctuations of corticosteroids are moderated or lost during aging. Persistent elevation of corticosteroid levels might be responsible for the documented age-related downregulation of glucocorticoid receptors in the brain, most obviously in hippocampus (Sapolsky et al. 1983). Their diminution—along with that of the sex steroids—might become permissive for exaggerated and prolonged activation of microglia. Excessively high levels of glucocorticoids may play a particularly important role in cognitive decline during aging because data suggest that chronic stress during mid- and late life can increase the risks for depression, cognitive impairment, and AD (Rothman and Mattson 2010). Moreover, recent findings suggest a role for adrenal glucocorticoids in the impaired neuroplasticity and cognitive deficits caused by diabetes and insulin resistance (Stranahan and Mattson 2008; Stranahan et al. 2008b).

1.6 INNATE IMMUNE EFFECTORS IN THE BRAIN DURING AGING

In the elderly, systemic infection is associated with an increased frequency of behavioral and cognitive complications (Penninx et al. 2003; Evans et al. 2005). Stimulation of the peripheral immune system in aged mice causes exaggerated neuroinflammation (Henry et al. 2008) that is paralleled by prolonged sickness behavior (Godbout et al. 2005), impaired working memory (Chen et al. 2008), and protracted depressive-like behavior (Godbout et al. 2008). In the following paragraphs we will describe the roles of TLRs and complements in mediating such adverse effects of aging on the brain.

1.6.1 TLRs

Aging of the brain is associated with changes in the expression of innate immune receptors. The expression of TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, and CD14 is upregulated during aging, with TLR4 showing the strongest response. In contrast, TLR9 expression in the brain decreases during aging, whereas levels of TLR8 are unchanged (Letiembre et al. 2007). A recent study assessed the response of microglial cells from aged mice to systemic LPS challenge (Henry et al. 2009). Peripheral LPS injection causes exaggerated microglial mRNA and protein induction of both inflammatory IL-1β and anti-inflammatory IL-10 in aged mice compared to young adult mice. Moreover, a large fraction of microglial cells in aged mice coexpress MHC-II and high levels of IL-1β, implying that these cells are in a primed or reactive state.

The reason why the expression of these innate immune receptors is altered in brain aging despite the absence of any overt pathology is unclear. During normal

brain aging, altered protein turnover is believed to contribute to the aggregation of multiple proteins (e.g., β, τ, and α-synuclein), and oxidized lipofuscin-ceroid accumulation (Kato et al. 1998; Terman and Brunk 1998, 2006). The accumulation of these proteins and lipids is, in part, the consequence of oxidative stress and believed to contribute to the dysfunction and damage to neurons that occurs in normal aging and, more dramatically, in AD, PD, and other age-related neurodegenerative disorders (Keller et al. 2004). Recently, several studies demonstrated the involvement of TLR2 and TLR4 in oxidative stress (Frantz et al. 2001; Miller et al. 2003; Walton et al. 2003a, 2003b; Holvoet et al. 2006), and that endogenous heat shock proteins (HSPs) that are elevated in brain aging (Calabrese et al. 2005) can interact with CD14, TLR2, and TLR4 (Asea et al. 2000, 2002; Dybdahl et al. 2002; Vabulas et al. 2001, 2002a, 2002b, 2002c). Thus, aging-induced oxidative stress generates HSPs that could potentially activate different TLRs, including TLR2 and TLR4. In addition, an exercise program resulted in a reduction in TLR4 signaling in circulating lymphocytes of human subjects (Stewart et al. 2005), which may contribute to the beneficial effects of exercise on the aging brain (van Praag 2009). Similarly, alternate-day fasting suppressed age-related and stroke-induced increases in pro-inflammatory cytokines in the brains of mice (Arumugam et al. 2010).

Downregulation of TLR9 in the brain during aging might be a mechanism that dampens inflammatory responses previously reported in brain aging (Blalock et al. 2003; Godbout and Johnson 2004; Lu et al. 2004). This would be consistent with a microglial dystrophy characterized by deramification, spheroid formation, and fragmentation of processes in brains of the elderly (Streit et al. 2004). An altered profile of innate immune receptors on these cells might be a correlate of such age-related microglial dystrophy. Interestingly, a TLR4 polymorphism was also associated with successful aging (Candore et al. 2006), which further indicates a role of innate immune receptors in aging.

1.6.2 The Complement System

The role of the complement system during brain aging is not clear. A DNA microarray study in mice demonstrated that cellular immunity and inflammation are elevated during brain aging (Lee et al. 2000). Analysis of gene transcription from the neocortex and cerebellum of aged mice showed increased transcription of complement C4, C1qa, C1qb, and C1qc. The mRNA increase of those selected markers during aging was confirmed by quantitative real-time PCR, which indicates that concurrent production of complement proteins in the brain might lead to the generation of pro-inflammatory peptide fragments contributing to functional alterations in the brain during aging or in age-related disease. Expression of several specific immune response genes was also elevated in the cortex, cerebellum, and hippocampus with aging (Lee et al. 2000). The complement C1q B and C chains also showed a steady increase in the aged hippocampus (Terao et al. 2002). In addition, expression of both the classical and alternative complement pathway components, such as C1q, C3, C4, C5, and factor B mRNA, shows an age-dependent increase in control mice. The protein level of C1q in the

brain was elevated in 15-month-old mice compared to young mice, which was correlated with a similar elevation of C1q mRNA levels (Reichwald et al. 2009).

It was reported that levels of C3a and MAC are higher in the CSF of elderly subjects than in younger subjects (Loeffler et al. 1997). The C3a concentration in normal aged subjects was threefold higher than in normal younger subjects. There was also a trend toward increased MAC levels in the brain during normal aging (Loeffler et al. 1997). In another study, levels of C4d and C3b fragments were elevated in hippocampal tissue samples from aged compared to young individuals, suggesting that early components of the complement cascade increase in the brain during normal aging (Loeffler et al. 2004). A finding from the comparison of brain complement activation between young and aged rhesus monkeys shows significantly higher activation of the early component of complement cascade, but with no detectable terminal component activation. Since the activation of terminal complement pathway is tightly controlled by regulatory proteins, as described above, lack of activation of a terminal component suggests that the complement cascade is restrained in brain cells during normal aging in the absence of pathology. In the case of pathology, such as intracerebral hemorrhage (ICH), levels of complement factor C9 and clusterin in ipsilateral basal ganglia were elevated more in aged rats than in young rats (Gong et al. 2008). More C9- and clusterin-positive cells were found around the hematoma in aged rats. However, myeloperoxidase (a marker for the detection of neutrophil infiltration)-positive cells in ipsilateral basal ganglia were fewer in aged rats after ICH. This suggests that ICH causes more severe complement activation and less neutrophil infiltration in aged rats (Gong et al. 2008).

1.7 OXIDATIVE STRESS AND INFLAMMATION IN THE BRAIN DURING AGING

Reciprocal, cross-amplifying interactions between cellular oxidative stress and inflammation occur in many tissues during usual aging. Many of the same oxidative and inflammatory cascades are clearly activated excessively in many different neurological disorders, and these pathological processes are often associated closely (in space and time) with hallmark histopathological lesions, including Aβ aggregates in AD and Lewy bodies in PD (Mattson 2002; Nunomura et al. 2007). In normal aging there is an accumulation of oxidized proteins, DNA, and lipids in brain cells (Cutler et al. 2004; Haripriya et al. 2005; Markesbery et al. 2005; Poon et al. 2005). DNA bases within the promoter regions of several important neuronal genes, including those that encode proteins involved in synaptic plasticity and mitochondrial function, are prone to oxidative modification during brain aging in humans (Lu et al. 2004). Several genes critical for inhibitory GABAergic transmission are markedly downregulated in brain cells during normal aging (Loerch et al. 2008). Reduced inhibition with aging may result in excessive activity in some neuronal circuits, which would be expected to promote cellular Ca^{2+} overload (Bezprozvanny and Mattson 2008). Moreover, genes encoding proteins involved in DNA protection and repair are suppressed in multiple brain regions during aging (Xu et al. 2007).

Oxidative stress in brain cells activates innate and humoral immune systems in the following ways. Oxidative modification of cell surface and secreted proteins by lipid peroxidation products and glycation can be recognized by receptors on microglia and lymphocytes (Wang et al. 2008; Yun et al. 2008). Oxidative stress activates several cellular signaling pathways in glial cells that result in the induction of genes encoding pro-inflammatory proteins. One such pathway involves the transcription factor NF-κB, which induces the expression of TNF-α, IL-1β, and IL-6 by microglia, cytokines that may damage neurons, particularly under conditions (metabolic, oxidative, and proteotoxic stress) that occur in aging (Fine et al. 1999; Kaushal and Schlichter 2008). However, it should be appreciated that activation of NF-κB in neurons upregulates the expression of several cytoprotective proteins, including Mn superoxide dismutase and Bcl-2, as well as proteins involved in synaptic plasticity (Mattson and Meffert 2006). In addition, oxidative stress upregulates the expression of several TLRs, including TLR2 and TLR4, in neurons (Tang et al. 2007).

Activation of innate and humoral immune systems promotes oxidative stress in brain cells in the following ways. As described above, TLRs 2 and 4 are coupled to NF-κB and the production of pro-inflammatory cytokines, which can promote oxidative stress in neurons and glial cells (Scirocco et al. 2010; Qin et al. 2005). Activation of the complement cascade induces oxidative stress by elevating intracellular Ca^{2+} levels (Xiong and McNamara 2002; Luo et al. 2003), resulting in generation of superoxide by the activity of oxidases and the mitochondrial electron transport chain (Hongpaisan et al. 2004; Ibi et al. 2008). In addition, infiltrating macrophages and lymphocytes produce pro-inflammatory cytokines and also reactive oxygen species (ROS) and excitotoxins (Guo et al. 2004). Figure 1.4 illustrates the involvement of TLRs and complement cascades in the inflammatory process during aging.

1.8 THE CONTRIBUTION OF INFLAMMATION TO AGING-ASSOCIATED COGNITIVE DECLINE AND ALZHEIMER'S DISEASE

This section reviews the evidence that innate and humoral immune signaling pathways are aberrantly activated in brain regions involved in learning and memory processes in Alzheimer's disease (AD), and to a lesser extent in age-related mild cognitive impairment. Similar alterations in these inflammatory pathways are also believed to occur in several other neurodegenerative disorders, including PD, HD, stroke, and amyotrophic lateral sclerosis. For information on the latter disorders, the reader is referred to previous articles (Singhrao et al. 1999a, 1999b; McGeer and McGeer 2005a, 2005b; Moisse and Strong 2006; Tang et al. 2007; Wang et al. 2007; Holmoy 2008; Stone et al. 2009; Tansey and Goldberg 2010). AD is a progressive neurodegenerative disease characterized by gradual onset and advancement of memory loss and other cognitive deficits. Definitive diagnosis of AD is based on the presence of extracellular amyloid plaques comprised of neurotoxic amyloid β-peptide (Aβ), which is generated by proteolysis of the β-amyloid precursor protein (APP), and intracellular neurofibrillary tangles composed of hyperphosphorylated insoluble forms of τ protein (Mattson 2004). Genetic factors that either cause or predispose

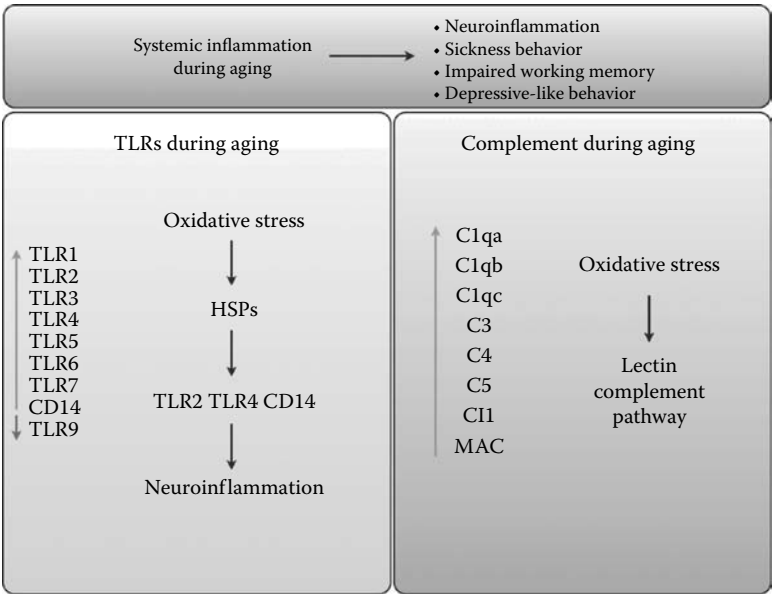

FIGURE 1.4 Aging-induced oxidative stress activates TLRs and complement cascades. Aging-related oxidative stress and inflammation result in impaired working memory and depressive-like behavior. Multiple mechanisms are responsible for these alterations, including increased expression of TLRs 1–7 and CD14, and decreased expression of TLR9. Oxidative stress activates heat shock proteins (HSPs), which in turn activate TLR2, TLR4, or CD14 to induce neuroinflammation. In addition, increased expression of the complement components C1qa, C1qb, C1qc, C3, C4, C5, and C11 occurs in the brain during aging, along with increased levels of the membrane attack complex (MAC). Oxidative stress associated with aging activates the lectin complement pathway contributing to neuroinflammation.

to AD include mutation in APP and presenilins 1 and 2 (which cause early-onset autosomal-dominant inherited AD) and polymorphisms in apolipoprotein E (ApoE4 increases the risk of AD). Activation of the innate immune response by reactive glia in association with Aβ and neurofibrillary tangles is a consistent pathological feature of AD. Neuroinflammation in the AD brain is concentrated at sites of Aβ plaques, which exhibit increased levels of pro-inflammatory cytokines, complement components, and proteases (Akiyama et al. 2000; McGeer et al. 2006). Aβ plaques are surrounded and infiltrated by activated astrocytes and microglia, which are believed to be the major source of local inflammatory components. Neuroinflammation is proposed to play a major role in AD pathogenesis, because long-term treatment with nonsteroidal anti-inflammatory drugs reduces AD risk and may delay disease progression (Stewart et al. 1997; in t'Veld et al. 2001).

1.8.1 TLRs

The expression of several TLRs is elevated in the AD brain. TLR2 and TLR4 expression is increased in the brain of AD patients (Walter et al. 2007). Further,

multiple TLR genes (1–8) are expressed in microglia in postmortem tissue from AD patients, with varying levels of expression (Bsibsi et al. 2002). A screening of TLRs in murine models of AD revealed an upregulation of TLR2 and TLR7 transcription levels compared to nontransgenic control mice (Letiembre et al. 2009). Activated glia surrounding Aβ plaques express high levels of TLR4 and TLR2 (Letiembre et al. 2007; Walter et al. 2007). The increased expression of TLRs in association with the disease process in the brains of AD patients and animal models of AD suggests roles for TLR signaling in neurodegenerative mechanisms and disease progression.

Interestingly, the TLR4 gene has emerged as a candidate susceptibility gene for AD. A common missense polymorphism occurs at the TLR4 gene locus resulting from an adenine-to-guanine substitution 896 nucleotides downstream of the transcription start site. This substitution causes the replacement of glycine for aspartic acid at amino acid 299 (Asp299Gly), and alters the structure of the extracellular domain of TLR4. This mutation attenuates TLR4 signaling in response to LPS and diminishes the ability to induce inflammation (Arbour et al. 2000). Accordingly, this polymorphism is associated with decreased cardiovascular disease and successful aging (Balistreri et al. 2004). The Asp299Gly polymorphism was associated with a decreased risk of late-onset AD in an Italian population cohort, independent of the susceptibility gene ApoE 4 (Minoretti et al. 2006). However, a mechanism by which the Asp299Gly polymorphism may suppress the disease process remains to be determined.

Studies in which TLR signaling is increased or decreased in cell culture and animal models have provided evidence that TLR4 signaling accelerates neurodegenerative processes in AD. Young APP transgenic mice treated for 12 weeks with LPS (a TLR4 ligand) exhibit high numbers of activated microglia and astrocytes throughout the neocortex and hippocampus. Further, following LPS treatment there was significant accumulation of intraneuronal Aβ, and these cells were in close proximity to activated microglia (Sheng et al. 2003). We have examined the role of TLR4 in AD using primary neuronal cultures from TLR4 mutant mice (Tang et al. 2008). Aβ damaged neurons by causing membrane-associated oxidative stress and the production of the lipid peroxidation product 4-hydroxynonenal (HNE). We found that TLR4 expression increases during exposure of neurons to Aβ and HNE. In addition, JNK and caspase-3 activity levels are augmented in neurons exposed to Aβ and HNE. Selective elimination of TLR4 function significantly suppresses the abilities of Aβ and HNE to induce activation of JNK and caspase-3 (Tang et al. 2008), suggesting that TLR4 expression increases neuronal vulnerability to Aβ-induced damage. Consistent with this, we found that levels of TLR4 are decreased in AD brain tissue samples compared to control subjects. Taken together, this suggests that neurons expressing TLR4 have increased sensitivity to Aβ and are vulnerable to degeneration in AD.

On the other hand, it has been suggested that activation of TLR4 is required for clearance of Aβ in AD. For example, in both young and old APP/PS1 double-mutant transgenic mice, an acute intrahippocampal injection of LPS significantly decreases Aβ deposition, dependent upon microglial activation (Herber et al. 2007). Mice carrying mutations in both APP and PS1, together with a point mutation in TLR4, exhibit augmented Aβ deposition in both the neocortex and hippocampus (Tahara

et al. 2006). Cultured microglial cells from these mice are unresponsive to TLR4 ligands, while activation of TLR4 in cultured microglial cells from wild type littermates induces phagocytosis of Aβ (Tahara et al. 2006). In addition to TLR4, activation of other TLRs may also contribute to Aβ clearance. Activation of TLR2 by peptidoglycan (PGN) initiates Aβ uptake by transformed microglial cells (Chen et al. 2006; Tahara et al. 2006). TLR9 activation by CpG also results in significant clearance of Aβ from cultured mouse microglia (Prat and Antel 2005; Prinz et al. 2006).

Whereas TLRs are activated by exogenous pathogens, mounting evidence indicates that Aβ itself activates TLRs and mediates microglial activation. Aβ stimulates microglia, as assessed by nitric oxide and TNF-α production, at similar levels to LPS (TLR4 agonist), Pam (TLR2 agonist), and CpG (TLR9 agonist) (Lotz et al. 2005). In mouse microglial cultures aggregated Aβ stimulates production of nitrite and TNF-α (Walter et al., 2007). However, Aβ stimulation of microglial cultures from mice bearing a point mutation in TLR4 was significantly diminished, suggesting that microglial activation by Aβ at least in part requires functional TLR4 (Walter et al. 2007). Consistent with this, TLR4 mutant AD mice exhibit diminished TNF-α, IL-1β, IL-l0, and IL-7 compared to AD mice with wild type TLR4, suggesting that the upregulation of these cytokines by activated microglia in AD is dependent upon TLR4 signaling (Jin et al. 2008). Aβ sensitizes microglia to stimulation by LPS (Gasic-Milenkovic et al. 2003), and administration of Aβ together with LPS increases activation of TLR4, leading to increased release of nitric oxide and TNF-α (Lotz et al. 2005). Costimulation of TLRs with Aβ and Pam3CSK results in hyperactivation of TLR2. However, Aβ does not potentiate the activation of all TLRs as Aβ depresses TLR9 activation by CpG (Lotz et al. 2005). This may be due to the location of the TLR9 receptor in the endoplasmic reticulum or different signaling mechanisms of TLR receptors (Latz et al. 2004; Wagner 2004). Nevertheless, the overall effect of Aβ on TLRs promotes inflammation because activation of TLR9 by CpG can have anti-inflammatory activities (Krieg 2003).

The mechanism by which TLR activation results in Aβ clearance is unclear. The human G-protein-coupled formyl peptide receptor-like 1 (FPRL1) is essential for human macrophage phagocytosis of Aβ (Iribarren et al. 2005). Its mouse homologue, mFPR2, has recently been identified as important in microglial clearance of Aβ following TLR activation in mouse models of AD. The phagocytosis of Aβ protein by both TLR2 and TLR9 is dependent upon activation of mFPR2 in cultured mouse microglia. Activation of both TLR2 and TLR9 initiates p38 MAPK signaling, expression of mFPR2, and subsequent Aβ internalization (Iribarren et al. 2005; Chen et al. 2006). Consistent with this, Aβ clearance following TLR4 activation is blocked by the G-protein inhibitor pertussis toxin (Tahara et al. 2006), suggesting that TLR4-induced Aβ phagocytosis occurs through a similar signaling pathway and requires mFPR2. Therefore, cell surface expression of mFPR2 may serve as a sensor of Aβ in TLR-activated microglia (Iribarren et al. 2005; Chen et al. 2006).

It remains to be determined if the activation of TLRs by Aβ contributes to or inhibits AD progression. Contrasting data exist on the precise role of TLRs in Aβ deposition. One interpretation of the available data is that mild activation is beneficial by promoting Aβ uptake and breakdown, whereas excessive activation of TLRs on microglia may lead to the accumulation of cytotoxic compounds such as reactive

oxygen species, cytokines, complement proteins, and proteases, causing damage and eventual neuronal loss (Akiyama et al. 2000). In addition, the potentiation of Aβ neurotoxicity by TLR4 activation (Tang et al. 2008) may override the potentially beneficial effects of TLR-mediated removal of Aβ by activated microglia. In any case, and similar to peripheral inflammatory disorders that involve TLR signaling in immune cells (Krishnan et al. 2007), TLR signaling pathways in brain cells are potential therapeutic targets in AD.

1.8.2 Complement Cascade

The role of a complement system in retarding or contributing to chronic neuronal degeneration during aging remains unresolved. For example, a neuroprotective role was suggested in a study of APP mutant mice with C3 deficiency or inactivation by overexpressing a soluble peptide inhibitor of C3 (Wyss-Coray et al. 2002; Maier et al. 2008). However, considerably more evidence suggests deleterious roles for complement proteins in AD. Complement components are deposited at sites of Aβ accumulation in the brains of AD patients (Eikelenboom et al. 1989; McGeer et al. 1989; Shen et al. 1997). Levels of early complement activation products are elevated in AD brain lesions, suggesting that the first steps in the complement cascade may mediate pro-inflammatory actions of Aβ (Eikelenboom et al. 1996; Veerhuis et al. 1996). The levels of mRNAs encoding C1q, C1r, C1s, and C2-C9 were markedly upregulated in affected areas of AD brain compared with non-AD brain. An activated complement system was confirmed by immunoblot analysis of AD hippocampus, consistent with an active role in the development of AD pathologies (Yasojima et al. 1999; Matsuoka et al. 2001; McGeer and McGeer 2002). Other studies revealed the presence of MAC proteins in AD brain tissue samples using several different methods, including immunohistochemistry, immunoblots, enzyme-linked immunosorbent assay (ELISA), and electron microscopy (Terai et al. 1997; Webster et al. 1997). Glial cells from AD patients secrete more C1q than do glial cells from nondemented elderly control subjects (Lue et al. 2001). These findings suggest that microglia are a source of complement proteins that may then damage neurons (Barnum 1995; Morgan and Gasque 1996; Shen et al. 1997). A pivotal role for the complement cascade in the AD process is suggested by a study showing that C1q deficiency reduces Aβ pathology in APP mutant mice (Fonseca et al. 2004).

1.9 IMPACT OF DIET AND EXERCISE ON INFLAMMATORY PROCESSES IN THE BRAIN

As with the cardiovascular system, diet and exercise can modify the aging process in the brain and may thereby modify vulnerability to disorders such as AD, PD, and stroke (Mattson 2004). Data from studies of animal models of aging and neurodegenerative disorders have demonstrated multiple beneficial effects of lifelong dietary energy restriction and exercise on the brain, including enhanced synaptic plasticity, neurogenesis, cognitive performance, and resistance of neurons to toxins

(Bruce-Keller et al. 1999; Lee et al. 2002a; Maswood et al. 2004; Fontan-Lozano et al. 2007; Adams et al. 2008). A recent study of elderly human subjects demonstrated a beneficial effect of caloric restriction on memory retention (Witte et al. 2009). Similarly, regular exercise (e.g., running) can stimulate neurogenesis and improve cognitive and motor function (van Praag et al. 2007). These beneficial effects of dietary energy restriction and exercise are believed to be mediated, in part, by activation of adaptive cellular stress response pathways, resulting in increased production of neurotrophic factors, protein chaperones, and antioxidant enzymes (van Praag et al. 2007; Stranahan et al. 2009; Arumugam et al. 2010).

Both dietary energy restriction and exercise have been shown to suppress multiple inflammatory processes in peripheral tissues (Chung et al. 2006; Woods et al. 2009). Studies of the brains of laboratory animals suggest that energy restriction and exercise also suppress inflammation in the brain. For example, gene microarray analysis of brains of old mice that had been maintained throughout their adult life on a diet with 40% fewer calories than mice on an *ad libitum* control diet exhibited reduced expression of multiple pro-inflammatory genes (Lee et al. 2000). Similarly, alternate-day fasting reduced levels of TNFα, IL-1β, and IL-6 in the cerebral cortex and striatum of mice (Arumugam et al. 2010). The latter study further showed that mice maintained on the alternate-day fasting diet exhibit reduced upregulation of the expression of pro-inflammatory cytokines following an experimental ischemic stroke. When mice that had been subjected to an ischemic stroke were exercised on a treadmill for 30 minutes every day for 3 weeks, they exhibited reduced levels of TLR4 and pro-inflammatory cytokines in the ischemic cortex (Zwagerman et al. 2010). Rats maintained for 7 weeks on a physical training regimen exhibited reduced levels of IL1β and IL-6 in the hippocampus and cerebellum, respectively (Chennaoui et al. 2008). Studies of the effects of short-term voluntary wheel running in control and AD mice demonstrated reductions in markers of inflammation in the brains of exercised compared to sedentary AD and control mice (Parachikova et al. 2008). Performance on a learning and memory test was improved in runner AD mice compared to sedentary AD mice, suggesting a potential role for reduced brain inflammation in the beneficial effect of exercise on cognition.

1.10 CONCLUDING REMARKS

During aging there are progressive increases in the amounts of oxidatively modified proteins, lipids, and DNA in brain cells, and parallel increases in levels of activated glial cells and pro-inflammatory cytokines. However, there are only modest decrements in brain function during normal aging, suggesting that neurons can tolerate a considerable amount of inflammatory and oxidative stress. In individuals who develop a neurodegenerative disorder such as AD, levels of oxidative stress and activation of both innate and humoral immune cells are greatly increased in association with the disease process. In AD, and possibly other age-related neurodegenerative conditions, activation of some TLRs (TLRs 2 and 4) and complement cascade components likely plays a role in amplifying disease processes that ultimately result in neuronal dysfunction and degeneration of neurons. Hyperactivation of intrinsic microglia and the recruitment of circulating leukocytes to sites of neuronal injury may

exacerbate oxidative stress and inflammatory processes. However, many individuals live into their eighth, ninth, and even tenth decades of life with a well-functioning brain—"sharp as a tack." Moreover, emerging findings suggest that everyone can improve their chances for successful brain aging through regular vigorous exercise, moderation in dietary energy intake, and maintaining an intellectually challenging lifestyle. The latter environmental factors may suppress inflammation and oxidative stress by engaging adaptive cellular stress response pathways in neurons. Finally, the innate and humoral immune signaling pathways involved in inflammatory processes in the brain provide abundant targets for the development of drugs with the potential to protect neurons and preserve or enhance brain function in aging and neurodegenerative disorders.

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2 Immune Modulation of Inflammation in Neurodegenerative Diseases

Alzheimer's Disease and Amyotrophic Lateral Sclerosis

Michelle Mahanian, Milan Fiala, Mathew T. Mizwicki, and Antonio La Cava

CONTENTS

2.1 INTRODUCTION

Misguided immune or phagocytic responses to misfolded proteins, amyloid-β (Aβ), superoxide dismutase-1 (SOD-1), α-synuclein, huntingtin, and prion are contributing factors to neurodegenerative diseases such as Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, Huntington's disease, and prion diseases. The process of phagocytic clearance of misfolded proteins is in delicate balance with the process of inflammation, such that one or the other predominates. For instance, dysfunctional clearance of misfolded proteins can lead to plaque formation and overstimulation of inflammatory signaling by eicosanoids, cytokines, and chemokines (i.e., chronic inflammation). Thus, clearance of misfolded protein is crucial in preventing chronic inflammation and maintaining overall human health (Fiala et al. 2007a).

This chapter will focus on two major neurodegenerative diseases, Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS), both of which have misfolded proteins as triggers of inflammatory immune dysfunction in the patients. In this chapter we also describe the potential of small molecules, ligands of the vitamin D receptor, such as curcumin and vitamin D_3, to inhibit inflammation and enhance phagocytic responses.

The major pathology in AD has been linked to the misfolded protein Aβ in neuritic plaques in the AD brain—related to defective clearance by macrophages in humans (Fiala et al. 2005) and microglia in transgenic models (Paresce et al. 1997). The reduced clearance of Aβ and increased inflammation lead to progression of the disease (Fiala et al. 2007a; Fiala 2010).

Similarly, ALS pathology may be due to an inflammatory cascade in response to the misfolded enzyme SOD-1 (Vucic and Kiernan 2009). Recently, we reported that in mononuclear cells of sporadic ALS patients, aggregated wild-type SOD-1 activated the cytokines IL-1β, IL-6, and IL-23. These results likely replicate the immune process occurring in the ALS spinal cord as induced by aggregated SOD-1 in neurons, leading to high serum levels of IL-17 in a majority of patients with ALS (Fiala 2010). Likewise, in mouse microglia, endocytosis of mutant SOD-1 was observed to activate caspase-1 and IL-1β in a process antagonized by autophagy (Meissner et al. 2010). It is hoped that further clarification of the inflammatory mechanisms induced by SOD-1 may lead to the design of new neuroprotective strategies, possibly through the inhibition of selected cytokines.

2.2 INFLAMMATION AND INNATE IMMUNE DYSFUNCTION IN ALZHEIMER'S DISEASE

2.2.1 Clinical Overview of AD

AD is the leading cause of dementia, affecting over 30 million people worldwide, and it is expected to triple or quadruple by mid-century, as the population ages.

This late-life neurodegenerative disease is characterized clinically by a progressive decline in memory and cognitive abilities, including impairment in language, visuospatial skills, and problem solving. The most important risk factors for AD are old age and positive family history (Bird and Miller 2008).

Diagnostically, it is often difficult to distinguish AD symptoms from manifestations of normal aging. The primary screening tool currently in use for AD is the Mini-Mental State Examination (MMSE), a brief questionnaire that assigns patients a maximum score of 30 (normal) based on evaluation of cognitive function. As performance on standard memory tasks declines, the patient is defined to have mild cognitive impairment (MCI), which has a 50% chance of progressing to AD within 5 years. Neuroimaging with computed tomography (CT) and magnetic resonance imaging (MRI) often shows atrophy of the hippocampus in AD patients, whereas functional imaging studies indicate hypoperfusion and hypometabolism in the posterior regions of the temporal-parietal cortex (Bird and Miller 2008). Examination of the brain of patients in autopsy reveals neuritic plaques containing aggregated amyloid-β (Aβ), along with neurofibrillary tangles composed of hyperphosphorylated tau (τ) (Rafii and Aisen 2009). These lesions occur in small amounts during normal aging, but accumulate extensively in Alzheimer's disease. Both Aβ and τ are central to the currently accepted hypothesis for AD pathogenesis. Other pathological changes in the brain include neuron loss, gliosis, decreases in neurotransmitter levels, and inflammation (Lemere and Masliah 2010).

2.2.2 Pathogenesis of AD and Genetic Factors

The leading model for AD pathogenesis—called the amyloid hypothesis—identifies Aβ as the primary factor setting off neural toxicity and degeneration in AD. Aβ is produced when β- and γ-secretases proteolytically cleave the transmembrane amyloid precursor protein (APP) (Yamin et al. 2008). In AD patients, Aβ accumulates due to defective clearance or production and aggregates in oligomeric and fibrillar forms. When Aβ deposits as plaques in the brain, it initiates a neurodegenerative process that involves inflammation, oxidative stress, and apoptosis of neurons, especially in the hippocampus (which is involved in memory consolidation) (Lemere and Masliah 2010).

In addition to Aβ pathology, silver staining of AD samples reveals neurofibrillary tangles in the cytoplasm of neurons made up of hyperphosphorylated τ. The τ proteins interact with tubulin to stabilize microtubules, but τ hyperphosphorylation destabilizes microtubules and leads to formation of paired helical filaments. The amyloid hypothesis suggests that the hyperphosphorylation of τ occurs downstream of the Aβ neurodegenerative cascade (Querfurth and LaFerla 2010).

As for the genetic susceptibility to familial and sporadic AD, the Apo ε gene on chromosome 19 appears as the single most important biological marker associated with late-onset forms of the disease. This gene has three alleles. The Apo ε4 allele has a strong association with AD, even though it is not necessary or sufficient to cause it. There is also some evidence that the Apo ε2 allele could be protective against the development of AD (Bird and Miller 2008).

Patients with Down's syndrome (trisomy 21) have an extra copy of the amyloid precursor protein (APP) gene that leads to increased Aβ formation and deposition in the brain (Bird and Miller 2008). This specific form of AD is caused by overproduction of Aβ. However, in the majority of cases, including late-onset forms of sporadic and familial AD, deficient clearance of Aβ may be the main culprit behind AD pathology.

2.2.3 Defective Phagocytosis of Aβ by Macrophages of AD Patients

Plaque-associated and intraneuronal Aβs appear to have different origins (Wegiel et al. 2007). The Aβ hypothesis attributes neural degeneration in AD to the accumulation of oligomeric Aβ, which accumulates in neurons and interferes with synaptic transmission (Querfurth and LaFerla 2010). The emphasis on mouse models in lieu of genuine AD brain has led to the belief that microglia are important in Aβ clearance in AD, and this has stirred controversy among researchers of mouse models on the role of resident versus blood-derived microglia (Paresce et al. 1997; Simard et al. 2006; Prinz and Priller 2010; Garcia-Alloza et al. 2007). Yet, immunohistochemical and confocal microscopic studies of AD brain suggest that blood-derived macrophages and monocytes are critical in handling Aβ in AD brain (Zaghi et al. 2009). Overall, it appears that both microglia and macrophages may participate (macrophages and monocytes of AD patients are defective in clearance of Aβ) (Fiala et al. 2007b), but microglia cannot be investigated in living patients.

In healthy human subjects, macrophages are able to completely phagocytize Aβ and transport it to lysosomes for degradation, thereby preventing it from building up. In AD patients, Aβ instead seems to bind only to the surface of macrophages. Due to this defect, AD macrophages do not phagocytize Aβ and cannot transport it into lysosomes for clearance and degradation (although they are still able to phagocyte and degrade bacteria). The phagocytic defect in AD macrophages is paralleled by a defect in clearance of neuronal autophagic vacuoles with Aβ (Nixon 2007). See Figure 2.1.

The cognitive defect of AD patients correlates with levels of soluble oligomeric Aβ in the brain (Lue et al. 1999), and the pathological consequences of intraneuronal accumulation of oligomeric Aβ are believed to relate to disruption of synaptic plasticity (although some authors do not find a relation between interneuronal Aβ and neurodegeneration (Wegiel et al. 2007)).

2.2.4 AD Is Associated with Transcriptional Alterations in MGAT III and Toll-Like Receptors

Monocytes and macrophages of AD patients not only show ineffective phagocytosis of Aβ, but also show transcriptional defects in key genes related to phagocytosis. The most prominent transcriptional defect observed in AD mononuclear cells involves downregulation of MGAT3 and Toll-like receptor (TLR) genes. (Fiala et al. 2007b). Monocytes and macrophages of healthy human subjects respond by upregulating

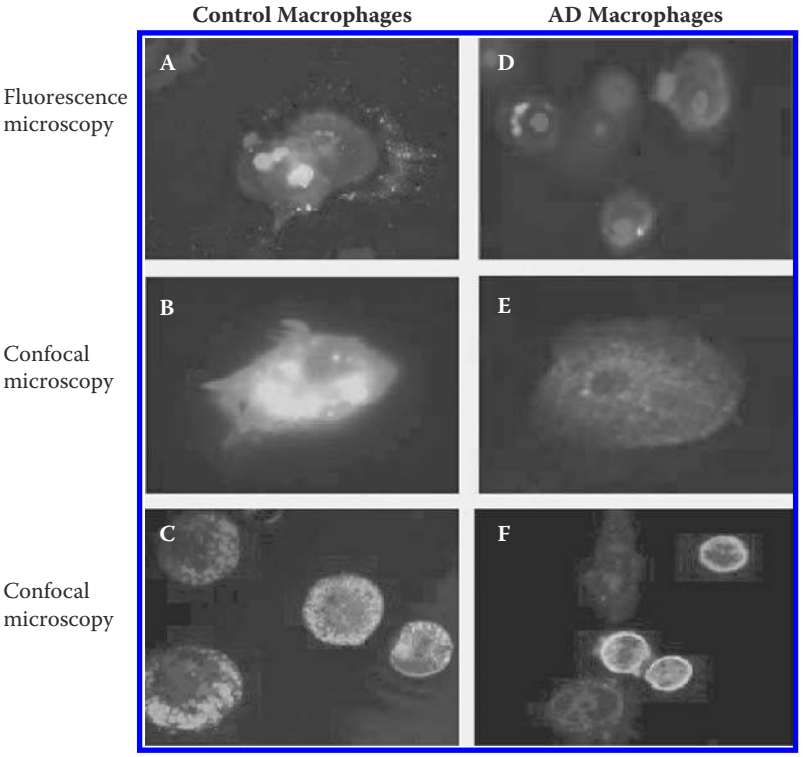
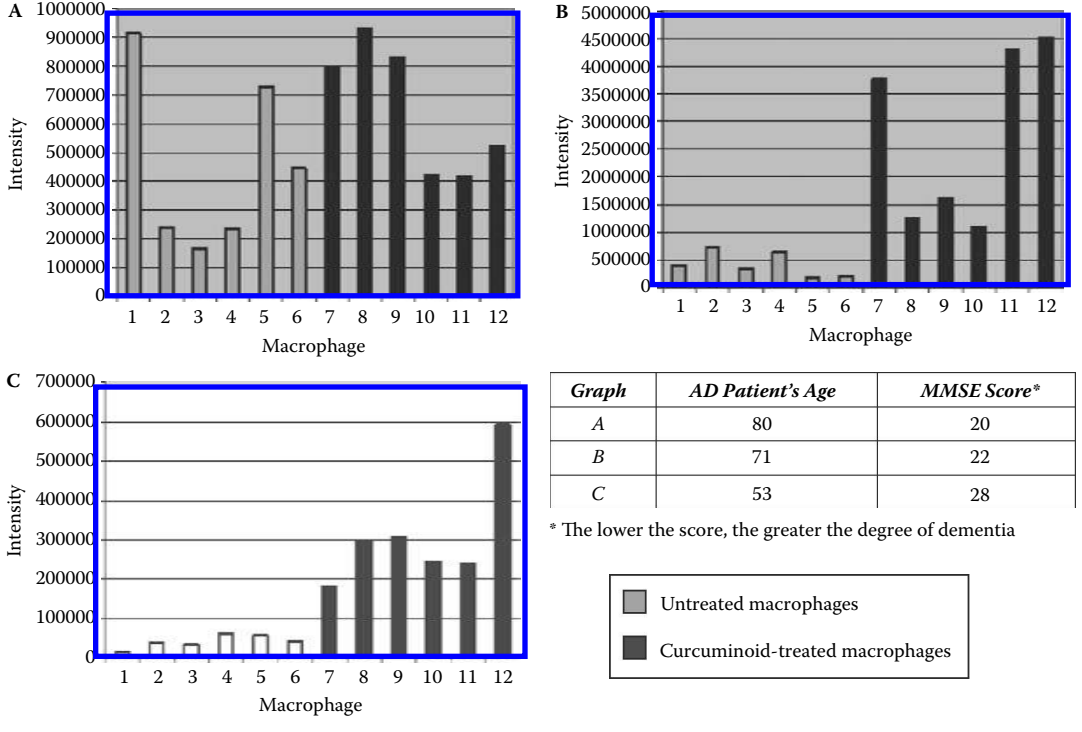


FIGURE 2.1 (See color insert.) Aβ phagocytosis by healthy control and AD-derived macrophages. Healthy control macrophages (a–c) are capable to bind and completely engulf Aβ, whereas AD macrophages (d–f) may bind Aβ to the surface but can neither phagocytize nor degrade it. Aβ is stained green, the cell cytoskeleton red, and the nucleus blue. Macrophages were viewed using both fluorescent (100×) and confocal microscopy (40×).

transcription of MGAT3 and TLRs in response to Aβ. In contrast, Aβ-stimulated mononuclear cells of AD patients (type I patients) generally downregulate these genes. This transcriptional downregulation may be reversed by the curcuminoid bisdemethoxycurcumin, which we will discuss in subsequent sections. See Figure 2.2.

MGAT3 encodes the enzyme β-1,4-mannosyl-glycoprotein 4-β-*N*-acetylglucosaminyltransferase (GlcNAc-TIII), which modulates cell–cell interactions through N-glycosylation of key proteins and receptors involved in Aβ phagocytosis. It is reasonable to expect that an inadequate transcription of this enzyme in AD patients may interfere with the clearance of Aβ by the innate immune system, and that silencing the transcription of MGAT3 in macrophages in healthy subjects can inhibit Aβ phagocytosis, as observed (Fiala et al. 2007b).

Innate immune cells can recognize misfolded amyloid proteins through TLRs, such as TLR2 (Tukel et al. 2009) and the NALP3 inflammasome (Halle et al. 2008). However, AD patients display a downregulation of the transcription of TLR genes in response to Aβ stimulation (Fiala et al. 2007b).



Graph	*AD Patient's Age*	*MMSE Score**
A	80	20
B	71	22
C	53	28

* The lower the score, the greater the degree of dementia

FIGURE 2.2 Treatment with bisdemethoxycurcumin enhances uptake of Aβ by macrophages of AD patients. AD macrophages from three patients were analyzed for phagocytosis of Aβ. Identical AD macrophage cultures in eight-chamber slides were exposed to FITC-Aβ overnight (25 mg/ml). The treated group additionally received 0.1 μM curcumin complex, whereas the untreated group received no curcumin. ImagePro scanning determined the intensity (density × area) of Aβ fluorescence in macrophages photographed at 100× magnification in a vertical strip from the middle of each well. Results analyzed by the *t*-test showed significant differences in Aβ uptake between untreated AD macrophages and those that were treated with curcuminoids.

2.2.5 AD Macrophages Contribute to Amyloid Angiopathy by Shuttling Aβ to Vessels

Amyloid angiopathy, a condition often associated with AD, refers to the deposition of Aβ in the walls of leptomeningeal and cortical blood vessels. The vessels that are most affected are small and mid-size arteries. Vasculopathic changes that occur due to amyloid angiopathy include both ischemic and hemorrhagic presentations, with primary intracerebral hemorrhage being the best recognized (Pezzini et al. 2009). An impairment of physiological perivascular drainage of Aβ is considered to underlie amyloid angiopathy in experimental animal models (Carare et al. 2008). In patients with AD, blood-borne macrophages have an important role in shuttling Aβ. Macrophages are attracted by chemokines, like CCL5 (RANTES) produced by neurons, to the site of Aβ accumulation, where they may upload oligomeric Aβ and then migrate toward blood vessels. At the periphery of vessels, the macrophages—which are engorged with Aβ—undergo apoptosis, and oligomeric forms of Aβ assemble into the fibrillar form, which is released upon apoptosis onto the vessel walls (Zaghi et al. 2009).

2.2.6 Balancing the Role of Immune Dysfunction and Inflammation in AD Pathogenesis

During progression of AD, the buildup of misfolded Aβ as neuritic plaques in the brain parallels the increased chronic inflammation in affected brain regions (Eikelenboom et al. 2010). AD neuroinflammation may be associated with an increased number of incompetent memory T cells and inflammatory cytokines produced by macrophages (Franceschi et al. 2007), whereas decreased Aβ clearance is likely due to defective transcription of MGAT III and TLRs (Fiala 2010).

Another major source of inflammation in AD that contributes to neurodegeneration is the chronic activation of the complement pathway. The components of the classical and alternative complement pathways have been colocalized with Aβ plaque aggregates and neurofibrillary tangles (Rogers et al. 1992). Recent evidence suggests that stimulation with oligomeric Aβ may induce activation of certain pro-inflammatory cytokines and chemokines, including interleukins IL-1β and IL-8. Furthermore, affected areas of the AD brain also show overexpression of genes involved with inflammation, including NF-κB, tumor necrosis factor (TNF)-α, and CCL20 (Parachikova et al. 2007).

2.3 CURRENT AND POTENTIAL THERAPIES FOR ALZHEIMER'S DISEASE

We have postulated that therapeutic strategies correcting the imbalance between increased inflammation and decreased Aβ phagocytosis might reduce inflammation-induced neurodegeneration (Fiala et al. 2007a). Curcuminoids and vitamin D_3 seem to offer a therapeutic potential in achieving this end.

2.3.1 Pharmacological Agents Currently in Use

Two major classes of drugs are currently FDA approved for the symptomatic treatment of AD. It is important to note that these pharmacological agents do not offer neuroprotective or curative benefits, but simply alleviate symptoms for a limited period of time during disease progression.

Acetylcholinesterase inhibitors that are clinically used in AD management include donepezil, rivastigmine, and galantamine. This class of drugs works by inhibiting the enzyme acetylcholinesterase in neuronal synapses, thereby increasing cortical levels of acetylcholine. How does this help to alleviate cognitive symptoms of AD? From a biochemical perspective, it has been noted that AD patients have decreased cortical levels of acetylcholine, nicotinic acetylcholine receptors, and choline acetyltransferase Thus, replenishing the levels of this neurotransmitter in the brain helps to slow cognitive decline, even though it does not cure the underlying pathology of the disease.

Another drug, memantine, inhibits overstimulated *N*-methyl *D*-aspartate (NMDA) receptors in the AD brain and helps to reverse some impairments induced by oligomeric Aβ. Most AD patients taking memantine alone or in combination with acetylcholinesterase inhibitors maintain the same MMSE score for a year, whereas placebo-treated AD patients will typically deteriorate by two to three MMSEs over the same period (Bird and Miller 2008).

2.3.2 Future Directions of AD Therapy

Based on the Aβ hypothesis (Hardy and Selkoe 2002), future directions for AD therapy focus on reducing the total amount of Aβ; this can be accomplished by either decreasing Aβ assembly or increasing Aβ clearance by macrophages. One path that is being explored is the Aβ vaccine, which would involve active or passive immunization to increase Aβ clearance. In mouse models, this technique was efficacious in the prevention and clearance of Aβ deposition in the brain (Schenk et al. 1999). In active immunization by Aβ antigens, Aβ-specific antibody production by the patient's humoral immune system was induced. In human trials, this approach led to clearance of Aβ from plaques, but also meningoencephalitis in 6% of vaccinated patients (Nicoll et al. 2006). An alternative approach uses passive immunization of patients with exogenously produced antibodies (Salloway et al. 2009).

A number of studies have shown that oxidative injury is present in the brains of AD patients, and that it may contribute to cognitive decline, so antioxidants, including selegiline, vitamin E, and vitamin A, are under evaluation to slow the progression of AD (Pratico and Delanty 2000; Smith et al. 2000). Furthermore, preclinical and clinical studies have suggested that extracts from *Ginkgo biloba* leaves, which have been used in traditional Chinese medicine, can modestly improve cognitive function in patients with AD and vascular dementia. It has been suggested that *Ginkgo biloba* may alter Aβ levels and improve blood flow and metabolism (Luo et al. 2002), but a 6-year study sponsored by NCCAM did not show benefits in the prevention of dementia.

Aside from the above-described methods, nonsteroidal anti-inflammatory drugs (NSAIDs) have been reported to inhibit aggregation of Aβ *in vitro*, likely by binding

to a site on Aβ and preventing oligomerization and amyloid plaque formation (Yamin et al. 2008). The results with COX-1 inhibitors have been positive in mice and show promise in human studies (McGeer and McGeer 2007). Other promising directions of AD therapy rely on enhancing the clearance of Aβ by macrophages of the innate immune system. This strategy involves the natural compounds curcumin and vitamin D_3, as detailed below.

2.3.3 Immune Therapy of AD with Curcumin and Vitamin D_3

Because innate immune dysfunction and chronic inflammation have been increasingly considered contributing factors in AD pathogenesis, a number of immune-boosting, anti-inflammatory substances are being evaluated for the therapy of AD. One such substance is curcumin, the active principle in the Indian curry spice turmeric, which influences a wide range of biomolecular cascades, and has been suggested as having therapeutic value in diseases such as arthritis, inflammatory bowel disease, ulcerative colitis, and psoriasis (Goel et al. 2008). Curcuminoids suppress the inflammatory response by inhibiting pro-inflammatory mediators such as TNF-α and IL-1β (Moon et al. 2010).

Bisdemethoxycurcumin (BDC) is the most potent and effective curcuminoid in stimulating uptake and clearance of soluble Aβ (Fiala et al. 2007b). Curcuminoids function by binding to and modulating the activity of the transcription factor vitamin D receptor (VDR), thereby regulating expression of inflammatory enzymes, cytokines, and molecules for adhesion and cell survival (Masoumi et al. 2009). BDC therefore addresses both inflammation and defective macrophage function to serve as a potential preventive therapy for AD.

As mentioned earlier, AD patients have defective transcription of MGAT III and TLRs that likely contributes to the innate immune dysfunction in AD. Curcuminoid compounds (i.e., BDC and synthetic forms) improve the transcription of both MGAT III and TLRs, and the phagocytosis of Aβ by macrophages in most AD patients (Fiala et al. 2007a). Phase I clinical trials have shown that curcumin supplements are safe and well tolerated even when taken at high daily doses for 3 months (Ringman et al. 2005, 2008). Since curcumin has poor bioavailability and poor absorption from the gut, several research groups have investigated ways to enhance its bioavailability by combining curcumin with other compounds. Although animal models and human studies have not been conclusive, combinations of curcumin with piperine have been suggested as potentially increasing bioavailability when administered orally (Goel et al. 2008).

More recently, BDC and its synthetic derivatives were observed to bind specifically to the nuclear vitamin D receptor (VDR) (Masoumi et al. 2009). This has led to the hypothesis that the beneficial therapeutic properties of curcuminoids in maintenance of macrophage phagocytosis or reducing inflammation may be regulated, at least in part, via VDR-regulated expression of inflammatory enzymes, cytokines, and molecules for adhesion and cell survival (Masoumi et al. 2009). Consistent with this hypothesis, the effects of BDC are mimicked or bettered by the hormonal form of vitamin D_3 (1α,25$(OH)_2$-vitamin D_3 (1,25D3)) (Masoumi et al. 2009).

Thus, 1,25D3 and its synthetic derivatives are currently under investigation for their immune-boosting potential in AD. Interestingly, 1,25D3 is synthesized in the body from 25(OH)-vitamin D_3 (25D3), and increasing evidence suggests that low-serum 25D3 levels (i.e., low vitamin D_3 dietary intake or synthesis in the skin) may be associated with AD onset and progression. 1,25D3 regulates 3.5% of human genes via activation of genomic and nongenomic cell signaling (Mizwicki and Norman 2009).

Curcumin, BDC, and 1,25D3 all bind specifically to the VDR; however, they differ in their stability when bound to the two binding sites defined for the VDR molecule that have been physicochemically linked to initiation of genomic and nongenomic signaling through extensive structure–function studies (Mizwicki and Norman 2009). Molecular mechanics calculations demonstrate that 1,25D3 shows preferential binding for the genomic pocket of the vitamin D receptor, whereas bisdemethoxycurcumin preferentially binds to the nongenomic pocket. In experiments with a 1,25D3 genomic antagonist analogue MK, the effects (i.e., phagocytosis of Aβ) of 1,25D3 were inhibited, but those of synthetic curcuminoids were not, implying that VDR *cis*-regulation of genes is required for 1,25D3 but not curcuminoids to recover phagocytic function in AD macrophages (Masoumi et al. 2009).

Two classes of AD macrophages have been defined based on their responsiveness to curcuminoids and 1,25D3 (Masoumi et al. 2009) and curcuminoid expression of MGAT III (Fiala et al. 2007b). A majority of AD patients have type I macrophages, which improve their phagocytic function with curcuminoids and 1,25D3. Combined treatment has additive effects in patients with this type of macrophages, which respond by increasing transcription of MGAT III. In contrast, type II macrophages respond positively (with increased transcription of MGAT III) to vitamin 1,25D3, but not to curcuminoids. In both type I and type II macrophages, 1,25D3 strongly stimulates Aβ clearance and protects from apoptosis (Fiala et al. 2007b).

2.4 ROLE OF INFLAMMATION IN ALS

2.4.1 Clinical Overview and Current Treatment

ALS, commonly known as Lou Gehrig's disease, is a fatal neurodegenerative disease that leads to progressive paralysis through loss of upper and lower motor neurons. Ninety percent of ALS patients die within 6 years of diagnosis, typically by respiratory failure, and age of onset is usually between 40 and 70 years old. The vast majority of ALS cases arise sporadically, but about 10% arise as familial ALS, which is inherited as an autosomal dominant trait (Brown 2008). Sporadic versus familial cases of ALS are virtually indistinguishable in terms of pathological and clinical presentation (Papadimitriou et al. 2010).

Clinical presentation of ALS will depend on the involved spinal cord segments. Patients may first notice weakness in the limbs without sensory involvement, most noticeable as twitching, muscle wasting, easy fatigability, stiffness, or cramps. If ALS first affects bulbar regions, then the patient may initially present with difficulty swallowing, breathing, coughing, chewing, or speaking. As the disease progresses toward increasing paralysis, the patient may begin to experience cognitive and behavioral changes (Simon et al. 1999).

The only pharmacological treatment option currently in use is riluzole, which is taken orally to block stimulation of glutamate receptors, sodium channel currents, and excessive calcium influx. This drug has a minimal effect in slowing progression of ALS, as it may increase survival by only 2–3 months. It is also associated with a wide range of side effects that may include dizziness, gastrointestinal problems, reduced liver enzymes, and reduced pulmonary function (Simon et al. 1999).

2.4.2 Familial ALS Is Linked to a Mutation in SOD-1 That Results in a Misfolded Enzyme

The familial form of ALS is associated with a mutation in the gene that encodes for superoxide dismutase-1 (SOD-1), a critical enzyme involved in protection of mitochondria from oxidative stress. In normal function, this enzyme catalyzes conversion of the superoxide anion to hydrogen peroxide, to eliminate harmful free radicals. In familial ALS, mutant SOD-1 enzyme aggregates in motor neurons in the spinal cord and elicits a neuroinflammatory response through the activation of astrocytes and microglia (Papadimitriou et al. 2010)

Transgenic mice expressing human SOD-1 mutation develop pathological and clinical hallmarks of ALS, including selective spinal motor neuron degeneration and glial activation. In contrast, control transgenic mice expressing wild-type human SOD-1 are healthy (Gurney et al. 1994), suggesting that the ALS phenotype is caused by mutant SOD-1, potentially through mitochondrial dysfunction, oxidative stress, aberrant protein aggregation, impaired axonal transport, or neuroinflammation (Papadimitriou et al. 2010).

2.4.3 Sporadic ALS Is Linked to the Aggregated Form of Wild-Type SOD-1

The findings in familial ALS led researchers to more closely examine whether wild-type (WT) SOD-1 enzyme could play a similar role in the *sporadic* form of ALS. When the enzyme is not mutated, how can it contribute to ALS pathogenesis? Our recent findings suggest that in sporadic ALS, WT SOD-1 becomes pro-inflammatory when it is aggregated in the fibrillar form, which induces an autoimmune inflammatory cascade (induced by macrophages and other immune cells). In our study, fibrillar WT SOD-1 caused induction of inflammatory cytokines and prostaglandins, similar to those induced in familial ALS by mutant SOD-1. In contrast, the soluble form of WT SOD-1 did not stimulate a comparable degree of inflammatory responses (Fiala 2010).

2.4.4 Inhibiting the Inflammatory Cascade in ALS May Provide Future Directions for Therapy

According to the autoimmune inflammatory model of ALS pathogenesis delineated in the previous section, an inhibition of inflammation in the ALS spinal cord may provide therapeutic benefit to patients. In mutant SOD-1 mouse models, a number of

anti-inflammatory strategies have been tested with mild success. For example, the COX-2 inhibitor celecoxib delayed onset of ALS (Drachman et al. 2002), while a TNF-α antagonist increased survival of mutant SOD-1 mice (West et al. 2004), but these strategies have not been useful in human studies.

Curcuminoids improve symptoms of autoimmune diseases such as ulcerative colitis and Crohn's disease (Goel et al. 2008). In our laboratory, curcumin inhibited IL-1β induction in the macrophages of ALS patients but not autoimmune activation. The therapeutic potential of curcuminoids in ALS needs further investigation, particularly studies aimed at optimizing its combination with other anti-inflammatory therapies.

2.4.5 Inhibition of Inflammatory Cascade in ALS Patients

Our recent results by immunofluorescence of the ALS spinal cord point to a crucial role of inflammatory damage to motor neurons by TNF-α and IL-6 –producing macrophages and IL-17A-positive CD8 T cells (Fiala et al. 2010). The omega-3 docosahexanenoic acid (DHA) has strong inhibitory effect on IL-6 produced by ALS macrophages (Fiala et al. 2010) and on IL-17A produced in PBMC's, suggestive of a new approach to immune therapy of ALS by DHA.

2.5 CONCLUSION

In many neurodegenerative diseases, including both Alzheimer's disease and amyotrophic lateral sclerosis, buildup of misfolded protein triggers overstimulation of eicosanoids, cytokines, and chemokines that results in chronic inflammation. Inflammation set off by amyloid-β in AD and misfolded SOD-1 in ALS thus seems to play a pivotal role in the neuropathology. Anti-inflammatory substances, particularly ligands of the vitamin D receptor such as curcuminoids and vitamin D, could offer therapeutic potential through a wide variety of transcriptional and signaling mechanisms. Further research is needed to discover the role of the ligands of the vitamin D receptor in downregulation of inflammation and upregulation of favorable phagocytic mechanisms for clearance of misfolded proteins. This may point to bisdemethoxycurcumin and vitamin D as having a potential role in preventing or halting progression of these important neurodegenerative diseases.

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3 Environment and the Role of Inflammation in Chronic Pulmonary Diseases

The Silent Mediator

Hongwei Yao and Irfan Rahman

CONTENTS

3.1 INTRODUCTION

The lung is sensitive to oxidative injury triggered by reactive oxygen and nitrogen species (ROS/RNS) due to its large epithelial surface area and blood supply. These oxygen/free radicals can be generated endogenously (e.g., from mitochondrial electron transport during respiration and during activation of phagocytes) as well as exogenously (e.g., air pollutants, silica, or cigarette smoke), leading to oxidative damage/modification of proteins, lipids, and DNA. Epidemiological study shows that tobacco smoking accounts for 66% of cases of chronic obstructive pulmonary disease (COPD), while biomass burning and high levels of ambient air pollution are other contributing risk factors for this disease (Christiani, 2010). Increased levels of ROS/RNS have been implicated in initiating inflammatory responses in the lungs through the activation of plasma membrane receptors e.g., Toll-like receptors (TLRs), kinases—IκB kinase (IKK), mitogen-activated protein kinase (MAPK), and protein kinase C (PKC), and transcription factors e.g., nuclear factor-kappaB (NF-κB), resulting in gene expression of pro-inflammatory mediators (Rahman et al., 2002; Asehnoune et al., 2004; Marwick et al., 2004; Moodie et al., 2004; Kode et al., 2006; Yang et al., 2006, 2008; Medicherla et al., 2008; Yao et al., 2008, 2010; Moretto et al., 2009). Lung inflammation not only is a common characteristic, but also plays an important role in the pathogenesis of chronic pulmonary diseases, such as COPD, asthma, pulmonary fibrosis, and lung cancer (Figure 3.1). In certain conditions, these diseases can occur simultaneously and affect each other. For example, fibrosis around the small airway is a major reason for irreversible airway narrowing in patients with COPD (Hogg et al., 2004a). Moreover, COPD has been shown to increase the susceptibility for lung tumorigenesis up to 4.5-fold (Yao and Rahman, 2009). Therefore, understanding the cellular and molecular mechanisms for inflammation would identify the targets in

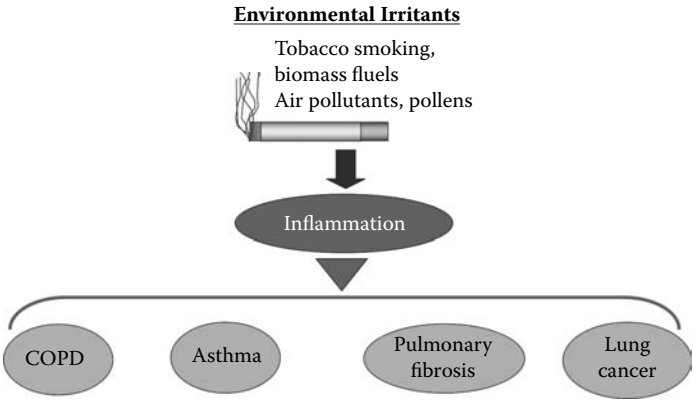

FIGURE 3.1 Involvement of inflammation in chronic lung diseases. Environmental irritants, such as tobacco smoking, biomass fuel burning, and air pollutants, cause lung inflammation by generating oxidative stress and activating a variety of receptors, kinases, and transcription factors. Lung inflammation not only is a common feature, but also is involved in the pathogenesis of chronic pulmonary diseases, such as chronic obstructive pulmonary disease (COPD), asthma, lung fibrosis, and cancer.

interfering with the progression of these chronic diseases. In this chapter, we discuss the role of inflammation as a silent mediator in the pathogenesis of these chronic pulmonary diseases.

3.2 INFLAMMATORY RESPONSE IN CHRONIC PULMONARY DISEASES

3.2.1 Cellular Infiltration

Inflammatory cell influx is significantly increased in lungs of patients with COPD, asthma, lung fibrosis, and lung cancer (Figure 3.2). The chemokines responsible for inflammatory cell trafficking can be released from activated lung structural cells (e.g., epithelial cells and fibroblasts) or accumulating inflammatory cells themselves in response to environmental pollutants, tobacco smoking, asbestos fibers, and allergens. Recruitment of inflammatory cells, such as macrophages, neutrophils, and lymphocytes, particularly in the smaller airways, is a feature of chronic inflammation in

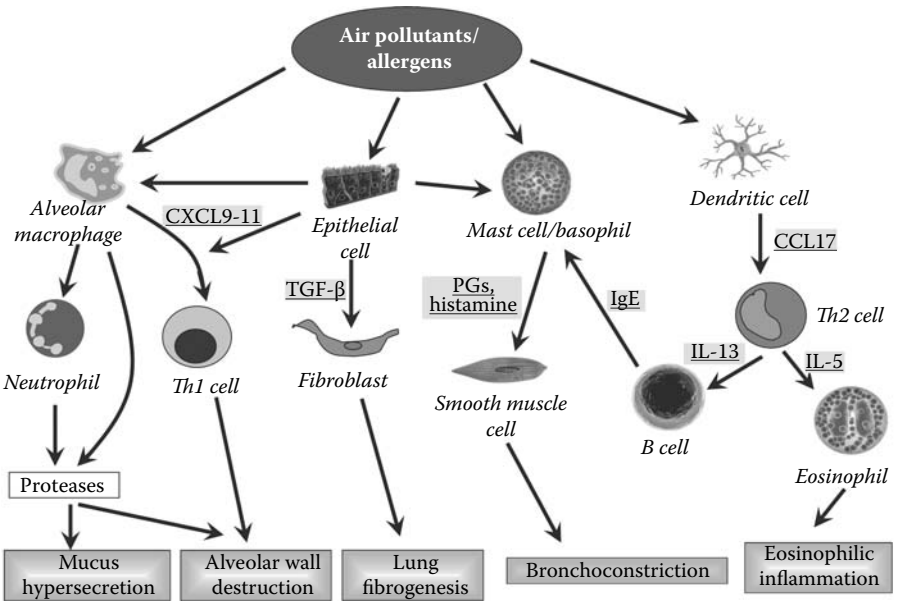

FIGURE 3.2 (See color insert.) Cellular mechanisms underlying lung inflammatory response to environmental pollutants/allergens. Exposure to air pollutants activates epithelial cells and macrophages to release chemokines, which attract other inflammatory and immune cells, including neutrophils and Th1 cells, into the lungs. The release of protease, perforin, and granzyme from these macrophages, neutrophils, and Th1 cells causes mucus hypersecretion and alveolar wall destruction. Activated epithelial cells release transforming growth factor-β (TGF-β), which contributes to lung fibrogenesis. Dendritic cells present antigens to memory Th2 cells, which induces IgE production by B cells or drives the differentiation of eosinophils and mast cells via releasing Th2 cytokines (e.g., IL-5 and IL-13). Activation of mast cells causes airway smooth muscle hyperresponsiveness by releasing prostaglandins (PGs), histamine, and leukotrienes.

COPD. Oxidants, proteinases, perforins, and granzymes released from these inflammatory cells contribute to alveolar wall destruction and mucus hypersecretion. The infiltration of these inflammatory-immune cells into lungs correlates with the severity of COPD. Furthermore, mature lymphoid follicles with a germinal center and separated T and B cell zones are observed in lungs of COPD patients, compared to in nonsmokers (Hogg et al., 2004a; van der Strate et al., 2006; Hogg and Timens, 2009). This may be due to increased antigen loading by bacterial and viral infections, as well as neoantigen formation from degraded extracellular matrix and carbonylated proteins (Hogg et al., 2004a; Voelkel and Taraseviciene-Stewart, 2005; van der Strate et al., 2006; Lee et al., 2007).

Asthma is characterized by mucus hypersecretion and smooth muscle hyperresponsiveness along with infiltration of mast cells, eosinophils, Th2 lymphocytes, and basophils in the central airway. Dendritic cells are responsible for presenting the processed peptides from inhaled allergens to Th2 cells, which can release mediators, such as interleukin (IL)-4, IL-5, and IL-13, causing a Th2 inflammatory response. In addition to dendritic cells, mast cells, eosinophils, and basophils also have the ability to present antigens to Th2 cells under different circumstances. Examination of eosinophil infiltration in sputum is an easy way to distinguish asthma from COPD (Tattersfield et al., 2002; Barnes, 2008a, 2008b; Ichinose, 2009). Interestingly, the number of neutrophils and eosinophils is also increased in lungs of asthmatics, and a negative correlation has been reported between FEV_1 and neutrophil/eosinophil numbers in patients with active asthma (Jatakanon et al., 1999; Green et al., 2002; Monteseirin, 2009). Furthermore, there is a mixture of Th1 and Th2 cells in lungs of patients with severe asthma and during exacerbation, which may be due to microbial infections. The number and function of T-regulatory (Treg) cells, $FoxP3^+CD4^+CD25^+$ cells, are decreased and functionally impaired in asthmatics (Provoost et al., 2009). This may contribute to allergic airway inflammation since Treg cells have an ability to inhibit Th2 cell differentiation and Th17-cell-mediated inflammation. This effect was mediated by the production of suppressive cytokines and cell surface molecules, including TGF-β, IL-10, and cytotoxic T lymphocyte antigen 4 (Paik et al., 2008; Girtsman et al., 2010). Furthermore, antigen-specific Th2 memory cells can be redifferentiated into $CD4^+CD25^+FoxP3^+$ cells in the presence of TGF-β, which further attenuates Th2-mediated allergic inflammation (Kim et al., 2010). Therefore, adoptive transfer of functional Treg cells would be a promising therapeutic approach in the management of asthma.

Infiltration of macrophages, neutrophils, eosinophils, and lymphocytes into the lungs is seen in patients with idiopathic pulmonary disease (IPF) (Reynolds et al., 1977; Peterson et al., 1987; Standiford et al., 1993; Obayashi et al., 1997; Papiris et al., 2005; Tabuena et al., 2005). It is negatively correlated with the survival and prognosis of interstitial pulmonary disease (Reynolds et al., 1977; Papiris et al., 2005; Tabuena et al., 2005; Parra et al., 2007). However, the role of inflammation in IPF is controversial since immunosuppression treatment was not effective in interfering with the progression of IPF (Mason et al., 1999; Gauldie, 2002; Strieter, 2002). A hypothesis is proposed that inflammation is a critical event in the pathogenesis of IPF. This is due to the release of elastase and cytokine, thereby damaging tissues, prolonging and amplifying defective wound repair, releasing growth factor, leading

to proliferation of fibroblasts, promoting the differentiation from one cell to another (e.g., transition of epithelial cells to mesenchymal cells), and damaging endothelial cells (Bringardner et al., 2008). Hence, prevention of an early inflammatory response may halt the progression of subsequent fibrogenesis.

Immune cells exhibit antitumor properties via eradicating aberrant cells, which is termed immunosurveillance. However, these inflammatory/immune cells (e.g., macrophages, neutrophils, and T lymphocytes) can cross-talk with tumor cells through a reciprocal and self-perpetuating manner, resulting in increased growth and resistance to immune destruction (Goswami et al., 2005; Liu et al., 2006; Reiman et al., 2007). For example, the ability of alveolar macrophages to induce T cell and antitumor immune responses is significantly impaired in many patients with lung cancer. Hence, the studies on how cancer and immune/inflammatory cells interact may provide therapeutic options in augmenting antitumor immune response.

3.2.2 Pro- and Anti-Inflammatory Mediators

3.2.2.1 Cytokines

Cytokines and chemokines play an important role in inflammation by recruiting and activating inflammatory cells, such as macrophages, neutrophils, T cells, and B cells, into the lungs (Figure 3.2). These cytokines and chemokines are intertwined in the pathogenesis of chronic pulmonary diseases, including COPD, asthma, lung fibrosis, and lung cancer. The transcription of these cytokine and chemokine genes is induced following TLR and NF-κB activation in response to environmental pollutants, cigarette smoke, and allergens (Figure 3.3). Furthermore, these cytokines are the contributing factors in activating NF-κB via binding to their receptors.

The levels of IL-1β, IL-6, CXCL8/IL-8, GM-CSF, and TNF-α are increased in induced sputum, BAL fluid, and alveolar macrophages in patients with COPD (Keatings et al., 1996; Pesci et al., 1998; Vlahos et al., 2006; Saha et al., 2009). The levels of IL-8 and TNF-α are the markers for the severity of COPD (Franciosi et al., 2006). Administration of antibody against GM-CSF significantly attenuated cigarette smoke-induced lung inflammation, suggesting neutralization of GM-CSF may be a useful adjunct therapeutic strategy for management of COPD/emphysema (Vlahos et al., 2006, 2010). However, further studies are required to extrapolate this finding in humans. IL-18 protein is strongly expressed in alveolar macrophages, $CD8^+$ T cells, and both the bronchiolar and alveolar epithelial cells in the lungs of COPD patients (Kang et al., 2007; Petersen et al., 2007; Imaoka et al., 2008). Serum IL-18 level is negatively correlated with predicted FEV_1 in patients with COPD. Importantly, knockout of the IL-18 receptor α subunit attenuated cigarette smoke or cigarette smoke/poly (I:C)-mediated emphysema, whereas constitutive overproduction of IL-18 in the lungs induced emphysema in mice, suggesting the pro-emphysematous effect of IL-18 (Hoshino et al., 2007; Kang et al., 2007, 2008). This is due to upregulation of IL-13 since disruption of IL-13 prevents IL-18 transgenic mice from emphysema (Hoshino et al., 2007). Overexpression of IL-13 or interferon (IFN)-γ also caused mouse emphysema (Zheng et al., 2000; Hoshino et al., 2007). The expression of Th17-related cytokines, including IL-17A, IL-22, and

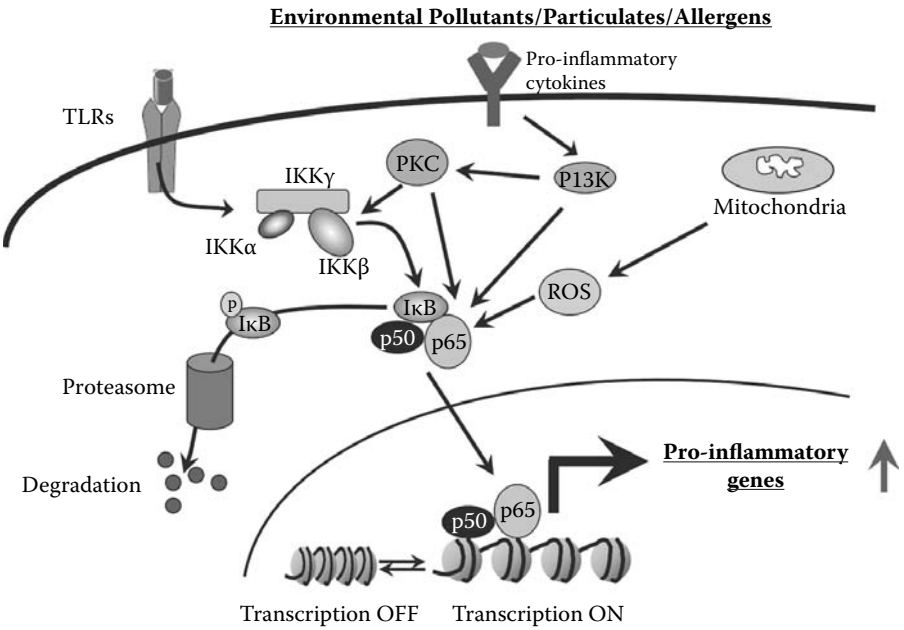

FIGURE 3.3 Signaling pathways for pro-inflammatory gene transcription after exposure to environmental agents/pollutants. In response to environmental stimuli, Toll-like receptors (TLRs) are activated, leading to NF-κB activation in a MyD88-dependent or -independent manner. Furthermore, NF-κB is activated by a variety of kinases, such as phosphatidylinositol-3 kinase (PI3K kinase), P38 kinase, IκB kinase (IKK), and protein kinase C (PKC). Endogenous and exogenous reactive oxygen species (ROS) in response to environmental agents/pollutants exposure is another mechanism for NF-κB activation in lungs. The activated subunits of NF-κB (p50/p65) are translocated into the nucleus, where they form a complex with coactivators on the promoters of pro-inflammatory genes, thereby increasing gene transcription.

IL-23, is increased in bronchial epithelial cells of patients with COPD (Di Stefano et al., 2009). The level/expression of IL-32 is increased in lung tissue of patients with COPD, where it is colocalized with TNF-α and correlated with the degree of airflow obstruction (Calabrese et al., 2008) suggesting the involvement of IL-32 in pathogenesis of COPD. Nevertheless, blocking specific cytokines (e.g., IL-8 and TNF-α antagonists) has so far been disappointing in clinical studies with COPD patients.

There is an increase in Th2 cytokines, such as IL-4, IL-5, IL-9, and IL-13, in lungs of asthmatics (Barnes, 2008a). These cytokines released from Th2 cells either induce immunoglobulin E (IgE) production by B cells (e.g., IL-4 and Il-13) or drive the differentiation of eosinophils (e.g., IL-5) and mast cells (e.g., IL-9), which leads to airway hyperresponsiveness and eosinophilia (Kay, 2006). The expression of both IFN-β and IFN-λ is reduced in epithelial cells of asthmatics, which is associated with increased asthma exacerbation induced by viruses (Wark et al., 2005; Contoli et al., 2006). Although the soluble Th2 cytokine receptor or humanized monoclonal antibodies against Th2 cytokines are effective in attenuating eosinophilia, and airway hyperresponsiveness and airway remodeling in animal models of asthma,

these interventions yielded disappointing results in clinical studies (Leckie et al., 2000; Kips et al., 2003; Simon, 2006; Corren et al., 2010).

Cytokines, including IL-1, TNF-α, and IL-8, are involved in the progression of pulmonary fibrosis via regulating inflammation and fibrogenesis. Transient overexpression of IL-1β induces acute lung injury and subsequent pulmonary fibrosis, which is due to upregulation of IL-17A (Kolb et al., 2001; Wilson et al., 2010). Administration of IL-1 receptor antagonist prevented bleomycin- or silica-induced pulmonary fibrosis (Piguet et al., 1993). Transgenic overexpression of IL-13 caused lung fibrosis, whereas inhibition of IL-13 protected against lung fibrogenesis in a rodent model (Zhu et al., 1999; Kolodsick et al., 2004). Similarly, knockout of TNF-α protected against bleomycin-induced lung fibrosis, whereas transgenic TNF-α on SPC promoter caused alveolitis and subsequent fibrogenesis in lung (Miyazaki et al., 1995; Liu et al., 1998). Although the levels of these cytokines are altered in the lungs of patients with IPF, attempting to inhibit these cytokines, such as TNF-α, has met with little success in patients with IPF (Vassallo et al., 2002; Selman et al., 2004).

3.2.2.2 Chemokines

Chemokines are divided into four subfamilies in light of their structural homology around four cysteine residues: -C-, -CC-, -CXC-, and -CX3C-, in which X substitutes for any amino acid. They bind to specific membrane-bound receptors, resulting in cellular chemotaxis, proliferation, differentiation, and survival. These chemokines are involved in the pathogenesis of chronic pulmonary diseases, such as COPD, asthma, lung fibrosis, and lung cancer.

Several chemokines, including -CXC- (CXCL1, CXCL5, CXCL7–11) and -CC- (CCL2–5, CCL7, CCL8, CCL11, CCL13) subfamilies are involved in the recruitment of inflammatory cells in COPD (Lukacs et al., 2005; Donnelly and Barnes, 2006). The levels of CXCR1, CXCR5, and CXCR8 are significantly increased in induced sputum and BAL fluid of COPD patients compared with normal smokers and nonsmokers (Keatings et al., 1996; Morrison et al., 1998; Soler et al., 1999; Traves et al., 2002). Neutralization of CXCL8 with an antibody significantly reduced the neutrophil chemotactic activity of sputum from patients with COPD (Beeh et al., 2003). However, this antibody has little clinical effect in improving the lung function in COPD patients (Mahler et al., 2004). This resulted in the study of small molecular inhibitors of CXCR2 for slowing the progression of COPD (Widdowson et al., 2004). In mice exposed to acute cigarette smoke, a CXCR2 antagonist (SCH-N) decreases the neutrophilic inflammatory response in lungs; however, the compound itself causes neutropenia (Thatcher et al., 2005). At present, CXCR2 antagonists are undergoing phase I and II trials; the efficacy of these treatments will become clear once these trials are completed. The levels of CXCL9 (monokine induced by IFN-γ), CXCL10 (IFN-γ-inducible protein 10 (IP-10)), and CXCL11 (IFN-inducible T cell α chemoattractant), as well as their receptor (CXCR3), are significantly increased in lungs of patients with COPD when compared with nonsmokers (Saetta et al., 2002; Hardaker et al., 2004; Costa et al., 2008). This increase, along with CX3R1 activation, may contribute to the infiltration of T and B lymphocytes in the lungs of COPD patients (Saetta et al., 2002; Ning et al., 2004; Hogg et al., 2004b; McComb et al., 2008; Porter et al., 2008). CCL2 (monocyte chemotactic protein-1 (MCP-1))

has been shown to activate monocytes and T cells through an autocrine/paracrine manner. MCP-1 level is increased in the sputum, BAL fluid, and lungs of patients with COPD, which may contribute to the matrix remodeling (Capelli et al., 1999; de Boer et al., 2000; Traves et al., 2002). CCR5 level is increased in the lungs of COPD patients, and CCR5 deficiency attenuated cigarette smoke-induced lung inflammation and airspace enlargement in mice (Bracke et al., 2007; Costa et al., 2008). However, it remains to be determined whether CCR5 plays the same role in other animal models of COPD/emphysema, and CCR5 antagonist has any beneficial effects in this disease.

In the lung of asthmatics, the expression of chemokines and their receptors (e.g., CCR3, CCR4, CXCR4, CCL5, CCL11, CCL13, CCL17, CCL22, CCL24, CCL26, CXCL8, and CX3CL1) is increased (Jatakanon et al., 1999; Ying et al., 1999, 2005; Rimaniol et al., 2003; Pilette et al., 2004). In addition to their chemotactic ability, these chemokines are also important in eosinophil differentiation and release of eosinophil progenitor from bone marrow (Dorman et al., 2005). Inhibition of these chemokine receptors using pharmacological and genetic approaches significantly attenuated airway inflammation and hyperresponsiveness in sensitized mice exposed to inhaled allergens (Lukacs et al., 2002; Ma et al., 2002; Das et al., 2006; Wegmann et al., 2007; Gauvreau et al., 2008; Suzaki et al., 2008). Disappointingly, several CCR3 antagonists failed in clinical trials due to their toxicity.

The levels of chemokines, including CCL2, CCL3, CXCL-8/IL-8, and CXCL5/ENA-78, are significantly increased in patients with IPF (Car et al., 1994; Keane et al., 1997, 2001). Deficiency of CCR2 attenuated bleomycin-induced release of growth factor TGF-β and fibrogenesis in lung (Gharaee-Kermani et al., 2003; Okuma et al., 2004). Circulating fibrocytes are thought to be progenitors for fibroblasts, and are involved in the pathogenesis of lung fibrosis. CXCL12 is essential for recruiting circulating fibrocytes into lung, leading to fibrogenesis (Phillips et al., 2004). However, administration of CXCL11 reduced lung damage, vascular remodeling, and neoangiogenesis in mice exposed to bleomycin (Burdick et al., 2005). Knockout of CXCR3 increased the mortality in response to bleomycin exposure, compared to wild-type mice (Jiang et al., 2004). The ability of CXCR3 to limit lung fibrosis was associated with increased production of IFN-γ, although treatment with IFN-γ failed to halt the progression of clinical IPF (Raghu et al., 2004; Strieter et al., 2004a). Further study is required to investigate the differential roles of these chemokines in lung fibrogenesis.

Cancer cells can be regulated by chemokines via an autocrine/paracrine manner (Strieter et al., 1995; Hartmann et al., 2004; Koizumi et al., 2007). Chemokines are involved in the progression and metastasis of cancer by regulating angiogenesis and leukocyte trafficking (Strieter et al., 2004b; Arenberg, 2006). Activation of CXCR4 chemokine receptor and integrins on small-cell lung cancer cells promotes their adhesion to accessory cells (e.g., stromal cells) and extracellular matrix molecules within the tumor microenvironment (Hartmann et al., 2004). Neutralization of CXCR4 ligand in preclinical *in vivo* studies results in a significant decrease of non-small-cell lung cancer metastases (Otsuka and Bebb, 2008). Hence, further elucidation of the biology of -CXC- chemokines in the context of neovascularization of

non-small-cell lung cancer will permit novel targeted therapy aimed specifically at attenuating the tumor growth and metastasis.

3.2.2.3 TGF-β

The levels of TGF-β1 mRNA and protein are increased in the airway and alveolar epithelial cells in patients with COPD, and its mRNA level is positively correlated with the smoking history and degree of small airway obstruction (Takizawa et al., 2001). An animal study showed that TGF-β activation contributes to MMP-12-mediated emphysema in integrin αvβ6 knockout mice (Morris et al., 2003). These results suggest the pro-fibrogenic and pro-remodeling role of TGF-β in COPD. A separate study demonstrated that reduced expression of TGF-β type II receptor occurs in bronchial glands of COPD patients, which may promote mucus hypersecrection, suggesting a cell-specific function of TGF-β (Baraldo et al., 2005).

The expression of TGF-β1 mRNA and protein is significantly increased in lungs of asthmatics and lungs of asthmatic rodents (Bosse and Rola-Pleszczynski, 2007). This was associated with subepithelial fibrosis and extracellular matrix remodeling. Inhibition of TGF-β signaling using anti-TGF-β antibody or TGF-β receptor I kinase inhibitor attenuated chronic allergic airway inflammation and remodeling (McMillan et al., 2005; Leung et al., 2006). A recent study showed that TGF-β1 and TGF-β2 exhibit specific and shared roles in the regulation of allergen-induced airway inflammation and remodeling (Bottoms et al., 2010), suggesting targeting TGF-β may be beneficial in the treatment of asthma.

TGF-β is highly associated with and is a dominant pro-fibrotic mediator in promoting pulmonary fibrosis. Transgenic delivery of active TGF-β induced severe and progressive lung fibrosis, whereas inhibition of TGF-β with soluble TGF-β receptor or a TGF-β receptor 1 (ALK5) inhibitor ameliorated pulmonary fibrosis (Sime et al., 1997; Wang et al., 1999; Bonniaud et al., 2005; Tarantal et al., 2010). Furthermore, the mice null for the TGF-β signaling molecule Smad3 are protected from pulmonary fibrosis (Zhao et al., 2002). The mechanism of pro-fibrogenesis of TGF-β was due to its ability to induce fibroblast migration, matrix protein synthesis, epithelial–mesenchymal transition, epithelial cell apoptosis, myofibroblast differentiation, and repression of matrix-degrading proteases. Therefore, strategies that inhibit activation of TGF-β in a cell- or disease-specific manner are an attractive option for the treatment of lung fibrogenesis.

In light of the inhibitory effect in normal epithelial cell proliferation and repair, TGF-β activation may prevent a proliferative response to environmental carcinogens, such as cigarette smoke. However, human lung cancer cells have an ability to escape from the autocrine growth inhibitory effect of TGF-β due to the loss/deficiency of TβRII. Restoration of TGF-β/TβRII signalling may be a potential chemotherapeutic strategy for lung cancer since the majority of non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) show weak or no expression of TβRII. However, (at the late stages of lung cancer) TGF-β promotes tumor spreading by enhancing invasion and angiogenesis (Fong et al., 2009). Not much information is currently available regarding the TGF-β pathways as a potential strategy for chemotherapeutic intervention in clinics for lung cancer.

3.2.2.4 Cyclooxygenase-2 (COX-2)

COX-2 and its product prostaglandins have been shown to play a critical role in inflammation in respiratory diseases. The levels of COX-2 and PGE2 are significantly increased in lungs of patients with COPD, and the level of PGE2 is inversely correlated with FEV_1 in COPD patients (Xaubet et al., 2004; Chen et al., 2008; Profita et al., 2010). Cigarette smoke has been shown to activate COX-2, and increase PGE2 synthesis by activating NF-κB (Martey et al., 2004). Treatment with 15d-PGJ2, a natural ligand of peroxisome proliferator-activated receptor γ (PPARγ), increased cigarette smoke extract-induced IL-8 release in monocyte/macrophages through a PPARγ-independent mechanism to increase ROS generation (Caito et al., 2008). The activation of COX-2 induced by cigarette smoke may create a pro-inflammatory microenvironment, which will amplify the inflammatory response. Interestingly, *in vivo* administration of 15d-PGJ2 significantly attenuated oxidative stress and lung inflammation, as well as improved lung mechanical properties in the rat model of COPD (Li et al., 2009). Therefore, activation of PPARγ may be beneficial in controlling chronic airway diseases, including COPD and asthma (Belvisi et al., 2006; Spears et al., 2006).

The role of COX-2 in the pathogenesis of asthma is complex, since its activity/level is unchanged, reduced, or increased in lungs of asthmatics in the different studies. This may be due to its fluctuating expression during the course of this disease. Inhibition of COX-2 attenuated bronchovascular inflammation, but increased airway hyperreactivity, in allergic mice (Torres et al., 2008), suggesting a differential role of COX-2 in regulating airway inflammation and lung function. The differential role of COX-2 may be due to the specific function of its derived products in asthma. For example, PGI2 has been shown to exhibit an antiasthmatic effect, while PGD2 is mainly considered to favor asthma (Matsuoka et al., 2000; Jaffar et al., 2002). Exogenous PGE2 exhibited a protective role in asthmatic response induced by aspirin, exercise, and allergens, as well as in house dust mite-sensitized mice (Gauvreau et al., 1999; Torres et al., 2008). The PGE and PGI receptors mediate the protective role of PGE2 and PGI2 against asthmatic response, respectively (Soberman and Christmas, 2006).

Previous studies show the role of COX-2 in limiting pulmonary fibrosis (Bonner et al., 2002; Hodges et al., 2004; Lovgren et al., 2006). Administration of 15d-PGJ2 significantly reduced bleomycin-induced lung injury (Genovese et al., 2005). The mechanism of this protective effect was associated with the inhibition of myofibroblast differentiation and induction of antioxidant defense (Burgess et al., 2005; Ferguson et al., 2009). A separate study showed that deficiency of COX-2 aggravated lung function decline, but did not affect the progression of pulmonary fibrosis, in response to bleomycin instillation (Card et al., 2007). The reasons for these discrepancies are unknown and remain to be investigated. Recently, Oga et al. (2009) demonstrated another prostaglandin, PGF2α, is an important inducer in facilitating bleomycin-induced lung fibrosis. Furthermore, the pro-fibrogenesis of PGF2α is independent of TGF-β, suggesting the potential therapeutic implication of targeting PGF2α-FP receptor pathway in attenuating lung fibrosis. COX-2 plays an important role in lung tumorigenesis, cancer invasion, and metastasis via regulating cellular

apoptosis, angiogenesis, and host immunity (Lee et al., 2008). Targeting COX-2 in lung cancer therapy and chemoprevention is under investigation.

3.2.2.5 Anti-Inflammatory Lipid Mediators

Acetylated COX-2 induced by aspirin does not produce prostaglandins, but retains its enzymatic activity to induce the synthesis of 15-hydroxyeicosatertraenoic acid (15*R*-HETE) from arachidonic acid, which is then converted into the lipoxins (i.e., 15 epi-lipoxin A4 (LXA4) and 15 epi-lipoxin B4 (LXB4)). These lipoxins have potential anti-inflammatory properties, which are involved in resolution of acute inflammation in rodent models of asthma, lung fibrosis, and acute lung injury (Levy et al., 2002; El Kebir et al., 2009; Martins et al., 2009). Thus, selective COX-2 inhibitors could prevent the generation of these anti-inflammatory 15-epi-lipoxins, thereby augmenting inflammatory response. Furthermore, omega-3 polyunsaturated fatty acid-derived lipid mediators, such as resolvins, protectins, and maresins, also have an ability to inhibit granulocyte infiltration and monocyte recruitment, thereby resolving active inflammation (Serhan et al., 2008, 2009). The levels of protectin D1 and resolvin E1 are significantly decreased in lungs with inflammation, whereas administration of protectin D1 protects against airway inflammation and hyperresponsiveness in mice (Arita et al., 2005; Levy et al., 2007; Haworth et al., 2008). Hence, these anti-inflammatory lipid mediators would be the potential biomarkers for monitoring the efficacy of therapeutic interventions in inflammatory airway diseases.

3.2.2.6 MicroRNA

MicroRNA (miRNA) is a recently identified family that regulates gene expression at the posttranscriptional level via an RNA interference mechanism. There are around 700 known miRNA genes, and each miRNA may modulate a variety of protein-coding genes. In addition to its role in regulation of stem cell differentiation, organ development, and metabolism, miRNA is also involved in inflammatory response. The expression of specific miRNA has been shown to be increased in inflammatory lungs and *in vitro* in cells exposed to inflammatory stimuli (Moschos et al., 2007; Perry et al., 2008; Bazzoni et al., 2009; Lu et al., 2009). Thus, miRNA would be a potential biomarker of chronic pulmonary diseases where lung inflammation occurs. It is interesting to note that the expression of miRNA is cell specific in lungs of asthmatics (Williams et al., 2009). The mechanism for induction of miRNA in the inflammatory condition is associated with increased TLR4-activated NF-κB pathway (Williams et al., 2008; Bazzoni et al., 2009). Antagonism of altered miRNA attenuated Th2 inflammation, mucus hypersecretion, and airway hyperresponsiveness (Mattes et al., 2009). This may be due to regulation of miRNA on inflammatory signaling pathways since miR-9 miRNA has an ability to reduce NF-κB1/p105 protein expression (Bazzoni et al., 2009). Hence, there may be a feedback loop between specific miRNA and transcription factor activation to fine-tune the inflammatory status. Furthermore, regulation of specific miRNA expression (e.g., anti-miRNA probe/antagomir or mimetic) would be a possible therapeutic approach in intervention of chronic lung inflammatory response.

3.2.3 Signaling Pathways in Lung Inflammation

3.2.3.1 Toll-Like Receptors (TLRs)

TLRs play a critical role in innate and adaptive immune responses to a variety of pathogens, such as viruses, bacteria, and fungi. TLRs are expressed in lung structure cells (e.g., epithelial cells, fibroblasts, and endothelium) and immune/inflammatory cells (e.g., macrophages, neutrophils, T cells, and dendritic cells), and their function is associated with the induction of antimicrobial response. Hence, TLRs agonists are promising immunomodulators and may enhance nonspecific immune response to attenuate bacterial and viral infection (Baldridge et al., 2004). TLRs are also involved in the pathogenesis of chronic lung disease, and their functions in these diseases are dependent on the lung microenvironment and specific regulation of different isoforms.

The role of TLR4 in development of experimental COPD/emphysema is studied by Zhang et al. (2006). Deficiency of TLR4 caused airspace enlargement without lung inflammation in mice, suggesting a role of TLR4 in maintaining lung integrity (Zhang et al., 2006). Interestingly, TLR4/MyD88 and IL-1R1/MyD88 signaling pathways were required for cigarette smoke- and elastase-induced lung inflammation and emphysema (Karimi et al., 2006; Doz et al., 2008; Couillin et al., 2009). The discrepancies between these findings are currently not known, which remains to be investigated.

TLR (e.g., TLR4, TLR2/6, TLR7, and TLR9) agonists have been shown to attenuate eosinophilia and airway hyperresponsiveness in mice by increasing Th1 response (Sur et al., 1999; Eisenbarth et al., 2002; Fuchs et al., 2009; Xirakia et al., 2010). However, a low dose of LPS through TLR4 activation increased the response to Th2 phenotype, leading to asthma (Eisenbarth et al., 2002). Sustained activation of TLRs will lead to a pro-inflammatory response via activating MAPK, and phosphoinositide 3 kinase (PI3K), and NF-κB in a MyD88-dependent or -independent manner. Furthermore, these kinases and NF-κB activation are involved in the development of allergic asthma (Duan et al., 2004, 2005; Lim et al., 2009). Therefore, one has to be cautious in employing TLRs as a therapeutic target in controlling allergic asthma.

TLR9 is highly expressed in the lungs of patients with idiopathic interstitial pneumonia and IPF (Meneghin et al., 2008). A recent study showed that activation of TLR2/TLR4 by hyaluranan in B cells leads to the production of pro-fibrogenic cytokines and autoantibodies, which contributes to bleomycin-induced lung fibrosis (Yoshizaki et al., 2008). However, it is unclear whether regulation of specific TLRs attenuates the animal model of lung fibrogenesis. TLRs have been shown to promote lung cancer initiation, progression, and chemoresistance (Tsan, 2006; Chen et al., 2007; Cherfils-Vicini et al., 2010). The mechanism is associated with activation of NF-κB, leading to increased expression of antiapoptotic protein BCL-2 (Cherfils-Vicini et al., 2010). However, the TLR2/4 receptor agonists have been shown to prevent cancer relapse under chemotherapy via inducing TNF-α and inducible nitric oxide synthase, leading to tumor cell apoptosis (Garay et al., 2007). Hence, further study is required to investigate the regulation of differential TLRs in lung cancer.

3.2.3.2 NF-κB Activation

The NF-κB signaling pathway plays a crucial role in pathogenesis/development of chronic pulmonary diseases by increasing the release of pro-inflammatory mediators

leading to chronic inflammation. The mechanism of NF-κB activation is associated with the activation of TLRs, IKK, MAPK, and PKC in response to environmental stimuli (Figure 3.3). Redox regulation is another mechanism for NF-κB activation since oxidative stress or ROS can directly activate the NF-κB nuclear translocation. However, the DNA binding activity of oxidized NF-κB is significantly decreased, and is restored by reducing enzyme thioredoxin (Kabe et al., 2005).

The number of RelA/p65-positive epithelial cells and macrophages, as well as RelA/p65 nuclear expression, is significantly elevated in patients with COPD (Di Stefano et al., 2002; Yagi et al., 2006; Rajendrasozhan et al., 2008). Furthermore, the number of RelA/p65-positive epithelial cells and macrophages is positively correlated with the degree of airflow limitation in COPD patients (Di Stefano et al., 2002). These findings suggest the importance of the NF-κB-dependent inflammatory response in the pathogenesis of COPD. The activation of NF-κB in lungs of patients with COPD is associated with increased oxidative stress, due to the fact that NF-κB-activating upstream kinases are redox sensitive (Bowie and O'Neill, 2000; Pantano et al., 2006). Inhibition of NF-κB using PHA-408 (8-(5-chloro-2-(4-methylpiperazin-1-yl)isonicotinamido)-1-(4-fluorophenyl)-4,5-dihydro-1H-benzo(gamma)indazole-3-carboxamide) attenuated cigarette smoke-induced acute lung inflammation in rodents (Rajendrasozhan et al., 2010), suggesting a potential therapeutic target of NF-κB in COPD. However, further studies are required to determine whether NF-κB inhibitor is effective in controlling chronic inflammation in long-term cigarette smoke-exposed animals, as well as overcoming the steroid resistance and subsequent pathological changes. Interestingly, the deficiency of p50, another subunit of NF-κB, enhanced cigarette smoke-induced lung inflammation and emphysema, suggesting the differential role of p50 and RelA/p65 in inflammation (Cao et al., 2006; Rajendrasozhan et al., 2010).

The importance of NF-κB in the pathogenesis of asthma is highlighted in several preclinical and clinical studies. The levels of RelA/p65 protein, IκB phosphorylation, and IKKβ protein are significantly increased in peripheral blood mononuclear cells in severe and moderate asthmatics, compared to normal individuals (Gagliardo et al., 2003). Similarly, both nuclear RelA/p65 and p50 are activated in sputum cells and bronchial epithelial cells from stable and untreated asthmatics in higher levels than those in nonasthmatic subjects (Zhao et al., 2001). Target disruption of IKKβ in Clara epithelial cells attenuates ovalbumin sensitization and challenge-induced airway inflammation, mucus hypersecretion, and airway remodeling (Broide et al., 2005). Administration of IKKβ inhibitor TPCA-1 significantly attenuated the expression of IL-β, IL-4, IL-5, IL-13, eotaxin, and TNF-α in the ovalbumin asthma model (Birrell et al., 2005). Thus, NF-κB is a potential target in controlling the development of lung allergic inflammation.

NF-κB activation has been shown to be a critical step in the production of early pro-inflammatory cytokines in bleomycin-induced lung inflammation and subsequent fibrogenesis (Gurujeyalakshmi et al., 2000). Administration of NF-κB inhibitors, such as IMD-0354 and SP100030, suppressed bleomycin-induced lung inflammatory response and fibrogenesis (Inayama et al., 2006; Fujimoto et al., 2007). Silica-induced lung inflammation and fibrogenesis were also lowered by systemic administration of NF-κB inhibitor, BAY 11-7085, suggesting the therapeutic

potential of NF-κB inhibitor in manipulating early inflammation seen in lungs of patients with pulmonary fibrosis.

NF-κB-regulated genes, such as cytokines, adhesion molecules, antiapoptotic factors, and matrix metalloproteinases, are associated with tumor progression and metastasis. Overexpression of NF-κB in lung epithelium induces the influx of inflammatory cells, thereby potentiating lung adenocarcinoma metastasis. Treatment with NF-κB inhibitor (e.g., pyrrolidine dithiocarbamate) and IκB protease inhibitor (tosylphenylalanylchloromethane) reduces TGF-β1-induced human lung cancer cell migration (Fong et al., 2009). Therefore, downregulation of NF-κB activation may improve the efficacy of first-line therapy in lung cancer.

3.3 INFLAMMATION-ASSOCIATED PROCESS: APOPTOSIS IN CHRONIC PULMONARY DISEASES

Inflammation and apoptosis are involved and intertwined in the pathogenesis of chronic lung diseases. Both processes affect each other. For example, lung inflammation can be triggered and amplified by increased lung structure cell apoptosis and reduced clearance of apoptotic cells (Henson et al., 2006). Increased apoptosis of epithelial/endothelial cells is observed in lungs of patients with COPD, and in lungs of mice exposed to cigarette smoke, as well as in cells treated with cigarette smoke extract, suggesting a critical role of apoptosis in the pathogenesis of COPD (Henson et al., 2006; Plataki et al., 2006). Induction of endothelial cell apoptosis has been shown to cause emphysema-like changes in mice (Giordano et al., 2008). The mechanism underlying these observations is unknown, but it might be associated with increased oxidative stress and impaired VEGF-VEGF receptor 2 signaling pathway in the lung (Kasahara et al., 2000; Marwick et al., 2006; Scherz-Shouval et al., 2007). Pharmacological inhibition and genetic disruption of cathepsin S attenuated the emphysematous response by reducing lung cell apoptosis (Zheng et al., 2005). Hence, prevention of lung structure/endothelial cells from apoptosis may have implications in the intervention of COPD/emphysema (Yoshida et al., 2010).

A defect in eosinophil apoptosis may contribute to the chronic tissue eosinophilia associated with asthma (Xu et al., 2007). There is a negative correlation between sputum eosinophil apoptosis and clinical severity of chronic stable asthma, suggesting the importance of eosinophil apoptosis in the resolution of eosinophilic airway inflammation in asthma (Duncan et al., 2003). Removal of apoptotic eosinophils is delayed in the lungs of patients with asthma (Kankaanranta et al., 2005). This may be due to defective phagocytosis of alveolar macrophages in asthmatics.

Alveolar epithelial cell apoptosis is increased, whereas apoptosis of myofibroblasts and inflammatory cells is decreased in pulmonary fibrosis (Moodley et al., 2004; Plataki et al., 2005; Drakopanagiotakis et al., 2008). The level of antiapoptotic protein Bcl-2 is significantly increased in neutrophils of patients with IPF (Mermigkis et al., 2001). Indeed, the interaction between lung structure cells and inflammatory cells affects their survival and fate. For example, release of cytokines and growth factors (e.g., TNF-α and TGF-β) from macrophages induces epithelial cell apoptosis. Furthermore, macrophage-derived insulin-like growth factor is shown to inhibit

myofibroblast apoptosis. Hence, blockade of epithelial cell apoptosis or induction of myofibroblast apoptosis would be a promising approach in interfering with the progression of idiopathic pulmonary fibrosis.

Lung cancer is resistant to the pro-apoptotic effect of antineoplastic agents due to the defective apoptotic pathway in lung cancer cells. Autophagy is known as type II programmed cell death, and prolonged autophagy causes cancer cell death, suggesting that manipulation of autophagy can be exploited as a therapeutic target for cancer (Gozuacik and Kimchi, 2004; Cao et al., 2006). Indeed, mTOR inhibitor (RAD001)-induced autophagy enhanced radiosensitization in a mouse model of lung cancer in the presence of caspase-3 inhibitor. In addition, knockdown of pro-autophagic proteins (i.e., Beclin-1 and ATG5) prolonged the survival of H460 lung cancer cells under irradiation. Haploinsufficiency of Beclin-1 significantly increased the incidence of lung lymphomas and carcinomas in mice (Yue et al., 2003). These findings implicate the therapeutic potential of autophagy inducers in lung cancer, particularly in the cells that are defective in the apoptosis pathway or resistant to pro-apoptotic agents.

3.4 HISTONE DEACETYLASES IN CHRONIC PULMONARY DISEASES

3.4.1 Sirtuin 1 (SIRT1)

SIRT1, a class III histone deacetylase (HDAC), is shown to modulate inflammation, cellular senescence/aging, and autophagy/apoptosis via deacetylating histones/non-histone proteins, including transcription factors, coactivators, and other signaling molecules, such as NF-κB, FOXOs, and p53. The anti-inflammatory effect of SIRT1 is attributed to its ability to deacetylate RelA/p65 on lys310 residue, thereby decreasing NF-κB transcription activity (Chen et al., 2002; McBurney et al., 2003; Yeung et al., 2004; Yang et al., 2007) (Figure 3.4). Inhibition of SIRT1 enhances NF-κB activation, whereas SIRT1 activators attenuate pro-inflammatory mediator release in response to cigarette smoke exposure (Yang et al., 2007; Rajendrasozhan et al., 2008). We and others have shown a significant reduction of SIRT1 level/activity in lungs of patients with COPD and in rodent lungs exposed to cigarette smoke (Yang et al., 2007; Rajendrasozhan et al., 2008; Nakamaru et al., 2009). This is due to SIRT1 posttranslational modifications, which mark the protein for proteasomal degradation (Caito et al., 2010). Hence, activation of SIRT1 with specific activators or polyphenols (resveratrol and quercetin) may be a potential pharmacotherapy for COPD. However, further study is required to investigate whether SIRT1 activators protect the lung against long-term cigarette smoke lung inflammation, airspace enlargement, and decline in lung function.

The activity of SIRT1 is significantly decreased in peripheral blood mononuclear cells from patients with severe asthma, compared to controls. This reduction of SIRT1 has been shown to induce Th2 cytokine expression through acetylation of GATA3 (Colley et al., 2010). This is further confirmed by a study showing that activation of SIRT1 by SRT1720 leads to suppression of ovalbumin-induced asthma in mouse model (Ichikawa et al., 2010). However, a separate study showed that administration of SIRT1 inhibitor attenuated antigen-induced airway inflammation and

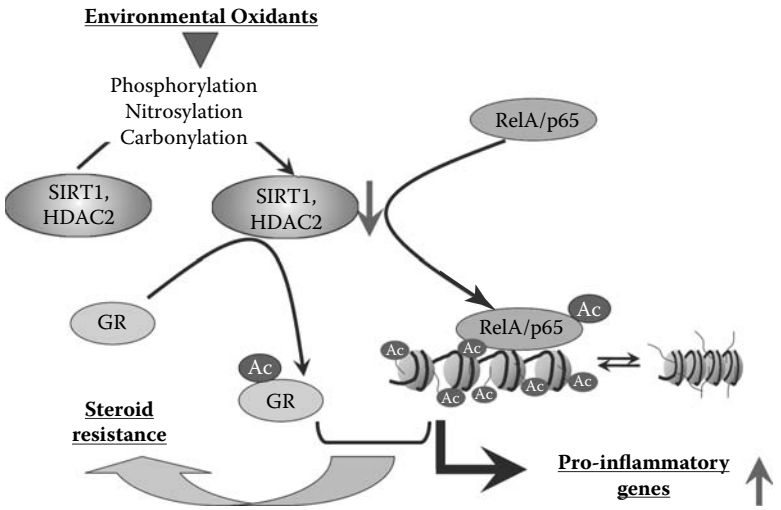

FIGURE 3.4 Role of SIRT1 and HDAC2 in lung inflammation and steroid resistance. Histone deacetylases (e.g., SIRT1 and HDAC2) are subjected to posttranslational modifications, such as phosphorylation, nitrosylation, and carbonylation, resulting in reduction in their level/activity in response to environmental stresses/oxidants. This reduction of HDAC2 and SIRT1 leads to increased acetylation of RelA/p65 and histone, thereby inducing transcription of pro-inflammatory genes. Inhibition of glucocorticoid on pro-inflammatory gene transcription is impaired since glucocorticoid cannot recruit enough HDAC2 on the promoters of these genes. Acetylation of glucocorticoid receptor (GR) further impairs the ability of glucocorticoid to inhibit NF-κB-dependent gene transcription due to reduction of HDAC2.

hyperresponsiveness via regulating vascular endothelial growth factor expression mediated by hypoxia-inducible factor 1α in mice (Kim et al., 2010). The discrepancies between these findings are unclear, and remain to be studied. No data are available on the involvement of SIRT1 in the pathogenesis of lung fibrosis.

SIRT1 is also involved in tumorigenesis via affecting cell survival and cell cycle progression. Its expression is upregulated in a variety of different types of cancers, including mouse lung carcinomas and human lung cancer. Downregulation of SIRT1 by antisense oligonucleotides or miR-34a induces cancer cell apoptosis, including lung cancer cells, suggesting its therapeutic use in lung cancer (Sun et al., 2007; Yamakuchi et al., 2008). The tumorigenic role of SIRT1 may be associated with deacetylation and inactivation of the antiapoptotic/tumor suppressors p53, p63, and p73, as well as deacetylation of histone H4 (lys16) on the promoters of these genes. Interestingly, SIRT1 also acts as a tumor suppressor *in vitro* and *in vivo* (Firestein et al., 2008; Wang et al., 2008). SIRT1 sensitizes tumor cells to TNF-α-induced cell death by reducing NF-κB transactivation (Yeung et al., 2004). Hence, the ability of SIRT1 to induce either apoptosis or cellular survival may depend on apoptotic stimuli or on whether p53 or NF-κB is deacetylated (Chung et al., 2010). Further studies on SIRT1 regulation and its role in tumorigenesis, invasion, and metastasis would provide the potential therapeutic targets for lung cancer.

3.4.2 HDAC2

The level and activity of HDAC2 protein and activity is significantly decreased in lungs of patients with COPD, and mouse lung as well as *in vitro* macrophages/lung cells exposed to cigarette smoke extract (Ito et al., 2005; Yang et al., 2006; Adenuga et al., 2009). The mechanism for HDAC2 reduction is attributed to its posttranslational modification, such as carbonylation, nitrosylation, and phosphorylation (Tsai and Seto, 2002; Marwick et al., 2004; Nott et al., 2008; Adenuga et al., 2009; Adenuga and Rahman, 2010). The HDAC2 activity is negatively correlated with increased production of inflammatory cytokines and a reduced effectiveness of the corticosteroid dexamethasone (Ito et al., 2002). This is corroborated by our recent findings that HDAC2 deficient mice were unresponsive to steroids in inhibiting lung inflammation (Adenuga et al., 2010). Upon activation, glucocorticoid receptor recruited the corepressor complexes containing HDAC2 to NF-κB-dependent gene promoters, leading to hypoacetylation and eventual repression of pro-inflammatory gene transcription. However, reduction of HDAC2 in lungs of patients with COPD leads to acetylation of both NF-κB and glucocorticoid receptor-α, which contributes to abnormal inflammatory response and steroid resistance (Ito et al., 2006) (Figure 3.4). Interestingly, SIRT1 activator SRT2172, but not dexamethasone, significantly inhibited cigarette smoke-induced MMP-9 expression in mouse lung (Nakamaru et al., 2009). Hence, restoration of HDAC2 or SIRT1 level/activity will enhance the efficacy of steroid in inhibiting chronic inflammation of COPD. This is corroborated by the study that curcumin and theophylline restore HDAC2 activity, thereby reversing steroid resistance (Ito et al., 2002; Cosio et al., 2004; Meja et al., 2008). Furthermore, inhibition of PI3K and protein kinase CK2 restored glucocorticoid function by attenuating HDAC2 posttranslational modifications, such as phosphorylation (Marwick et al., 2009; Adenuga and Rahman, 2010). Hence, development of specific PI3K and CK2 inhibitors may be effective therapies for improving steroid response in chronic inflammatory diseases where oxidative stress occurs. Interestingly, phosphodiesterase 4 (PDE4) inhibitors have an ability to reduce lung inflammation and parenchymal destruction in a steroid-resistant model of COPD (Martorana et al., 2005). Thus, PDE4 inhibitor would be an optional therapeutic approach in decreasing lung inflammatory response in chronic pulmonary diseases that are resistant to steroids.

HDAC2 expression and activity are reduced in lung macrophages, bronchial biopsy specimens, and blood cells from patients with severe asthma and smoking-induced asthma, suggesting the heightened inflammation and reduced steroid responsiveness that occur in these patients (Bhavsar et al., 2008). Differentiation of fibroblasts into myofibroblasts is an important event in pathogenesis of lung fibrosis. Knockdown of HDAC4 by siRNA attenuates TGF-β1-stimulated α-SMA expression and fibroblast differentiation into myofibroblasts, which may provide targets clinically for fibrosis treatment (Guo et al., 2009).

HDAC inhibitors and DNA demethylating agents synergistically induce apoptosis in lung cancer cells, and prevent lung cancer development in animals exposed to tobacco carcinogens. Several clinical data are available for HDAC inhibitors (e.g., vorinostat and N-acetyldinaline) in the treatment of advanced NSCLC (Gridelli et al., 2008), and these agents are being investigated in randomized phase III trails.

However, the specificity on a particular isoform of HDAC, optional therapeutic doses, timing, and mode of administration are still under investigation for these agents.

3.5 DIETARY NATURAL PRODUCTS/NUTRACEUTICAL POLYPHENOLS IN REGULATION OF INFLAMMATION

Polyphenols represent a group of compounds having aromatic ring(s), characterized by the presence of one or more hydroxyl groups with varying structural complexities. They are derived from a variety of fruits, vegetables, tea, and red wine. For example, resveratrol (3,5,4′-trihydroxystilbene) is a phytoalexin found in red wine and grapes, while curcumin, diferuloylmethane, is an active component of spice turmeric. Quercetin (3,3′,4′,5,7-pentahydroxylflavone) is a plant-derived flavonol found in apples, tea, capers, and onions, used as nutritional dietary supplements. The commonly studied dietary polyphenols, such as resveratrol, quercetin, catechins, sulforaphane, and curcumin, have been reported to possess direct antioxidant and anti-inflammatory properties via regulating NF-κB and Nrf2 activation, glutathione biosynthesis, and scavenging ROS (Rahman et al., 2006). In addition, HDAC2 and SIRT1 are also the targets of these polyphenols in regulating a variety of cellular functions, such as inflammation, senescence/aging, metabolism, and apoptosis (Chung et al., 2010). The preclinical studies show that these polyphenols are effective in interfering with lung inflammation, alveolar wall destruction, airway hyperresponsiveness, lung fibrogenesis, and tumorigenesis (Bani et al., 2006; Kim et al., 2006; Manna et al., 2009; Suzuki et al., 2009). Epidemiological studies suggested that increased dietary polyphenol intake correlates with improved symptoms and lung function in COPD patients, as well as the incidence of asthma (Tabak et al., 2001; Knekt et al., 2002; Walda et al., 2002). Therefore, dietary polyphenols have a potential therapeutic value against chronic inflammatory and epigenetically regulated diseases, such as asthma, COPD, and lung cancer. However, most polyphenols are poorly absorbed, rapidly metabolized and oxidized, and undergo sulfation and glucuronidation, and also lead to formation of their own oxidation products. Future studies are required to understand the pharmacokinetics, bioavailability, and *in vivo* effects of polyphenols in animal models of different diseases.

3.6 CONCLUSIONS

Inflammation plays an important role in the pathogenesis of chronic pulmonary diseases, such as COPD, asthma, pulmonary fibrosis, and lung cancer. The underlying mechanism is involved in the activation of a variety of receptors, kinases, and transcription factors, leading to increased transcription of pro-inflammatory genes in response to environmental pollutants. However, antagonism or blocking of these pro-inflammatory mediators has not produced satisfactory clinical efficacy in the intervention of these chronic lung diseases so far. Further investigations on signaling pathways in inflammation will facilitate the understanding of pathogenesis and provide the potential therapeutic targets for these chronic lung diseases where inflammation is a silent mediator. Dietary natural products, nutraceutical

polyphenols, and novel anti-inflammatory compounds would be the promising therapeutic candidates in reversing steroid resistance and in management of chronic pulmonary diseases in terms of their roles in regulating inflammation, oxidative stress, and epigenetic modifications.

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4 Role of Inflammation and Lifestyle in Chronic Asthma

Eugene W. X. Ho, Khai Pang Leong, Chang Cheng, and W. S. Fred Wong

CONTENTS

4.1 ASTHMA—PREVALENCE, ECONOMIC BURDEN, AND LIFESTYLE

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular mediators play a role. This chronic inflammation causes an increase in airway responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing (Masoli et al. 2004), and failure to use appropriate medications or to adhere to treatments can eventually lead to morbidity and mortality. Apart from being the most common chronic disease among children, asthma is estimated to affect over 300 million people worldwide, comprising all ages, ethnicities, and economic backgrounds, with an estimated additional 100 million people with asthma by the year 2025 (World Health Organization 2008). Approximately 180,000 deaths are attributable to asthma each year, with a notable record of 255,000 people dying of asthma in the year 2005 (World Health Organization 2008).

There is an increasing trend in the number of hospital admissions for asthma, reflecting an increase in severe asthma, poor disease management, and poverty (Locksley 2010). The associated economic cost of asthma is considerable in terms of both direct medical costs (such as hospital admissions and medication costs) and indirect medical costs (such as time lost from work and premature death). Globally, these direct and indirect economic costs associated with asthma exceed those of tuberculosis and HIV/AIDS combined (World Health Organization 2008). With over 80% of asthma deaths occurring in low and lower-middle socioeconomic sectors, it is essential not only to secure affordable and accessible medications, but also to understand the association of lifestyle and chronic asthma development.

The *Oxford English Dictionary* defines *lifestyle* as "a style or way of living (associated with an individual person, a society, etc.); especially the characteristic manner in which a person lives (or chooses to live) his or her life." About a third of the asthma disease severity can be explained by individual or environmental factors (Trupin et al. 2010). In a well-known diagram, Thomas Platts-Mills plotted the successive steps in the development of perennial rhinitis and asthma from genetic susceptibility, sensitization, and inflammation to overt disease (Platts-Mills 2003). In each step, various factors come into play. Inherent in this idea is that external or environmental factors act on the genetically predisposed individual not only in disease expression, but also in the preclinical stages (von Mutius 2009). There is a 30-fold difference in the prevalence of asthma around the world, and the only plausible explanation is the variation in regional environmental factors (Gold and Wright 2005). Of course, the individual cannot alter many environmental factors, especially if he or she is living under social or economic constraints. Therefore, it is imperative to elaborate how lifestyle choices may be associated with both disease initiation and disease exacerbation.

At present, data on the association of lifestyle and asthma come mostly from epidemiological studies. Thus, they demonstrate association but not causation. Many of these studies are all plagued by problems of disease definition, difficulty in measuring the exposure retrospectively, and the need for a large subject number due to the small effect size. In addition, the impact of these factors on disease expression takes years to manifest fully (Lynch and Smith 2005). Consequently, the association between lifestyle choices and the development and severity of asthma cannot be unequivocally established (Wright and Subramanian 2007). This is why a large, comprehensive project with a sufficiently long follow-up is necessary. When completed, URECA, a large prospective observational study on the effects of the urban environment on young children, will shed light on the contribution of multiple factors to the development of asthma (Gern et al. 2009). The environmental parameters being studied include allergen exposure, maternal stress, pollution, exposure to cigarette smoke, and diet.

In this chapter, we shall discuss the pathogenic features of chronic asthma and the various ways in which the choices individuals make about how they live affect airway inflammation in asthma. In addition, we recount the epidemiological evidence for the association of the particular factor on the development and exacerbation of asthma. Finally, we discuss some novel pharmacological approaches and lifestyle interventional studies that modify disease expression.

4.2 ALLERGIC ASTHMA—PATHOGENIC FEATURES

Asthma has many causes, including inhaled exposure to allergens and isocyanates, and often the inciting agents are not known. Inhaled allergens are identified as important environmental factors in the pathogenesis of asthma, and most likely contribute to its persistence (Hamilton 2005; Thomas et al. 2010). Extensive reviews on the genetics of asthma can only agree that there is a hereditary contribution to the underlying causes of asthma, and the inheritance pattern of asthma demonstrates that it is a "complex genetic disorder," such as arthritis and diabetes mellitus (Lemanske and Busse 2003). The interaction between host factors (genetics) and environmental stimuli can result in the development of airway inflammation, altered pulmonary physiology, and asthma symptoms in the susceptible host.

Asthma can be categorized into several phenotypes, including allergic asthma, severe steroid-resistant asthma, and asthma induced by cigarette smoke, air pollution, and so on (Kim et al. 2010). Both adaptive and innate immunities are involved in the shaping of various asthma phenotypes. For allergic asthma, airway dendritic cells are the main type of antigen-presenting cells in adaptive immunity that are involved in the induction of Th2 responses to allergens in asthma (Holgate et al. 2010). Severe persistent asthma usually represents a distinct pathological entity, characterized by steroid resistance or neutrophilic airway inflammation (The ENFUMOSA Study Group 2003). A recent study suggested that natural killer T cells play a critical role in severe persistent asthma, and could act as airway effector cells without pathoregulatory oversight by conventional Th2 cells (Meyer et al. 2008).

In most asthma phenotypes, eosinophils are increased in the tissues, blood, and bone marrow, and in general correlate with disease severity (Kay 2005). Notwithstanding, there are also noneosinophilic types of asthma triggered by bacterial endotoxin, particulate air pollution, ozone, and viral infections (Douwes et al. 2002). Eosinophils are produced in the bone marrow from CD34+ hematopoietic stem cells under the stimulation of macrophage-colony stimulation factors, interleukin (IL)-13, and IL-5 (Asquith et al. 2008). IL-5 is also responsible for the terminal maturation of eosinophils and prolongs eosinophil survival by delaying apoptotic death, possessing eosinophil chemotactic activity, increasing binding affinity between eosinophils and endothelial cells, and enhancing eosinophil effectors' function (Takatsu and Nakajima 2008; Rosenberg et al. 2007). The first step of eosinophil migration from the bone marrow is regulated by IL-5 and chemokines such as RANTES and eotaxin, whereas IL-4 and IL-13 are known to regulate transmigration of eosinophils from the vascular bed into the inflamed tissue through adhesion molecules expressed by the endothelium (Aceves and Broide 2008).

Upon reaching the site of inflammation, eosinophils release granule proteins, which contribute to the clinical manifestation of asthma. Eosinophil-derived neurotoxin acts as a chemotactic agent for dendritic cells to facilitate Th2 immune responses upon allergen challenge (Yang et al. 2008). Major basic protein (MBP) has been demonstrated to regulate activation of mast cells, basophils, and neutrophils (Piliponsky et al. 2001; Shenoy et al. 2003), and to mediate airway hyperresponsiveness (AHR) (Gleich 2000). Eosinophils could also induce asthma exacerbation through the release

of eosinophil-derived fibrogenic and growth factors. They amplify airway remodeling and mucus secretion, leading to deterioration in airway symptoms (Kay 2005).

4.3 CHRONIC SEVERE ASTHMA—PATHOGENIC FEATURES

In chronic severe, corticosteroid-resistant, and refractory asthmatics, in addition to pulmonary eosinophilia, significantly higher levels of neutrophils have been found. Prominent neutrophilic inflammation has also been observed during asthmatic exacerbation (Jatakanon et al. 1999), and in cases of sudden-onset fatal asthma (De Magalhaes Simoes et al. 2005). Studies have attributed neutrophilic inflammation in severe asthma to the use of high doses of corticosteroid, because steroids have been found to increase neutrophil survival by reducing apoptosis (Cox 1995; Meagher et al. 1996), but to promote apoptosis in eosinophils (Meagher et al. 1996). Therefore, neutrophilic inflammation may be a biomarker for the poor response of severe and refractory asthmatics to corticosteroids, and thus there is an urgent need for the discovery of steroid-resensitizing or steroid-sparing drugs for treating severe asthma.

Apart from neutrophilic inflammation, airway remodeling is a predominant feature of chronic asthma, which is represented by epithelial detachment, subepithelial fibrosis, airway smooth muscle hypertrophy and hyperplasia, goblet cell hyperplasia, proliferation of blood vessels, collagen deposition, and changes in the cartilage. Persistent inflammation causes repeated episodes of airway structural damage, repair, and proliferation, which eventually leads to the thickening of airways and reduction in the airway luminal diameter (Homer and Elias 2000; Bourdin et al. 2007). The small airways (2–4 mm) are commonly involved, and in fatal asthma, all the airways except the largest are affected. Studies have demonstrated that bronchodilator reversibility is impaired in corticosteroid-resistant asthmatic patients (Goleva et al. 2007). Therefore, prevention and reversal of airway remodeling is important in the management of chronic asthma.

Another characteristic feature of severe asthma is overproduction of mucus. Mucus can mechanically narrow the airway lumen and, in severe asthma, form tenacious plugs that will obliterate the airway (Lemanske and Busse 2003). Airway mucus protects the epithelial surface from injury and facilitates the removal of bacterial, cellular, and particulate debris from the lung. However, mucus production is also an important feature of asthma and contributes substantially to morbidity and mortality, especially in more severe conditions (Tagaya and Tamaoki 2007). Goblet cells and mucus glands are the sources of mucin glycoproteins (MUCs) and, to date, 13 MUC genes (MUC1–4, MUC5AC, MUC5B, MUC6–9, MUC11–13) have been identified in human airways. IL-13 is a potent inducer of goblet cell hyperplasia (Wills-Karp and Chiaramonte 2003). Besides, matrix metalloproteinase-9, an asthma-related protease, is involved in the elevation of MUC5AC expression via stimulation of epidermal growth factor receptor in human airway epithelial cells (Ohbayashi and Shimokata 2005).

Airway remodeling and inflammation eventually result in persistent AHR and airway obstruction, causing breathlessness and wheezing. Although the precise mechanisms that control AHR are poorly understood, the magnitude of AHR has been found to correlate with the level of airway inflammation (Boulet et al. 2006).

IL-13 is found to be most associated with airway AHR, where blockade of IL-13 in animal models of allergic asthma markedly inhibits allergen-induced AHR, and delivery of IL-13 to the airways is able to trigger AHR (Kuperman et al. 2002).

4.4 ASTHMA—ENVIRONMENT AND LIFESTYLE

4.4.1 Socioeconomic Status

Socioeconomic status is associated with the type and location of housing as well as psychosocial stress. Asthmatic children from low socioeconomic status overexpress genes associated with immune response and inflammation, gene ontology terms, chemokine activity, stress response, wound response, and antigen processing and presentation (Chen et al. 2009). In a cross-sectional survey carried out in New York City schools with 4,853 respondents, 21.8% of those living in public housing had asthma compared to 11 to 13% of those in private housing. The other characteristics associated with asthma were associated with poor housing quality: water leaks and reported sightings of cockroaches and rats (Northridge et al. 2010). In contrast, living on a farm from young age is associated with a lower risk of developing asthma, with the odds ratio about half that of city dwellers (Alfvén et al. 2006). Even youths who stay in the city but have exposure to a farm are less likely to develop asthma (Dimich-Ward et al. 2006).

4.4.2 Air Pollution

Air pollution comes from human activities such as vehicle exhaust, industry, and power generation, as well as from natural sources like volcanic activity. Pollutants include gaseous compounds (e.g., volatile hydrocarbons, carbon dioxide, carbon monoxide, sulfur dioxide, and nitrogen dioxides), particulate matter, and toxic metals. The component of air pollution that is most closely linked with asthma varies in different studies. Diesel exhaust particles, generated through the burning of fossil fuels, have been shown to enhance the production of IgE, and the infiltration of eosinophils, granulocytes, monocytes, and lymphocytes into the airways, and favor the Th2 inflammatory phenotype (Nel et al. 1998; Riedl and Diaz-Sanchez 2005).

Though it had been shown that pollution is associated with exacerbation of asthma, it could not be linked convincingly to asthma pathogenesis until recently (Heinrich and Wichmann 2004). In a significant study involving 3,863 children, the pollution level of the birth address was correlated with the presence of asthma at age 8 years (Gehring et al. 2010). The component of pollution most strongly associated with asthma was particulate matter less than 2.5 microns in diameter ($PM_{2.5}$). The temporary reduction in air pollution related to the curtailing of traffic activity in large cities has produced interesting data. During the Atlanta Olympic Games in 1996, the control of downtown traffic caused a 27.9% fall in the peak daily ozone concentration (Friedman et al. 2001). In the study group of patients aged 1 to 16 years, the number of emergency room visits or hospitalization for asthma decreased from 4.23 events per day during the baseline period to 2.47 events during the games. Similarly, during the 2008 games in Beijing, there was a fall in the concentration of particulate matter

and a decrease in the number of outpatient visits for asthma (albeit from the records of only one hospital) (Li et al. 2010).

4.4.3 Pets

The way we view the relationship between pets and asthma has changed and has been thoughtfully reviewed (Simpson and Custovic 2003; Chen et al. 2010). In the past, it was felt that sensitization to pet allergens led to the development of allergic airway disease. Lately, a diametrically opposite picture has emerged in that keeping pets seemed to protect the owners from allergic diseases. It was suggested that the environmental load of animal allergens, especially that of cats, might be associated with endotoxin. This endotoxin was thought to be protective. However, one study showed that there was, in fact, an inverse relationship between cat allergen and endotoxin level (Platts-Mills et al. 2005). Another hypothesis is that high levels of exposure alter the allergic response, in which the specific antibodies produced are IgG1 and IgG4, minus the IgE (Platts-Mills et al. 2004).

However, the data concerning pet ownership and asthma are not consistent (Chen et al. 2010). For instance, a study showed that exposure to more than one cat or dog in the first year of life protected against sensitization to environmental allergens and dog allergen (but did not reach statistical significance for cat allergen) at 6 years, but there was no effect on the development of asthma (Ownby et al. 2002). In a study of 224 Australian schoolchildren (albeit recruited only at school-going age), ownership of cats before age 18 protected against current asthma, while exposure after that predisposed to asthma (de Meer et al. 2004).

4.4.4 Smoking

There is good evidence that maternal smoking is associated with the development of asthma in children. In fact, *in utero* exposure is a stronger predisposing factor than postnatal exposure (Gilliland et al. 2001; Lannerö et al. 2006). There is a refinement to this understanding. In an interesting study of 1,314 German infants followed since birth, it was found that maternal smoking during pregnancy and after birth only increased risks in asthma in those with single or double parental history of allergic diseases (current and ever wheezing, asthma, allergic rhinitis, eczema, and food allergies). The risk of developing asthma in an infant with no family history but exposed to tobacco smoke was no different from that of an unexposed infant (Keil et al. 2009).

4.4.5 Diet

The study of diet and asthma is very difficult because of the complex nature of the way we consume food. A recent review discussed the roles of antioxidants, lipids, sodium, magnesium, the Mediterranean diet, and maternal diet, and found that the association between food and asthma could not be established conclusively (Kim et al. 2009). It has been hypothesized that decreased antioxidant and increased lipid intake led to the increased incidence of atopy and asthma (Devereux and Seaton

2005). A carefully conducted cross-sectional study uncovered the rather difficult to explain finding that a vegetarian diet was associated with asthma (Bakolis et al. 2010). A large cross-sectional survey of Spanish children showed that a food pattern consistent with the Mediterranean diet did not show an association with self-reported asthma and wheeze (Gonzalez Barcala et al. 2010).

4.4.6 Obesity

Obesity has been associated with the development of asthma in the pediatric age group (Noal et al. 2011) and in adulthood. In a prospective study of 9,810 adults aged 20 to 82 followed up for a mean of 11.5 years, obesity (defined as body fat percentage greater than or equal to 25% in males and 30% in women) was associated with the new onset of asthma (odds ratio 1.38) (Ortega et al. 2010). One of the theories to explain the association between obesity and asthma is insulin resistance, but one study found that this is not a contributory factor (Ma et al. 2010). Whether obesity leads to worsened asthma control is not clear. One study in adults showed an absence of correlation (Clerisme-Beaty et al. 2009), while another found association, particularly in female adolescents (Kattan et al. 2010).

4.4.7 Mental Stress

Stress is known to activate the adrenergic and hypothalamus–pituitary–adrenal system (Wright et al. 1998). Stress modifies the response of the asthmatic airways to environmental precipitants (Chen and Miller 2007). Even in older people, stress can lead to the development of asthma. In a study on the effects of stress sustained by Kuwaiti civilians during the Iraqi invasion of 1990, those reporting the highest level of stress were twice as likely to develop asthma as those who did not report any stress (Wright et al. 2010). An acute stressful event is associated with a higher likelihood of precipitating an asthma attack in children within a few weeks (Sandberg et al. 2000).

4.5 ASTHMA—CONVENTIONAL AND ALTERNATIVE THERAPIES

With asthma being a major and increasing global health problem, current therapies and treatments serve primarily to control the disease but not cure it. Based on the Global Initiative for Asthma (GINA) guidelines, current mainstream treatments for the disease focus on inhaled corticosteroids and β_2-adrenoceptor agonists. Corticosteroids reduce airway inflammation and asthmatic exacerbation through repression of inflammatory genes coding for cytokines, chemokines, and adhesion molecules and the concomitant activation of anti-inflammatory genes, such as annexin-1 and inhibitory κB (Barnes 2006). β_2 adrenoceptor agonists, such as salbutamol, increase the level of cAMP, leading to activation of protein kinase A, which alleviates bronchoconstriction by phosphorylating myosin light-chain kinase and by opening Ca^{2+}-dependent K^+ channels (Duffy et al. 2005).

Despite the availability of effective and relatively inexpensive treatments, approximately 5–10% of chronic asthmatic patients remain poorly controlled, and the number of chronic asthmatic patients has significantly increased in recent years

(Hermosa et al. 2010). A significant proportion of these poorly controlled asthmatics can develop severe exacerbations that are difficult to prevent, leading to time lost from work and admission to hospital, and utilize a highly disproportionate amount of healthcare costs (Beasley 2002).

In order to complement current mainstream treatments, many natural herbs and compounds have been found to possess potent therapeutic effects against asthma. Andrographolide is a labdane diterpene lactone isolated from the leaves of the *Andrographis paniculata* plant (Rao et al. 2004), a medicinal herb that has been long used for the prevention and treatment of upper respiratory tract infection in Asian countries (Poolsup et al. 2004). In a murine model of allergic asthma, andrographolide was found to possess potent anti-inflammatory effects in an ovalbumin (OVA)-induced allergic mouse asthma model (Bao et al. 2009). In that study, andrographolide was found to dose-dependently suppress airway inflammation, mucus hypersecretion, IL-4, IL-5, and IL-13 levels in bronchoalveolar lavage fluid, as well as airway hyperresponsiveness.

In a similar manner, resveratrol, a polyphenolic stilbene found in many fruits, nuts, flowers, seeds, and barks of many plants, especially in grapes, had anti-inflammatory activities comparable to those of corticosteroid drugs, in a murine model of allergic asthma (Lee et al. 2009). Curcumin, a substance derived from the *Curcuma longa* plant, commonly known as turmeric, has been widely used around the world, especially the Indian subcontinent, as a traditional spice and medicinal agent. Curcumin has also been found to reduce T cell production of IL-2, IL-5, GM-CSF, and IL-4, which is associated with bronchial asthma development (Ram et al. 2003). Although these compounds have yet to be investigated in multicenter clinical trials, they represent a wide variety of potent anti-inflammatory agents that can be eventually developed for the treatment of chronic asthma.

4.6 CONCLUSION

Asthma is a chronic airway inflammatory disorder that exists in many clinical phenotypes, and can be triggered by allergen exposure, viral infection, air pollution, and cigarette smoke via activation of a wide spectrum of cell types, including eosinophils, dendritic cells, mast cells, neutrophils, macrophages, natural killer T cells, epithelial cells, and Th2 cells (Shalaby and Martin 2010). Asthma development can be predisposed by genetic susceptibility in combination with environmental factors. Most of the data about the relationship between lifestyle and environment on the development and severity of asthma are based on epidemiological work. There are suggestions of association with some factors (for example, pollution, smoking, and stress), and less so with others (for example, diet and obesity), and even conflicting in some (such as pet ownership). Together with newly discovered cytokines such as IL-33, IL-25, and thymic stromal lymphopoietin (TSLP) (Kakkar and Lee 2008; Wang et al. 2007), it is clearly indicated that, apart from the Th2 paradigm, additional pathophysiological pathways should be incorporated into our understanding of different phenotypes and severity of asthma, a wide range of environmental and lifestyle factors predispose and precipitate asthma, and therapeutic approaches should be utilized to manage asthma.

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5 Inflammation in Rheumatic and Arthritic Diseases
Role of Dietary Phytochemicals

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CONTENTS

5.1 INTRODUCTION

Inflammation is the immune system's physiological response to injury or infection resulting in pain (*dolor*), redness (*rubor*), heat (*calor*), and swelling (*tumor*) in the affected area. It is a complex biological response to harmful external stimuli such as

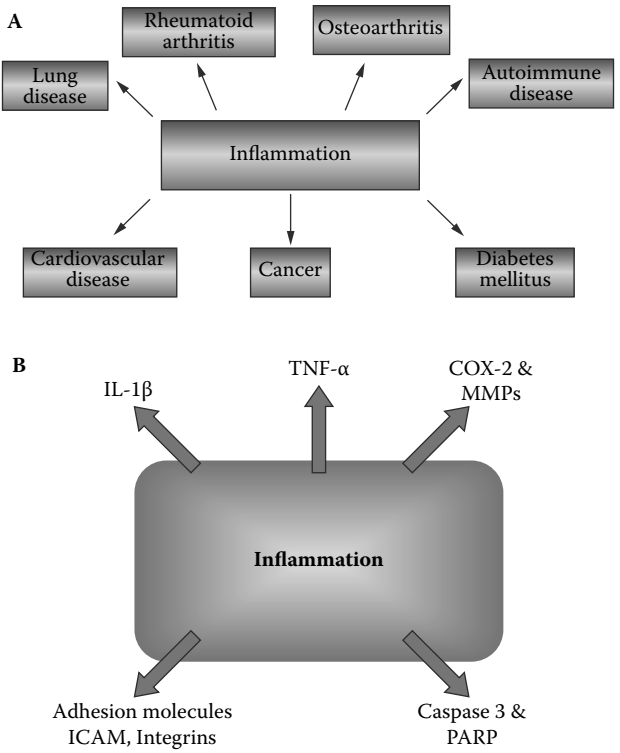

FIGURE 5.1 The role of inflammation in chronic disease. A: Inflammation as a key player in the pathogenesis of chronic disease. B: Inflammation results in the synthesis and secretion of various pro-inflammatory cytokines, including IL-1β, TNF-α, upregulation of cyclooxygenase 2 (COX-2) and matrix metalloproteinases (MMPs), activation of caspase-3 and polyADP ribose polymerase (PARP), and the turnover of adhesion molecules such as integrins and ICAM.

pathogens (bacteria, viruses) and chemical irritants or damaged cells. Effectively, inflammation is a protective attempt of the body to remove the injurious agent and initiate the healing process. Inflammation is often incorrectly confused with infection. Although it can be caused by infection, the two terms are not synonymous and should be appropriately used: infection is caused by an exogenous pathogen, while inflammation is one of the responses of the organism to a pathogen, insult, or injury.

Despite the fact that inflammation has long been known as a localized protective reaction of tissue to irritation, injury, or infection, characterized by pain, redness, swelling, and sometimes loss of function, there has been a new realization about its role in a wide variety of chronic diseases, including cancer (Aggarwal et al., 2006). While acute inflammation is a part of the body's natural defense response, chronic inflammation is thought to lead to or perpetuate cancer, diabetes, and cardiovascular, pulmonary, and neurological diseases (Soory, 2009; Khansari et al., 2009; Aggarwal et al., 2006) (Figure 5.1). Overweight and obesity are inflammatory health problems of epidemic proportions, increasing the risk of cardiovascular disease,

type 2 diabetes mellitus, and various types of cancer (van Kruijsdijk et al., 2009). Extensive research within the past two decades has revealed that obesity is also a pro-inflammatory disease and a major risk factor for type 2 diabetes, atherosclerosis, cancer, and other chronic diseases.

Many types of rheumatic diseases and arthritic conditions are inflammatory disorders. The term *arthritis* characterizes a group of conditions involving damage to synovial joints (Di Paola and Cuzzocrea, 2008). Arthritis literally means an inflammation (*itis*) of the joints (*arthros*). It involves pain, redness, heat, swelling, and other harmful effects of inflammation within the joint. There are over 200 different forms of arthritis. The most common form, osteoarthritis (OA) (also known as osteoarthrosis or degenerative joint disease), can result from trauma to the joint, infection of the joint, or simply as a consequence of age. Other forms of arthritis include psoriatic arthritis, and autoimmune diseases in which the body's immune system attacks itself, such as rheumatoid arthritis (RA). The major consequences of arthritis are pain and disability. Pain is a constant and daily feature in well-established forms of the disease. Arthritis pain occurs due to inflammation that occurs around the joint, damage to the joint from disease, daily wear and tear of joints, muscular strains caused by movement against stiff, painful joints, and fatigue. Disability in patients with arthritis is a consequence of degeneration in the joint and surrounding tissues and is further enhanced by inflammation-induced pain. There is currently no effective pharmacotherapy capable of restoring the structure and function of damaged synovial tissues in any form of arthritis. Consequently, one of the most important factors in treating arthritis is to understand the root causes and find ways to reduce the major risk factors.

OA affects large load-bearing joints such as the hip, shoulder, and knee. The disease is essentially one acquired from daily wear and tear of the joint. Its most prominent feature is the progressive destruction of articular cartilage (Buckwalter et al., 2005). The current consensus is that OA is a disease involving not only articular cartilage but also the synovial membrane, subchondral bone, and peri-articular soft tissues (Goldring and Goldring, 2007). OA may occur following traumatic injury to the joint, subsequent to an infection of the joint, or simply as a result of aging and the mechanical stresses associated with daily life.

RA, on the other hand, is a disorder where, for unknown reasons, the immune system begins an autoimmune attack on synovial joints and other tissues. In RA, most of the damage occurs to the joint lining (synovium) and cartilage, which eventually results in erosion of two opposing bones. Similar changes occur in the latter stages of OA, where the two opposing bones erode into each other.

5.2 ARTICULAR CARTILAGE: STRUCTURE, FUNCTION, AND DEGENERATION IN ARTHRITIS

Articular cartilage is a specialized connective tissue with unique biological and mechanical properties that depend on the structural design of the tissue and the interactions between its unique resident cells, the chondrocytes, and the extracellular matrix (ECM) that makes up the bulk of the tissue (Buckwalter and Mankin, 1998).

Chondrocytes are the architects of the ECM (Muir, 1995), building the macromolecular framework of the ECM from three distinct classes of macromolecules: collagens, proteoglycans, and noncollagenous proteins. Of the collagens present in articular cartilage, collagens type II, IX, and XI form a fibrillar meshwork that gives cartilage tensile stiffness and strength (Eyre, 2004; Buckwalter and Mankin, 1998; Kuettner et al., 1991), whereas collagen type VI forms part of the matrix immediately surrounding the chondrocytes, enabling them to attach to the macromolecular framework of the ECM and acting as a transducer of biomechanical and biochemical signals in the articular cartilage (Guilak et al., 2006; Roughley and Lee, 1994). Embedded in the collagen mesh are large aggregating proteoglycans (aggrecan) that give cartilage its stiffness to compression, its resilience, and contribute to its long-term durability (Dudhia, 2005; Kiani et al., 2002; Luo et al., 2000; Roughley and Lee, 1994). ECM proteins in cartilage are of great significance for the regulation of cell behavior, proliferation, differentiation, and morphogenesis (Kosher et al., 1973; Kosher and Church, 1975; von der Mark et al., 1977; Hewitt et al., 1982; Sommarin et al., 1989; Ramachandrula et al., 1992; Ruoslahti and Reed, 1994; Enomoto-Iwamoto et al., 1997; Gonzalez et al., 1993).

Small proteoglycans including decorin, biglycan, and fibromodulin are embedded in the ECM. Decorin and fibromodulin both interact with the type II collagen fibrils in the matrix and play a role in fibrillogenesis and interfibril interactions. Biglycan is mainly found in the immediate surroundings of the chondrocytes, where it may interact with collagen type VI (Buckwalter and Mankin, 1998; Roughley and Lee, 1994). Modulation of the ECM proteins is regulated by an interaction of a diversity of growth factors with the chondrocytes (Jenniskens et al., 2006; Trippel et al., 1989; Isgaard, 1992; Hunziker et al., 1994; Sah et al., 1994). IGF-I and TGF-β increase the surface expression of integrins, increasing the adhesion of chondrocytes to matrix proteins (Loeser, 1997). Other noncollagenous proteins such as cartilage oligomeric matrix protein (COMP), are less well studied and may have value as biomarkers of ECM turnover and degeneration (Di Cesare et al., 1996), while tenascin and fibronectin influence interactions between chondrocytes and the ECM (Buckwalter and Mankin, 1998; Burton-Wurster et al., 1997).

The ECM surrounds chondrocytes and protects them from biomechanical stress arising during normal joint motion, determines the types and concentrations of molecules that reach the cells, and helps to maintain the chondrocyte phenotype. Throughout life, cartilage undergoes continuous internal remodeling as chondrocytes replace matrix macromolecules lost through degradation. Evidence indicates that ECM turnover depends on the ability of chondrocytes to detect alterations in the macromolecular composition and organization of the matrix, including the presence of degraded macromolecules, and to respond by synthesizing appropriate types and amounts of new ECM components. It is known that mechanical loading of cartilage creates mechanical, electrical, and physicochemical signals that help to direct the synthesizing and degrading activity of chondrocytes (Mobasheri et al., 2002). In addition, the ECM acts as a signal transducer for the chondrocytes (Millward-Sadler and Salter, 2004). A prolonged and severe decrease in the use of the joint leads to alterations in the composition of the ECM, and eventually to a loss of tissue structure and

its specific biomechanical properties, whereas normal physical strain of the joint stimulates the synthesizing activity of chondrocytes and possibly the internal tissue remodeling (Buckwalter and Lane, 1997, Maffulli and King, 1992).

Ballistic, high-impact exercise may result in long-term disturbances in the structure and function of articular cartilage. Although the joint can tolerate a tremendous amount of intensive and repetitive physical stress, it manifests a striking inability to heal even the most minor injury (Buckwalter and Martin, 2004; Buckwalter, 2003; Newman, 1998; Buckwalter and Lane, 1997). Furthermore, aging leads to alterations in the ECM composition and alters the activity of the chondrocytes, including their ability to respond to a variety of stimuli, such as growth factors (Hudelmaier et al., 2001; Eckstein et al., 2001; Ralphs and Benjamin, 1994). All these alterations increase the probability of cartilage degeneration (Sarzi-Puttini et al., 2005; Buckwalter, 2003; Poole, 1999; Setton et al., 1999) and emphasize the importance of interaction between chondrocytes and their surrounding ECM, because this interaction regulates growth, differentiation, and survival of chondrocytes in normal and pathophysiological conditions (Shakibaei et al., 1999).

5.3 GLOBAL BURDEN OF OSTEOARTHRITIS (OA)

According to the United Nations and the World Health Organization (WHO), musculoskeletal conditions are leading causes of morbidity and disability, giving rise to enormous healthcare expenditures and loss of work throughout the world (Woolf and Pfleger, 2003) (http://www.arthritis.org/).*†

OA is one of the most prevalent and chronic diseases affecting the elderly (Aigner et al., 2004). The symptoms and signs characteristic of OA in the most frequently affected joints are heat, swelling, pain, stiffness, and limited mobility. OA is often a progressive and disabling disease, which occurs in the setting of a variety of risk factors, such as advancing age, obesity, and trauma, that conspire to incite a cascade of pathophysiologic events within joint tissues (Abramson and Attur, 2009). Other sequelae include osteophyte formation and joint misalignment. These manifestations are highly variable, depending on joint location and disease severity.

It is now generally accepted that OA must be viewed not only as the final common pathway for aging and injuries of the joint, but also as an active joint disease. As medical advances lengthen average life expectancy, OA will become a larger public health problem—not only because it is a manifestation of aging, but because it usually takes many years to reach clinical relevance. OA is already one of the ten most disabling diseases in industrialized countries. OA is rare in people under 40 but becomes more common with age—most people over 65 years of age show some radiographic evidence of OA in at least one or more joints. OA is the most frequent cause of physical disability among older adults globally. More than 20 million Americans are estimated to have OA (http://www.niams.nih.gov/). It is also anticipated that by the year 2030, 20% of adults will have developed OA in Western Europe and North America.

* http://www.who.int/healthinfo/statistics/bod_osteoarthritis.pdf.
† http://whqlibdoc.who.int/bulletin/2003/Vol81-No9/bulletin_2003_81(9)_630.pdf.

OA is not only a common problem among the elderly population, but it is also becoming more widespread among younger people. In the United States, RA and OA combined affect as many as 46 million people. This amounted to a healthcare cost of over $128 billion in 2003 (http://www.arthritis.org). This huge financial burden emphasizes the acute need for new and more effective treatments for articular cartilage defects, especially since there are no effective disease-modifying drugs or treatments for OA. Existing pharmaceuticals include analgesics, steroids, and NSAIDs, which only treat the symptoms of RA and OA by reducing pain and inflammation. Therefore, RA and OA represent a major opportunity for research innovation, development, and testing new therapies. Since RA/OA and related osteoarticular conditions of synovial joints are characterized by inflammation, a better understanding of anti-inflammatory nutrients and their biological actions on joint tissues and cells may facilitate the development of clinically safe, orally administered therapeutic agents for treating joint diseases.

5.4 BONE AND JOINT DECADE

The United Nations,* the World Health Organization,† and 37 countries have proclaimed 2000–2010 as the Bone and Joint Decade‡ (Woolf and Pfleger, 2003; McGowan, 2003). This global initiative is intended to improve the lives of people with musculoskeletal disorders, such as arthritis, and to advance understanding and treatment of musculoskeletal disorders through prevention, education, and research. The 10-year global initiative launched by the United Nations urges governments around the world to start taking action to draw attention to the growing pervasiveness and impact of musculoskeletal diseases, and to reduce the social and financial burdens to society. Support for this global initiative will raise awareness of musculoskeletal health, stimulate research, and improve people's quality of life.

Musculoskeletal diseases are one of the major causes of disability around the world, and have been a significant reason for the development of the Bone and Joint Decade (Woolf and Pfleger, 2003; McGowan, 2003; Brooks, 2002). RA, OA, gout, and back pain are important causes of disability-adjusted life-years in both the developed and developing worlds (Brooks, 2006).

The Arthritis Foundation§ in the United States plays a key role in coordinating efforts during the Bone and Joint Decade as a supporter. Its aims are to:

- Raise awareness and educate the world on the increasing societal impact of musculoskeletal injuries and disorders
- Empower patients to participate in decisions about their care and treatment
- Increase global funding for prevention activities and treatment research
- Continually seek and promote cost-effective prevention and treatment of musculoskeletal injuries and disorders

* http://www.un.org/.
† http://www.who.int/en/.
‡ http://www.arthritis.org/bone-joint-decade.php.
§ http://www.arthritis.org/.

In the following section, we explore the role of inflammatory agents in the pathogenesis of RA and OA. We will then discuss the nutritional targeting of inflammatory signaling pathways in arthritis as a realistic treatment in more detail.

5.5 RHEUMATOID ARTHRITIS (RA)

Rheumatoid arthritis (RA) is a chronic and progressive autoimmune disease that attacks synovial joints.*,†,‡ RA affects 0.8–1% of the adult population. It is a painful condition that can cause severe disability (this varies between individuals and depends on how severe and aggressive the disease is) and ultimately affects a person's ability to carry out everyday tasks. The disease can progress very rapidly (again, the speed of progression varies widely between individuals), causing swelling and damaging cartilage and bone around the joints. Any joint may be affected, but commonly the hands, feet, and wrists are affected. It is a systemic disease, which means that it can affect the whole body and internal organs, such as the lungs, heart, and eyes. Furthermore, RA is associated with an increased risk of coronary disease, infection, and lymphoma, as well as reduced life expectancy (Wolfe and Michaud, 2004, 2007; Pinals, 1987; Reilly et al., 1990; Mitchell et al., 1986). RA affects approximately three times more women than men, and onset is generally between 40 and 60 years of age, although it can occur at any age. There are studies that suggest RA is also associated with sex hormone production in the body. The peak incidence of RA in women coincides with the peri-menopausal age, and the juvenile form occurs mainly during puberty, suggesting a connection of RA with hormonal alterations (Goemaere et al., 1990). Although controversial, several studies have reported ameliorating effects on clinical measures of disease activity and inflammation, improved bone mineral density (BMD), and presented results pointing toward retardation of joint damage by hormone replacement therapy (HRT) (D'Elia et al., 2003a, 2003b; Mitchell et al., 1986).

The pathogenesis of RA is poorly understood, and little is known about the risk factors associated with it. Smoking is an important risk factor and makes the outlook much worse, but there is no mechanistic insight to explain why this is the case. There is no cure for RA, and more information is needed to help understand the inflammatory processes that occur in the disease and how to manage it. The effects of RA are not well publicized. Therefore, awareness of the severity of the condition tends to be restricted to those who are directly affected or their caregivers and relatives. The outlook for RA patients is significantly better now than it was 20–30 years ago. RA patients have a much better quality of life in spite of having RA, especially if the disease is diagnosed and treated with appropriate anti-inflammatory agents. We now know that uncontrolled RA increases mortality through an increased risk

* http://www.nras.org.uk/.

† http://www.nras.org.uk/about_rheumatoid_arthritis/what_is_ra/what_is_ra.aspx.

‡ http://www.arthritisresearchuk.org/arthritis_information/arthritis_types__symptoms/rheumatoid_arthritis.aspx.

of cardiovascular disease, such as heart attacks and strokes; again, the need for early treatment is imperative.

5.6 EFFECTS OF INFLAMMATION ON ARTICULAR CARTILAGE AND OTHER JOINT TISSUES

As outlined in the introduction, inflammation is a natural physiological response that provides protection against an adverse environment and is used to remove the agents causing the inflammation and promote the repair of damaged tissues. When the causative agent cannot be destroyed, for instance, in a RA joint, chronic inflammation results in extensive damage to joint tissues. Additionally, during inflammation, tissue-derived factors contribute to the destruction of joint tissues; these include lysosomal enzymes released by inflammatory cells and macrophages (Bartholomew et al., 1984; Cooke et al., 1985), reactive oxygen species (Schalkwijk et al., 1986, 1987), prostaglandins, and pro-inflammatory cytokines (Pettipher et al., 1986). These factors have been suggested to play a central role in the degeneration of articular cartilage during inflammation of the joint (Keiser et al., 1976). They degrade the core proteins of cartilage proteoglycans into fragments containing one and five chondroitin sulfate chains and leave the hyaluronate binding region bound to hyaluronate, thereby producing a chondroitin sulfate-free fragment, and so inhibit proteoglycan biosynthesis (Roughley, 1977). The main symptom of joint inflammation in OA is pain, which is actually a physiological signal to the brain and the immune system to protect the joint from extreme use.

Joint damage causes inflammation and stimulates the synthesis and release of more mediators that degrade joint tissues (Calin, 1989). Several studies have reported that pro-inflammatory cytokines induce hyperplasia of synovial cells (also known as synovitis) in joints. This is an important aetiology for RA; high concentrations of TNF-α and IL-1β have been reported within the synovial fluid and plasma of patients with RA (Eastgate et al., 1988, Saxne et al., 1988). Pro-inflammatory cytokines stimulate the synthesis of matrix metalloproteinases, activate caspase-3 (and downstream effector caspases), and stimulate osteoclasts, causing irreversible damage to soft and calcified tissues (i.e., subchondral bone) in joints (Csaki et al., 2009, Olsen and Stein, 2004). Furthermore, cytokines suppress the expression of cartilage-specific ECM components in chondrocytes, such as collagen type II and cartilage-specific proteoglycans, exacerbating the arthritis-associated loss of cartilage ECM (Kolettas et al., 2001, Murakami et al., 2000, Robbins et al., 2000).

Chondrocyte proliferation is considered to be an attempt to counteract cartilage degradation, but disease progression and secondary inflammation prove that this is generally unsuccessful. The short-lived hyperplasia (chondrocyte cloning) is followed by hypocellularity and apoptosis (Blanco et al., 1995, 1998; Clegg and Mobasheri, 2003; Kim et al., 2003; Mobasheri, 2002). Catabolic events responsible for cartilage matrix degradation comprise the release of catabolic cytokines such as IL-1β, IL-6, and TNF-α (Goldring, 1999; Westacott and Sharif, 1996), inducing matrix-degrading enzymes such as matrix metalloproteinases (MMPs) and aggrecanase (ADAM-TS4, ADAM-TS11) by chondrocytes and by synoviocytes in early OA (Goldring, 1999,

2000a, 2000b; Martel-Pelletier, 1998; Westacott and Sharif, 1996). An imbalance between MMPs and tissue inhibitors of MMPs occurs, resulting in active MMPs, and this may be important in cartilage matrix degradation. However, IL-1β may also contribute to the depletion of cartilage matrix by decreasing synthesis of cartilage-specific proteoglycans and collagen type II (Richardson and Dodge, 2000; Robbins et al., 2000; Studer et al., 1999; Goldring, 2000a). Systemic effects of elevated IL-1β levels include stimulation of glucose transport and metabolism, causing hypoglycemia and impairing glucose-induced insulin secretion (del Rey and Besedovsky, 1987). In articular cartilage, the acute effects of IL-1β also involve stimulated glucose uptake and metabolism (Hernvann et al., 1996; Shikhman et al., 2001). When the matrix is degraded, an inappropriate, inferior repair matrix is synthesized that cannot withstand mechanical load. Consequently, cartilage fibrillation and breakdown occur by the focal formation of vertical, oblique, and tangential clefts into the ECM, and are localized preferentially in areas of proteoglycan depletion. Apoptosis is another contributing factor to the loss of articular cartilage in RA and OA: apoptosis increases the cell loss observed in aging and OA cartilage (Adams and Horton, 1998; Blanco et al., 1998; Mobasheri, 2002). In addition to deregulated MMP activity and chondrocyte apoptosis, poor diets and malnutrition are also considered to be contributors to the pathogenesis of bone and joint disorders in humans and animals (Kealy et al., 1997; McAlindon and Felson, 1997).

Many of the biological effects of pro-inflammatory cytokines on chondrocytes have been shown to be regulated by the ubiquitous central transcription factor NF-κB (Largo et al., 2003; Liacini et al., 2003; Singh, 2007). In other cell types the expression of adhesion molecules such as cell adhesion molecule-I (I-CAM), vascular endothelial growth factor (VEGF), urokinase plasmin activator (uPa), Bcl-2, and pro-inflammatory cytokines has been shown to be regulated by NF-κB (Bharti and Aggarwal, 2002; Crawford et al., 2001; Shakibaei et al., 2008). NF-κB appears to be a common downstream target of multiple converging catabolic signaling pathways (e.g., those mediated by IL-1β and TNF-α) (Feldmann et al., 2002). NF-κB is present in the cytoplasm as an inactive heterotrimer complex consisting of two subunits and an additional inhibitory subunit: IκBα. Five different subunits exist: c-Rel, RelA (also known as p65), RelB, p50/p105, and p52/p100, which can form homo- or heterodimers in varying combinations. P65/p50 is one of the most prevalent combinations (Kumar et al., 2004). During the activation process, the inhibitory subunit IκBα is phosphorylated at Ser 32 and Ser 36 residues by IKK kinase (IκBα kinase) and is subsequently degraded. Once released, subunits of activated NF-κB translocate to the nucleus, where they bind NF-κB-recognition (κB) sites in the promoter regions of selected target genes, activating their expression (Ding et al., 1998, Largo et al., 2003). Dysregulation of NF-κB has been implicated in the pathogenesis of a wide spectrum of human diseases, including cancer, Alzheimer's disease, multiple sclerosis, cardiovascular disease, and RA (Kumar et al., 2004). Activation of NF-κB has been observed in synovial cells from patients with RA (Fujisawa et al., 1996). Although the cause of RA is unknown, autoimmunity plays a pivotal role in its chronicity and progression. Smoking and stress are thought to contribute to RA. RA is diagnosed chiefly on

symptoms and signs, but also with blood tests (especially a test called rheumatoid factor) and radiographs.

5.7 PHARMACOTHERAPY FOR ARTHRITIS AND RHEUMATIC DISEASES

In the following sections we discuss the currently available pharmacotherapies for OA and RA before moving on to natural compounds and their potential for targeting joint inflammation in arthritic and rheumatic conditions. RA and OA are treated with analgesics such as acetaminophen, opioids, NSAIDs, and intra-articular therapies, such as glucocorticoids and hyaluronans. Furthermore, antirheumatic drugs are used to treat and modify the clinical courses of RA (Smolen and Aletaha, 2008), for example, methotrexate, sulfasalazine, leflunomide, hydroxychloroquine, anti-TNF therapy (etanercept, infliximab, certolizumab pegol, golimumab, and adalimumab), anti-CD20 therapy (rituximab), and abatacept. Moreover, the major problems associated with these drugs are the side effects—impairment of the patient's immune and surveillance systems, which will increase susceptibility to opportunistic bacterial and viral infections and inducible malignant tumors (den Broeder et al., 2002; Elliott et al., 1994; Seymour et al., 2001; Smolen and Aletaha, 2008; Suryaprasad and Prindiville, 2003). The use of oral steroids is not recommended in the treatment of OA because of the low benefit and high rate of adverse effects, such as osteoporosis and obesity. The use of oral steroids is not recommended in the treatment of OA because of the low benefit and high rate of adverse effects, such as osteoporosis and obesity. In fact, high doses of steroids exert catabolic effects on cartilage and bone (Solomon, 1973).

5.7.1 Steroids and Anti-TNF-α Therapies

TNF-α belongs to the large and heterogeneous group of pro-inflammatory cytokines. It is mainly secreted by activated macrophages and fibroblasts, stimulating further production of additional chemokines, prostaglandins, proteases, and growth factors, and can activate neutrophils, B cells, and endothelial cells (Feldmann et al., 1994, 1995). In synovial joints, soluble TNF-α stimulates synovial cell proliferation and production of pro-inflammatory cytokines in an autocrine and paracrine fashion (Simmonds and Foxwell, 2008). Activation of TNF-α receptors results in the activation of the NF-κB signaling cascade, which controls the expression of additional pro-inflammatory cytokines, adhesion molecules, and matrix-degrading enzymes. Theoretically, utilizing anti-TNF-α as an RA therapeutic agent would block the TNF pathway, interrupting the inflammatory process and breaking the cycle of inflammation, and thus limiting joint damage (Segal et al., 2008). The efficacy of anti-TNF-α therapy has been well supported in randomized controlled trials in over 10,000 patients with RA, and quality of life and joint function have markedly improved (Bathon et al., 2006; Maini et al., 1998). However, several studies and a meta-analysis have suggested that the relative risk of serious infection is increased 1.3- to 2-fold (Bathon et al., 2006; Maini et al., 1998; Schneeweiss et al., 2007; Segal et al., 2008). Further, there is evidence suggesting increased risk of heart failure, and international

societies such as the American College of Rheumatology have produced guidelines for the usage of anti-TNF-α therapy in RA (Saag et al., 2008).

Injection with steroids is a therapy that has been extensively used for the treatment of RA. The intra-articular injection of glucocorticoids leads to short-term relief that lasts between weeks and months, but the intra-articular injection presents the risk of damaging cartilage if it is repeated often (Fubini et al., 2001). Furthermore, it has been reported that intra-articular hyaluronic acid injection could have protective effects on cartilage ECM and could reduce the production and activity of pro-inflammatory mediators and matrix metalloproteinases (Moreland, 2003). Indeed, the effects of hyaluronic acid last longer than glucocorticoid injections (Richette et al., 2009).

5.7.2 Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)

Nonsteroidal anti-inflammatory drugs (NSAIDs) have become the common choice for treating rheumatologic conditions like OA, RA, and gout. Most general practitioners issue prescriptions for NSAIDs for such conditions. NSAIDs effectively relieve pain by about half and increase mobility in about 60% of people with OA. Current treatment recommendations for RA include early use of disease-modifying antirheumatic drugs along with NSAIDs (Ross, 1997). Animal studies and data from human patients demonstrate that cyclooxygenase (COX)-2 upregulation in OA and RA is associated with the pain and inflammation of the disease state (Lipsky, 1999). The COX-1 isoform, however, is a constitutive enzyme with homeostatic functions. Most conventional NSAIDs inhibit both forms of the COX enzyme. However, drugs such as celecoxib inhibit COX-2 preferentially to COX-1 (Lipsky, 1999). In patients with OA of the knee, the drug aceclofenac decreases pain, reduces disease severity, and improves the functional capacity of the knee to an extent similar to that of diclofenac, piroxicam, and naproxen (Dooley et al., 2001). There is insufficient information to rank their effectiveness, but what information we have suggests that diclofenac (100–150 mg) and naproxen (500–750 mg) are much more effective than low doses of ibuprofen, and more effective than paracetamol or acetaminophen for treating OA. Gout is caused by monosodium urate crystals in the joints, bones, and soft tissues, and can be treated by NSAIDs or intra-articular glucocorticoids. All these drugs can block the acute immune reactions, but all of these drugs are associated with numerous side effects (Smolen and Aletaha, 2008). Therefore, the search is still on for safer, natural, and more selective pharmacotherapies for OA, RA and related rheumatic and arthritic conditions.

5.8 PROPHYLACTIC AND THERAPEUTIC APPLICATIONS OF PHYTOCHEMICALS IN OA AND RA

As highlighted above, current treatments for gout, OA, and RA are associated with unwanted side effects and are expensive. Natural products do not have such disadvantages and offer alternative treatment options for OA and RA (Hak and Choi, 2008; Sale et al., 2008). Traditional medicine is known to be fertile ground for the

sources of modern medicines (Corson and Crews, 2007). In many different chronic diseases (including RA) in which inflammation is known to play a central role, phytochemicals (i.e., curcumin or resveratrol) have been shown to exhibit therapeutic potential. The main aim of RA therapy is to counteract the systemic chronic inflammation and associated inflammatory symptoms in the joints, delay joint degradation, reduce and minimize disability, and provide a better quality of life for patients. It is recognized that current treatments for arthritis are insufficient and inefficient, cause substantial side effects, and tend to be expensive (especially when calculated and spread over the long time course of the disease). However, natural products do not have such disadvantages and offer novel treatment opportunities (Hak and Choi, 2008; Sale et al., 2008). A number of natural substances have been investigated for their anti-inflammatory capabilities, including omega-3 fatty acids (FA) (Curtis et al., 2002), curcumin (Henrotin et al., 2010), resveratrol (Lee and Moon, 2005), the polyphenolic green tea catechins (Annabi et al., 2007; Kim et al., 2007), and various flavonoids (Kong et al., 2008; Vijayababu et al., 2006).

5.9 TURMERIC AND CURCUMIN

Numerous studies have shown that natural compounds such as curcumin (a yellow coloring agent present in the spice turmeric, *Curcuma longa*, that belongs to the ginger (Zingiberaceae) family) have potential in the treatment of arthritis (Csaki et al., 2008, 2009). The turmeric is used in Ayurvedic medicine (traditional Indian medicine) to treat various common diseases, including stomach upset, ulcers, jaundice, arthritis, wounds, and skin and eye infections (Largo et al., 2003; Liacini et al., 2003; Singh, 2007). Curcumin was first isolated in 1815 by Vogel and Pelletier. Its chemical structure, 1,6-heptadiene-3,5-dione-1,7-bis(4-hydroxy-3-methoxyphenyl)-(1E,6E), was shown in 1910 by J. Milobedzka and V. Lampe (Germany). In addition to curcumin, turmeric contains demethoxycurcumin and bisdemethoxycurcumin. Commercial curcumin contains three major components: diferuloylmethane (82%), demethoxycurcumin (15%), and bisdemethoxycurcumin (3%), together referred to as curcuminoids (Aggarwal et al., 2003), all of which have anti-inflammatory activity.

Several preclinical and clinical studies have shown that curcumin has potential therapeutic value against most chronic diseases, including neoplastic, neurological, cardiovascular, pulmonary, metabolic, and arthritis diseases. Indeed, it has been reported that the treatment of the IL-1β-stimulated chondrocytes with curcumin inhibits activation of caspase-3 and PARP cleavage. Curcumin is able to antagonize the IL-1β- and TNF-α-dependent upregulation of MMPs and COX-2. Curcumin inhibits the inflammatory and apoptotic effects on IL-1β-stimulated chondrocytes, and this correlates with downregulation of NF-κB-specific gene products that are known to mediate inflammation, degradation, and apoptosis of chondrocytes in OA. Additionally, curcumin suppressed IL-1β-induced downregulation of both the cartilage-specific ECM component collagen type II and the cartilage-specific master transcription factor Sox-9. Furthermore, inhibition of NF-κB activation by curcumin occurs mainly through the IKK activation (Csaki et al., 2009; Liacini et al., 2003; Onodera et al., 2000; Shakibaei et al., 2007a).

5.9.1 Pro-Apoptotic and Anti-Proliferative Activity of Curcumin

Curcumin has been shown to suppress tumor cell growth and induce apoptosis in numerous cell lines. Therefore, it is suspected that curcumin also has anti-proliferative and pro-apoptotic effects on chondrocytes or synovial cells. Three independent papers have reported that curcumin dose-dependently decreased cell viability (in a range of 10–50 mM), estimated by tetrazolium salt reduction (MTT) assay, of adherent synoviocytes (Jackson et al., 2006; Thompson et al., 2004; Lev-Ari et al., 2006). In addition, curcumin (20 mM) enhanced celecoxib's apoptotic effects on OA synovial-adherent cells, whereas curcumin added alone had no apoptotic effect (Lev-Ari et al., 2006). The relevance of this inhibitory effect on cell growth in OA is that synovial fibroblasts secrete mediators of inflammation and joint destruction and are recognized as important factors in the pathogenesis of OA. Therefore, induction of apoptosis in these cells is an attractive therapeutic goal and could induce long-term remission, but this approach needs to be approached with caution.

5.9.2 Antioxidant Activity of Curcumin

The degradation of cartilage in OA results from a combination of inappropriate mechanical stress, inflammatory mediators, and biochemical factors, mainly MMPs and reactive oxygen species (ROS) (Henrotin and Kurz, 2007). The principal ROSs involved in the pathogenesis and progression of OA are NO, $ONOO^-$, and superoxide anion radicals. These factors not only are deleterious agents involved in cartilage degradation, but also act as catabolic cell signaling molecules (Henrotin et al., 2003). The activity of ROS is balanced by enzymatic and nonenzymatic antioxidants, that act by inhibiting oxidative enzymes, scavenging free radicals, or chelating ion metals (Henrotin et al., 2005a). A number of antioxidant supplements or drugs with antioxidant properties have been developed to reinforce the cellular antioxidant status. However, so far, there is no consistent or convincing evidence to support the notion that additional antioxidant supply is efficacious in relieving the symptoms of OA or preventing structural changes in OA cartilage (Henrotin et al., 2005a, 2005b; Henrotin and Kurz, 2007). Although further investigation is required to support the concept of antioxidant therapy in the management of joint diseases, basic research to find new and safe scavengers of ROS for the treatment of OA is justified.

Curcumin has been shown to be an effective scavenger of ROS and reactive nitrogen species *in vitro* (Sreejayan and Rao, 1996, 1997). However, it is still not established whether curcumin acts directly as an antioxidant *in vivo*. Due to its limited oral bioavailability in humans, plasma and tissue curcumin concentrations are likely to be very low. However, the finding that 7 days of oral curcumin supplementation (3.6 g/day) decreased the number of oxidative DNA adducts in malignant colorectal tissue suggests that curcumin taken orally may reach sufficient concentrations in the gastrointestinal tract to inhibit oxidative DNA damage (Garcea et al., 2005). In addition to direct antioxidant activity, curcumin may function indirectly as an antioxidant by inhibiting the activity of inflammatory enzymes, such as MMP-9 (Swarnakar et al., 2005), or by enhancing the synthesis of glutathione, superoxide dismutase, and catalase, important intracellular antioxidants (Nishinaka et al., 2007).

Recently, it was demonstrated that curcumin at concentrations ranging between 1 and 20 mM inhibited, in a dose- and time-dependent manner, IL-1β-induced inducible nitric oxide synthase (iNOS) gene expression and NO production by bovine chondrocytes in monolayer, human chondrocytes in alginate beads, and human cartilage explants (Mathy et al., 2007, personal communication from Y. Henrotin). They compared the effect of curcumin with those of two NSAIDs in bovine chondrocytes in monolayer culture. Curcumin at 10 mM inhibited IL-1β-stimulated NO production by 50%, whereas celecoxib and indomethacin IC_{50} values were superior at 20 mM. In bovine chondrocyte monolayer cultures, curcumin did not modify basal superoxide dismutase (SOD) activity but reduced IL-1β-stimulated SOD activity at concentrations of 15 μM (personal communication from Y. Henrotin). At these concentrations, curcumin also inhibited IL-1β-stimulated MnSOD gene expression. Thus far, there are few publications that support antioxidant properties for curcumin in cartilage. Clearly, this is an important area that is worthy of further investigation.

5.9.3 Anti-Inflammatory Activity of Curcumin

Arachidonic acid in cell membranes plays an important role in inflammatory responses by generating potent chemical messengers known as eicosanoids. Membrane phospholipids are hydrolyzed by phospholipase A_2 (PLA_2), releasing arachidonic acid, which may be metabolized by cyclooxygenases (COX), to form prostaglandins and thromboxanes, or by lipoxygenases (LOX), to form leukotrienes. Curcumin has been found to inhibit PLA_2, COX-2, and 5-LOX activity in cultured cells (Hong et al., 2004). Although curcumin has been shown to inhibit the catalytic activity of the enzyme 5-LOX directly, it inhibited PLA_2 by preventing its phosphorylation and COX-2 mainly by inhibiting its transcription. Nuclear factor-kappa B (NF-κB) is a transcription factor that binds DNA and enhances the transcription of the COX-2 gene and other pro-inflammatory genes, such as iNOS. Curcumin has been found to inhibit NF-κB-dependent gene transcription in colorectal cancer cells (Plummer et al., 1999), articular chondrocytes (Schulze-Tanzil et al., 2004; Shakibaei et al., 2005), and mesenchymal stem cells (MSCs) (Buhrmann et al., 2010). Curcumin also inhibits the induction of COX-2 and iNOS in culture models and animal studies (Brouet and Ohshima, 1995, Nanji et al., 2003).

Recent studies in our laboratories have shown that curcumin (1–20 mM) dose-dependently inhibited both IL-1β-stimulated COX-2 expression and PGE_2 production, but did not affect COX-1 expression in bovine chondrocyte monolayers (Mathy et al., 2007). PGE_2 production was inhibited by 50% at curcumin concentrations of 5 mM. In comparison, indomethacin and celecoxib IC_{50} values were below 2.5 mM. Curcumin has also been shown to reduce IL-1β-stimulated PGE_2 production from bovine chondrocytes cultured in agarose constructs (Chowdhury et al., 2008). In bovine chondrocytes cultured in monolayer, IL-1β-stimulated IL-6 and IL-8 gene expression was dose-dependently inhibited by curcumin. Furthermore, curcumin inhibited IL-6 and IL-8 protein synthesis in human chondrocytes grown in alginate beads and in human cartilage explants (Mathy et al., 2007). Altogether, these *in vitro* results strongly indicate that curcumin may reduce inflammation in OA by reducing the chondrocyte-mediated production of inflammatory mediators.

These findings provide a preclinical basis for the *in vivo* testing of curcumin, and suggest that this natural compound could be helpful in alleviating symptoms in OA patients (Mathy et al., 2007).

5.9.4 Anticatabolic Activities of Curcumin

Studies of synovial fibroblasts cultured from human RA patients have shown that macrophage migration inhibitory factor upregulates messenger RNAs encoding MMPs, a process that is inhibited by curcumin (Onodera et al., 2000). Oncostatin M (OSM), a member of the IL-6 superfamily of cytokines, is elevated in patients with OA and, in synergy with IL-1β, promotes cartilage degeneration by MMPs (Liacini et al., 2002). Curcumin has been shown to be able to suppress IL-1β and OSM-induced MMP-1, MMP-3, MMP-9, and MMP-13 gene expression by human chondrocytes via inhibition of NF-κB activation and nuclear translocation (Li et al., 2001; Schulze-Tanzil et al., 2004; Shakibaei et al., 2005). It seems likely that the anticatabolic effects of curcumin are mediated through its actions on cytokine receptors, subcellular signaling pathways, or a combination of both. It remains to be determined whether curcumin can downregulate the expression of cytokine receptors on the cell surface.

5.9.5 Potential Anabolic Effects of Curcumin

The apparent anabolic effects of curcumin may be attributed to its anticatabolic effects. Direct evidence for its involvement as a pro-anabolic compound is still lacking. IL-1β is well known for downregulating type II collagen and cartilage-specific proteoglycan (CSPG) expression in chondrocytes; we have shown that this process is reversed by curcumin treatment. In addition to its effects on type II collagen and CSPG expression, curcumin also reverses the IL-1β-induced downregulation of β1 integrin (Shakibaei et al., 2007a). Recently it was shown that curcumin at 10 mM decreased proteoglycan mRNA expression in bovine chondrocytes grown in monolayer, though this was not seen at the lower concentrations (Jackson et al., 2006).

5.9.6 Potential for Curcumin in Treating Arthritis

Curcumin has been shown to stimulate cortisol production by cells in the *zona fasciculata* of the bovine adrenal gland by inhibiting the bTREK-1 potassium channels that set the cells' resting membrane potential (Enyeart et al., 2008). Cortisol relieves inflammation, and if TREK-1 is expressed in chondrocytes or synoviocytes, then curcumin may act on these channels to reduce other pathways regulating inflammation. Thus, curcumin may combat the prostaglandin-mediated pain associated with arthritis. It is reported that curcumin may act like capsaicin, an active ingredient in cayenne pepper, by depleting nerve endings of the neurotransmitter substance P, also involved in pain and inflammation (Surh et al., 1998). It has also been demonstrated that curcumin is a potent inducer of heme oxygenase-1 (HO-1) in vascular endothelial cells, and that increased HO activity is an important component in curcumin-mediated cyto-protection against oxidative stress (Motterlini et al., 2000).

In an early study published in 1980 (Deodhar et al., 1980), RA patients who took 1,200 mg of curcumin a day experienced the same reduction in stiffness and joint swelling as those who took the prescription anti-inflammatory drug phenylbutazone. Unfortunately, the study was fundamentally flawed because the study design did not include a control or placebo group (Deodhar et al., 1980). Studies in rats have shown that oral administration of curcumin lowers the level of inflammatory markers in serum by 73%, with concomitant lowering of paw inflammation in arthritic animals (Joe et al., 1997).

5.10 OTHER DIETARY SUPPLEMENTS WITH ANTI-INFLAMMATORY PROPERTIES

Chemicals that have the ability to interfere with inflammatory processes and their mediators may reduce inflammation and damage to joint tissues and could be of prophylactic and therapeutic value. Therefore, naturally occurring compounds capable of blocking NF-κB-mediated catabolic activity may prove to be promising therapeutic agents for the treatment of OA and RA. This realization has resulted in the proliferation of new research aimed at understanding how nutrients and genes interact. This new field is known as nutrigenomics (van Ommen and Stierum, 2002; Müller and Kersten, 2003; van Ommen, 2004).

5.10.1 Resveratrol

Resveratrol or *trans*-3,5,4′-trihydroxystibene is a polyphenolic, antifungal natural phytoalexin found in grapevines (*Vitis vinifera*) and a variety of other plants. It occurs in the vines, roots, seeds, and stalks, but its highest concentration is in grape skins. Resveratrol has been shown to possess potent anti-inflammatory, antioxidant, and anticancer properties. In addition, it has immunomodulatory and cardioprotective effects, suppresses angiogenesis, prevents diabetes mellitus, and there are suggestions that it prolongs life span (Bertelli et al., 1999; Elliott and Jirousek, 2008; Soleas et al., 1997). Since resveratrol is a potent and specific inhibitor of cytokine-induced NF-κB activation, it may have potential for treating RA (Csaki et al., 2008, 2009; Elmali et al., 2005; Molnar and Garai, 2005; Penberthy, 2007).

In vitro studies have shown that IL-1β-induced suppression of chondrocyte proliferation and morphological alterations are relieved by resveratrol. Resveratrol inhibits its membrane-bound IL-1β and mature IL-1β protein production in chondrocytes. Furthermore, cotreatment of IL-1β-stimulated cells with resveratrol blocks activation of caspase-3, PARP cleavage, apoptosis, and accumulation of tumor suppressor gene protein p53, and induces ubiquitin-independent degradation of p53. Resveratrol suppresses IL-1β-induced, NF-κB-dependent pro-inflammatory and matrix-degrading gene products, including MMPs, caspase-3, VEGF, and COX-2. Resveratrol inhibits its IL-1β-induced IκBα degradation and consequently accumulated IL-1β-induced IkBα phosphorylation. Resveratrol suppressed IL-1β-induced NF-κB-dependent expression of apoptosis-related gene products by stimulating the accumulation of

phosphorylated IκBα, ubiquitinated IκBα, and inhibition of proteasome activity (Csaki et al., 2008, 2009; Shakibaei et al., 2007b, 2008).

The *in vivo* effects of intra-articular injections of resveratrol on cartilage and synovium have been studied in a rabbit model of OA (Elmali et al., 2005). Resveratrol reduces cartilage tissue destruction and may protect cartilage against the development of experimentally induced OA.

5.10.2 Omega-3 Fatty Acids

Omega-3 fatty acids have significant effects on the production of various pro-inflammatory cytokines. However, inconsistent results indicate that dose rates, treatment time course, and the choice of omega-3 fatty acids used in supplements significantly impact the effects of supplementation on individual inflammatory cytokines (Trebble et al., 2003; Kelley et al., 1999; Kew et al., 2004; Vedin et al., 2008). Nevertheless, most studies report reduced secretion of cytokines such as IL-1β, IL-6, and TNF-α, following oral administration of omega-3 fatty acids in humans (Vedin et al., 2008; Meydani et al., 1991; Caughey et al., 1996). Similar effects have been observed *in vitro* (Weldon et al., 2007). Further, decreased genetic expression, protein levels, and activity of MMP-2, -3, -9, and -13 following stimulation with omega-3 fatty acids have been reported *in vitro* (Curtis et al., 2002; Harris et al., 2001; McCabe et al., 1999).

5.10.3 Green Tea Catechins

Green tea catechins, such as (–)-epigallocatechin-3-gallate (EGCG), have been extensively researched for their antioxidant and anti-inflammatory capabilities, particularly as chemopreventive agents. A number of reports have demonstrated a role for various green tea catechins (particularly those that contain a 3′-galloyl group) in the suppression of MMP expression and inflammation (Syed et al., 2007; Annabi et al., 2007). Research indicates that EGCG functions in reducing inflammation in a manner similar to that of other phytochemicals, via the NF-κB pathway. Pretreatment of normal human bronchial epithelial cells with EGCG resulted in a significant reduction in cell proliferation, as well as NF-κB activity, in response to stimulation with cigarette smoke condensate (Syed et al., 2007). A reduction in MMP-9, IL-8, and various upstream signaling pathway molecules, such as ERK-1/2, p38 MAPK, PI3K, and Akt, was also observed (Syed et al., 2007).

The potential of polyphenolic compounds in medicinal therapy may also extend beyond MMP inhibition. For example, curcumin may exert both anticatabolic and antiapoptotic effects by reversing IL-1β-mediated collagen type II suppression and preventing caspase-3 activation in human chondrocytes (Shakibaei et al., 2005). Similarly, resveratrol inhibits caspase-3 activation in IL-1β-stimulated chondrocytes (Shakibaei et al., 2007b). These multifaceted effects, although not as potent as those of synthetic drugs, may be beneficial in the treatment of MMP-related conditions by affecting multiple pathways in the disease process (Evans et al., 2006). For example, a combination of anticatabolic, antiapoptotic, antioxidant, and anti-inflammatory

effects may synergistically suppress disease progression. By suppressing, but not totally inhibiting, these pathways, polyphenols also have the potential to reduce negative side effects observed with synthetic drugs.

The effects of polyphenols (both beneficial and detrimental) appear to be more pronounced *in vitro* than *in vivo* due to the higher concentrations that can be achieved *in vitro* (Rahman et al., 2006). This is likely due to poor bioavailability and absorption following oral ingestion. Further, interactions between various polyphenols and other food additives require comprehensive analysis to ensure that the effects of a single compound observed *in vitro* are in fact transferrable to the *in vivo* situation when other compounds are available. For example, green tea catechins are generally recognized as antioxidants with inhibitory effects on MMPs in various tissues (Dona et al., 2003). However, EGCG also appears to be capable of pro-oxidant effects by enhancing MMP-7 production in human colon cancer cell lines (Kim et al., 2007). These pro-oxidant effects were inhibited by stimulation with the polyphenols curcumin, benzyl isothiocyanate, and gallic acid (Kim et al., 2007). Therefore, the use of phytochemicals as therapeutic agents requires rigorous pharmacokinetic and toxicity studies, as well as clinical verification of results obtained *in vitro*. Nevertheless, this is an exciting new area of research, which holds much potential for the treatment of inflammatory-mediated pathologies.

5.11 CONCLUDING REMARKS

In modern developed countries, public concern about synthetic drugs and their unwanted side effects is growing. Some anti-inflammatory drugs can actually increase the risk of neoplastic disease by interfering with immune surveillance and destruction of cells that accumulate mutations. Consequently, there is increasing interest in natural remedies, particularly those of botanical origin. Plant-derived extracts, which possess therapeutic properties, such as curcumin, offer a potentially safer and cheaper alternative to conventional drugs. Curcumin has beneficial effects on numerous cell types *in vitro* and has already begun clinical trials in humans. However, the bioavailability of curcumin and its metabolites is debatable and has raised the question of whether these *in vitro* effects can be replicated *in vivo*.

In this chapter and a recent review article (Henrotin et al., 2010) we have used text mining and a systematic review of the literature to highlight the anti-inflammatory effects of curcumin on articular chondrocytes. We have also reviewed the literature relating to the effects of resveratrol, omega-3 fatty acids, and green tea catechins on arthritis and joint tissues.

The research conducted to date with curcumin suggests that it is safe and may offer considerable potential as an aid to preventing or at least delaying the onset of OA or RA. In fact, its anticatabolic effects, namely, reducing degradative enzyme expression and activity, and its positive influence on anabolic gene expression suggest that it may be a suitable adjunct to conventional pharmaceutical therapy. Curcumin is also a powerful inhibitor of inflammatory pathways and mediators. The schematic shown in Figure 5.2 summarizes the available information in PubMed on the effects of curcumin on the TNF-α signaling pathway. The available information suggests that curcumin could be used in conjunction with NSAIDs. In contrast to NSAIDs, curcumin has

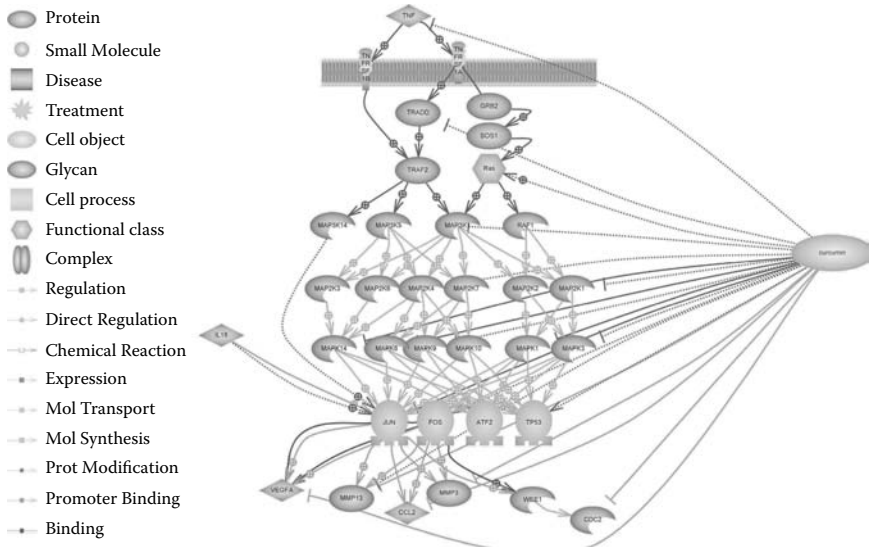

FIGURE 5.2 (See color insert.) Schematic of the effects of curcumin on the TNF-α receptor and its downstream signaling pathway. The biochemical pathway illustrated here was generated by text mining and makes use of a collection of canonical Ariadne pathways in addition to MedScan text mining.

no gastrointestinal side effects, and can even protect the gastric mucosa. Therefore, curcumin could be beneficial in the management of chronic inflammatory-related joint disease, including OA. However, despite this optimistic view, it must be recognized that there is a paucity of data regarding possible adverse effects of curcumin at concentrations that are biologically effective *in vitro*. Indeed, the absence of systemic adverse effects after oral administration of curcumin is probably the result of its poor bioavailability and chemical modification by the gut and liver. While some evidence exists for toxicity, at super-physiological concentrations, these are unlikely to be experienced or achieved *in vivo*. Nevertheless, we cannot exclude the possibility that increasing curcumin absorption, by chemical or natural process, could have unsuspected deleterious effects. It is now documented that curcumin at concentrations in excess of 50 mM shows cytotoxicity in a chondrocyte cell line (Toegel et al., 2008). In addition, there is no published information about the possible side effects of the metabolites of curcumin. Further work is therefore required to address the issues of bioavailability and tissue accumulation in order to calculate appropriate dose formulations to assess whether curcumin can be convincingly considered an aid to treating OA in both human and veterinary medicine.

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6 The Role of Inflammation in Skin Disease

Inflammation, Lifestyle, and Chronic Diseases—The Silent Link

Ricardo L. Berrios, Jigar R. Patel, and Jack L. Arbiser

CONTENTS

6.1 INTRODUCTION

Evidence of inflammatory mechanisms at play can be found in almost all skin diseases—either as primary culprits or as secondary features. Common clinical examples of inflammation as the prime mover include psoriasis, atopic dermatitis (eczema), contact and irritant dermatitis, the immunobullous disorders (pemphigus, bullous pemphigoid, dermatitis herpetiformis, and linear IgA disease), the dermatologic manifestations of autoimmunity (lupus spectrum disorders, rheumatoid arthritis, inflammatory bowel disease, dermatomyositis, and the sero-negative arthritides), and the newly described autoinflammatory disorders (neonatal onset multisystem inflammatory

disorder, familial cold urticaria, Schnitzler syndrome). In many of these conditions, inflammation is identified as primary in pathogenesis simply because the ultimate trigger is yet to be identified. Examples of inflammation as a secondary phenomenon include acne, rosacea, ulcers, stasis dermatitis, skin cancers, and the excoriations of pruritic disorders and psychodermatoses.

Inflammation in skin disease (just as in other organ systems) comes from two sources and occurs along a temporal spectrum. The sources of inflammation are the two major divisions of the immune system—innate and acquired—with a considerable degree of overlap between the two. Their respective cellular and humoral components translate into the variety of clinical presentations and differences in therapy. Temporally, inflammation can be either acute, subacute, or chronic; pathologic insults can recruit inflammatory mediators immediately and dismiss them much in the same way (e.g., allergic contact dermatitis), or they can be recruited to the skin after an indolent period (e.g., stasis dermatitis), oftentimes persisting in tissues after the insult has been dealt with (e.g., granulomatous disorders).

The aim of this discussion then is to guide the reader through a few examples of dermatoses viewed through the lens of primary and secondary inflammation, in the ultimate effort to illustrate common pathways and highlight new insights—both of which may in turn yield new therapeutic targets or explain old ones.

6.2 PRIMARY INFLAMMATORY DERMATOSES

6.2.1 Psoriasis

Psoriasis, a papulosquamous dermatosis with a prevalence of 2%, has five recognized clinical subtypes: chronic plaque disease, palmoplantar, guttate, erythrodermic, and pustular (von Zumbusch). Chronic plaque psoriasis is by far the most common type, accounting for 85 to 90% of all patients.[1] Psoriasis, in any form, has a high degree of morbidity, with an enormous impact on quality of life, including the mounting costs of long-term therapies.[2,3] Clinically, the lesions of chronic plaque psoriasis are described as raised, erythematous, well-delineated plaques with overlying silver scale[4] (see Figure 6.1); the result of a hyperproliferative epidermis, removal of scale, can lead to bleeding (Auspitz's sign). Psoriasis can have a variety of systemic manifestations, including psoriatic arthritis, hair and nail changes, and elevated cardiovascular disease risk. The exact cause of psoriasis remains unknown, but various treatments center around the mitigation of inflammation, usually with topical steroids, immunomodulators (methotrexate, cyclosporine), retinoids (isotretinoin), biologics (infliximab, adalimumab, efalizumab, etanercept), and phototherapy.

Psoriasis can have a variety of triggers, including stress, microorganisms, environmental agents, drugs, and trauma.[5] They occur, however, in the broader context of genetic susceptibility; monozygotic twins carry a psoriasis risk that is two to three times higher than that in dizygotic twins, and rates of psoriasis are higher among first- and second-degree relatives of patients when compared with the general population.[6] Genetic linkage studies have identified nine chromosomal loci collectively known as *PSORS* genes (*PSORS1* through *PSORS9*) that confer elevated risk. Two proteins encoded by PSORS1—HLA-Cw6 and corneodesmosin—have been the

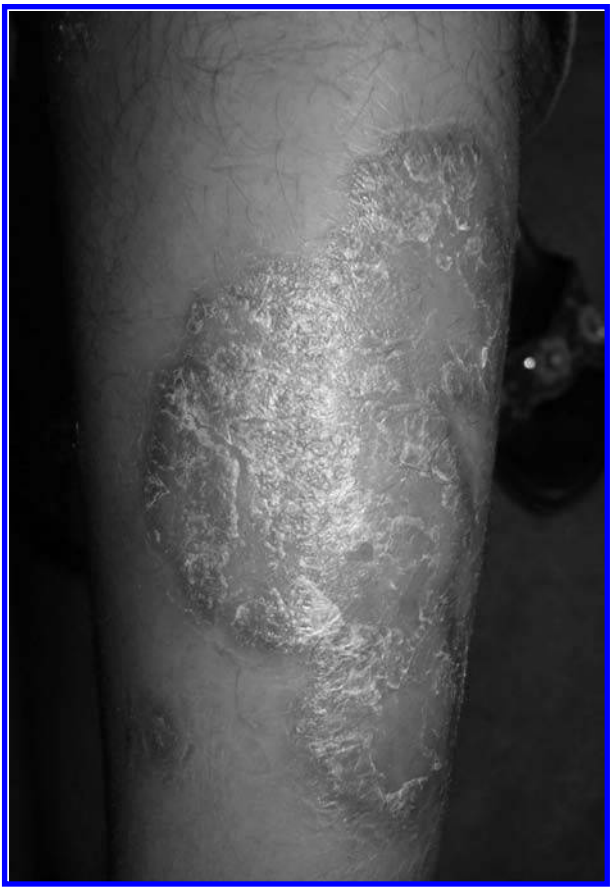

FIGURE 6.1 Chronic plaque type psoriasis. (Photograph courtesy of Dr. Ryan Wells.)

focus of much investigation.[7–10] Mutations have also been found in the interleukin-23 receptor (IL-23R) and genomic regions of IL-12B.[11,12]

The main stimulus for epidermal hyperproliferation in psoriasis is underlying inflammation. What draws the inflammation there is still in question, but pathological examination of diseased skin easily demonstrates infiltration with dendritic cells, macrophages, neutrophils, and T cells. Classified broadly as an autoimmune disorder, psoriasis is by definition a disorder of adaptive immunity, but interplay with innate immunity has also been described.[13] Mutations in HLA-Cw6 point to a pathogenic role for T cells in general, and a significant portion of the literature has centered around a subset of T cells known as type 1 helper T (Th1) cells and their associated cytokines, interferon-γ (IFN-γ) and IL-2, that together are responsible for regulating the immune response. IFN-γ is considered a central player in psoriasis; it and its downstream effectors mediate vasodilation via upregulation of inducible nitric oxide synathase as well as further recruitment of T cells.[14] But, growing bodies of evidence implicate dendritic cells, epidermal keratinocytes, and new subsets of T cells, natural killer (NK) T cells, and Th17 cells as well.

Dendritic cells, the innate, immune surveillance cells of the skin, are increased in psoriatic skin. They are further subdivided into two types: myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs). Included in mDCs are Langerhans cells (LCs) and inflammatory dendritic epidermal cells (IDECs). Often thought of as bridging the innate and adaptive responses, PDCs, for example, actively recruit T cells to the skin by way of IFN-α production[15,16]; what's more, IFN-α has been identified as a possible inducer of psoriasis.[17] Further IFN-γ production by NK T cells, as they interact with CD1d on keratinocytes, augments the adaptive immune response. In psoriatic skin and elsewhere, keratinocytes themselves elaborate a host of pro-inflammatory cytokines, including Toll-like receptors (TLRs), IL-1, IL-8, and tumor necrosis factor-α (TNF-α), a target of many effective, biologic agents. Keratinocytes also sustain inflammation by elaborating antimicrobial peptides (LL-37, β-defensins, S100A7); they act as chemotactic factors and modulators of naïve T cells.[18]

Dendritic cells also produce IL-23, a cytokine that activates interleukin-17-A-producing type 17 helper T (Th17) cells.[15] Th17 cells are a subset of adaptive T cells believed to participate in the immunosurveillance of epithelial tissue. The production of IL-17 and IL-22 by these T cells ultimately induces keratinocyte proliferation as well as production of antimicrobial peptides and chemotactic cytokines.[19] The recent introduction of ustekinumab, a human monoclonal antibody against the conserved p40 subunit of both IL-12 and IL-23, has spurred further interest in this particular T cell–cytokine pathway.[20–23]

Mechanisms to counter inflammation are also dysregulated in psoriasis. Regulatory T (Treg) cells are normally responsible for maintaining the immune response in check. While alterations in the number of Treg cells have not been observed in psoriasis, a CD18-knockout murine model deficient in these cells demonstrates a psoriasiform phenotype.[24,25] Also, IL-10, a suppressor of inflammation, is downregulated in psoriasis.[26,27] Administration of IL-10 as a therapy for psoriasis has shown promise in several early trials, but no confirmatory, placebo-controlled, randomized studies have been done to date.[28–34]

Psoriasis as an inflammatory disorder extends beyond the skin. Comorbidities associated with psoriasis indicate that it is probably more of a systemic disease than once thought. Over time, inflammatory cytokines diffuse into the general circulation and exert similar effects as other systemic inflammatory states, manifesting as increased risks for cancer, depression, metabolic syndrome, and psoriatic arthritis.[35] Ultimately, patients with moderate and severe psoriasis are at increased risk of cardiovascular and all-cause mortality.[36–38]

6.2.2 Atopic Dermatitis

Atopic dermatitis (AD) is a chronic, relapsing, inflammatory dermatosis characterized by pruritic, eczematous patches or plaques. The appearance and location of lesions tend to change over time. Acute inflammation and exudates with a predilection for face and extensor surfaces dominate in infancy, while chronic inflammation results in lichenification and scale in the flexoral surfaces in the years beyond (see Figure 6.2a and b). The prevalence of AD depends on age and is estimated to be between 15 and 30% in children and 2 and 10% in adults.[39] While 85% of cases begin before the

A

B

FIGURE 6.2A AND B Atopic dermatitis. (Photographs courtesy of Dr. Leslie P. Lawley.)

age of 5 years, up to 70% undergo spontaneous remission before adolescence.[40] AD has a variety of environmental triggers, including skin irritants, foods, dust mites, pollens, molds, and temperature extremes; it can also occur in association with allergic rhinitis and asthma in a triad known as atopy and in conjunction with a variety of food allergies. AD rates are higher in urban centers than in rural areas, possibly adding credence to the so-called hygiene hypothesis, which states that children who are not exposed to a variety of infectious and noninfectious agents early on are more susceptible to allergic disorders.[41,39,42] Treatment regimens center around avoidance of aggravating factors, skin hydration with emollients, and reduction of inflammation with topical steroids.

The exact cause of AD remains unknown but, like most diseases, involves an interplay between genetic susceptibility and environmental factors. The concordance

rate among monozygotic twins is over five times the rate in dizygotic twins.[43] Genetic linkage studies have identified six chromosomal loci associated with AD, mainly coding for epidermal differentiation factors and overlapping with regions altered in psoriasis.[44–48] An association has also been found between AD and chromosome 5q31–33. This region codes for a variety of cytokines that are involved in the regulation of IgE class switching: IL-4, IL-5, IL-12, IL-13, and granulocyte-macrophage colony-stimulating factor (GM-CSF)[49,50]; as is well known, IgE is a key mediator of type 1 hypersensitivities. Mutations have also been found in the filaggrin gene (*FLG*), which encodes a structural protein involved in epidermal maturation; this has been documented in a subset of European and Japanese patients with AD.[51–55,178] Whatever the ultimate pathway, investigations into atopic skin have identified alterations in the skin's barrier functions and the innate and adaptive immune responses.

In order for the skin to maintain its function as a barrier to outside insult, it must remain intact; this involves a delicate balance between degradation and buildup of both the "bricks" and "mortar." In AD, this balance is disrupted. Sphingomyelin deacylase, an enzyme responsible for the degradation of sphingomyelin, is upregulated, resulting in a deficiency of ceramide, an important component of the barrier's mortar. Ceramide deficiency ultimately leads to impaired barrier function and increased transepidermal water loss—a hallmark of AD.[55] As mentioned above, mutations in filaggrin, an important structural protein that bundles cytokeratin filaments, also result in disrupted keratinocye maturation and an impaired barrier. Via decreases in natural antimicrobial peptides (β-defensins, dermcidin), AD skin is more likely to be colonized with organisms, particularly *Staphylococcus aureus*[56–58]; moreover, levels of CD14 are reduced, impairing the ability of immune cells to respond to microbes.[59] Taken together, large pathogens such as pollens, molds, and foods that would normally have been blocked from entry are now more likely to pass through and interact with immune surveillance cells to begin the inflammatory cascade and the path to sensitization.

Once breached, AD skin does not respond to insult in the same way normal skin does. AD skin contains a predominance of LCs and IEDCs over the characteristic pDCs seen in psoriasis.[60] Additionally, these cells not only present IgE but are high in FcεRI, a high-affinity receptor for IgE.[61–64] Once activated, LCs go on to recruit CD4+ T cells to the skin via production of IL-16, and then favor a Th2-dominant response.[65] On the other hand, activated IEDCs go on to promote a Th1 response by producing IL-12 and IL-18. Allergen challenge leads to influx of IEDCs to AD skin and upregulation of FcεRI on both LCs and IEDCs.[66]

Antigen-presenting cells (APCs), including LCs, DCs, and macrophages, promote a Th2-dominant response in the early stages of AD via dendritic cell production of IL-4 and keratinocyte production of IL-7-like thymic stromal lymphopoietin.[67] Once activated, Th2 cells begin to secrete IL-4, IL-5, and IL-13, which promote IgE class switching in B cells; they also secrete IL-5, which stimulates increases in eosinophils.[68] Altogether, these factors promote high levels of IgE in the skin and serum. While levels of IgE do not necessarily correlate with disease severity or course, the fact that IgE levels quickly rise following the onset of AD suggests that the skin is the first site of sensitization.[40,69] Passing through an already compromised

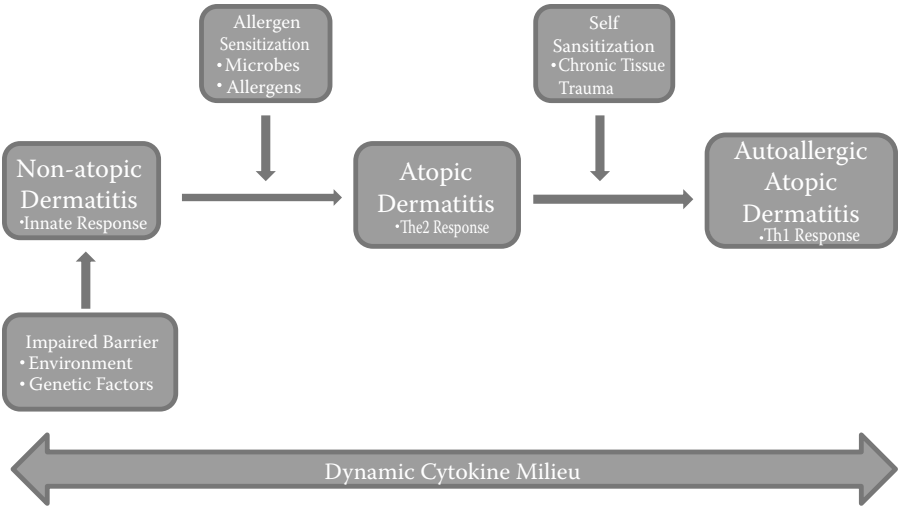

FIGURE 6.3 Model of the evolving immune response in atopic dermatitis. (Adapted from Bieber, T., *New Engl J Med.* 2008; 358(14):1483–94.)

barrier, allergens, on subsequent encounters with APCs, quickly elicit memory and further elaboration of IgE.

As mentioned above, AD patients are more likely to be colonized by fungi and bacteria such as *S. aureus* because of decreased levels of natural, defensive antimicrobial peptides (AMPs). Organisms like *S. aureus* not only cause superinfections of AD lesions, but also exacerbate pruritus, a hallmark feature of AD, which in turn worsens the compromised barrier and promotes further inflammation. AD patients also make IgE to enterotoxins produced by *S. aureus*, a feature not present in normal serum[70]; additionally, these enterotoxins induce expression of IL-31 by T cells, a strongly pruritogenic cytokine.[71–73]

The inflammatory picture of AD changes over time (see Figure 6.3). A combination of environmental factors and genetic susceptibility translating into an impaired barrier results in what Bieber terms nonatopic dermatitis.[69] With time, sensitization to various allergens and microbes develops, promoting an influx of proinflammatory cytokines and receptors consistent with a Th2-dominant response. This cytokine milieu worsens pruritus and diminishes natural defenses (scratching, altered barrier function, and fewer AMPs); Bieber terms this second stage atopic dermatitis.[69] As AD becomes more chronic in nature, the adaptive picture changes to a Th1-dominant response concurrent with the development of IgE to a variety of self-proteins from keratinocytes and endothelial cells.[74–77] Evident as early as 1 year of age, IgE-mediated autoimmunity promotes a change to Th1 and maintenance of inflammation via recurrent exposure to autoantigens.[75,77] Bieber terms this final, chronic stage autoallergic atopic dermatitis.

What this three-stage model of AD serves to illustrate other than the progressive nature of the disease is the clinical imperative to treat AD early and aggressively in order to control inflammation. This includes the use of emollients to maintain the

skin moist, the use of antimicrobials to reduce colonization, and the use of topical corticosteroids to reduce what inflammation does develop.

6.2.3 Autoinflammatory Disorders

While psoriasis and atopic dermatitis represent complex disorders of adaptive immunity, autoinflammatory disorders (hereditary periodic, or recurrent, fever syndromes) represent a group of diseases associated with defects in the innate immune system. With various frequencies depending on geographic location, these syndromes are associated with high levels of circulating proinflammatory cytokines from innate cellular elements that can be triggered by environmental exposures, body trauma, or seemingly nothing at all. Characterized by recurrent episodes of fevers and acute phase inflammation, autoinflammatory disorders include a significant number of relatively rare disorders caused by both inherited mutations and acquired deficiencies in several different pathways of innate immunity (see Table 6.1).

6.2.3.1 Cryopyrin-Associated Periodic Syndromes

Cryopyrin-associated periodic syndromes (CAPSs), or cryopyrinopathies, include familial cold urticaria (familial cold autoinflammatory syndrome (FCAS)), Muckle-Wells syndrome (MWS), and neonatal onset multisystem inflammatory disorder (NOMID). The three can be thought of as occurring on a spectrum of phenotype severity, with FCAS being the mildest and NOMID the most severe (see Figure 6.4).[78] Central to these disorders are disruptions in the structure and function of the inflammasome.

Inflammasomes are enzymes present in a variety of cells that act on other enzymes to eventually release active forms of acute phase cytokines. The Nalp3 inflammasome, found in macrophages, monocytes, fibroblasts, and dendritic cells, is activated by foreign material such as pyrophosphate crystals or bacteria; its function is to activate caspase-1, which in turn goes on to cleave pro-IL-1β to IL-1β, a proinflammatory cytokine. The Nalp3 inflammasome is a complex whose quaternary structure is made up of four components: cryopyrin, apoptosis-associated speck-like protein (ASC), cardinal, and procaspase-1.[79] This unit forms only after the cryopyrin portion has been activated by binding of materials to its LRR domain; potential triggers include muramyl dipeptide, adenosine triphosphate, toxins, *S. aureus*, *Listeria monocytogenes*, bacterial RNA, viruses, viral RNA, and gout or pseudogout crystals.[80–84] The final unit goes on to activate the caspase portion with eventual release of IL-1β. Cryopyrin is encoded by the cold-induced autoinflammatory syndrome 1 (*CIAS1*) gene on chromosome 1q44. First described in 2001, gain-of-function mutations in the gene result in constitutive activity of cryopyrin, oversecretion of IL-1β, and constitutive activation of innate immunity; three clinical syndromes associated with mutations in *CIAS1* have been described.[85,86]

Familial cold urticaria, or FCAS, is an autosomal dominant disorder characterized by the onset of systemic inflammation following prolonged, generalized exposure to the cold. Clinical features include an urticarial rash along with burning, itching, and edema; extracutaneous manifestations include polyarthralgias and myalgias without overt arthritis, fever, and chills. Attacks occur within 8 hours of cold exposure

TABLE 6.1
Inherited and Acquired Autoinflammatory Disorders with Known Genetic Mutations, Altered Protein Products, Recommended Initial Laboratory Testing, and Possible Treatments

Autoinflammatory Syndrome	Inheritance Pattern	Gene	Protein Product	Laboratory Testing	Treatments
FCVS	AD	*CIAS1*	Cryopyrin	CPR, ESR Genetic analysis	Cold Avoidance Antihistamines NSAIDs Epi-pen Anakinra
MWS	AD	*CIAS1*	Cryopyrin	ESR/CRP Genetic analysis Serum amyloid Creatinine Urinalysis	Antihistamines NSAIDs Anakinra
NOMID	AD	*CIAS1*	Cryopyrin	ESR/CRP Creatinine Urinalysis Genetic Analysis Neuro Testing Imaging	Anakinra
DIRA	AR	*IL1RN*	IL-1 Receptor Antagonist	ESR/CRP Imaging Genetic Analysis	Anakinra

continued

TABLE 6.1 (Continued)
Inherited and Acquired Autoinflammatory Disorders with Known Genetic Mutations, Altered Protein Products, Recommended Initial Laboratory Testing, and Possible Treatments

Autoinflammatory Syndrome	Inheritance Pattern	Gene	Protein Product	Laboratory Testing	Treatments
TRAPS	AD	*TNFRSF1A*	TNF Receptor 1	ESR/CRP Serum amyloid Genetic Analysis	Corticosteroids Etanercept (?) Colchicine Anakinra
PAPA	AD	*CD2BP1/PSTPIP*	CD2BP1/PSTPIP	ESR/CRP Genetic Testing	Corticosteroids Anti-TNF Biologics Anakinra
PFAPA	Acquired Prior Infection (?)	?	?	ESR/CRP Exclusion of other syndromes	Corticosteroids Colchicine Cimetidine Tonsillectomy (?)
Schnitzler	Acquired Prior Infection (?)	?	?	CBC SPEP/UPEP ESR/CRP Exclusion of other syndromes	Corticosteroids Antihistamines Immunomodulating Agents Anti-TNF Biologics Anakinra

AD = Autosomal Dominant, AR = Autosomal Recessive, CBC = Complete Blood Count, SPEP = Serum Protein Electrophoresis, UPEP = Urine Protein Electrophoresis, ESR = Erythrocyte Sedimentation Rate, CRP = C-Reactive Protein.

Source: Adapted from Shinkai, K., et al. *Clin Exp Dermatol.* 2008; 33(1): 1–9.

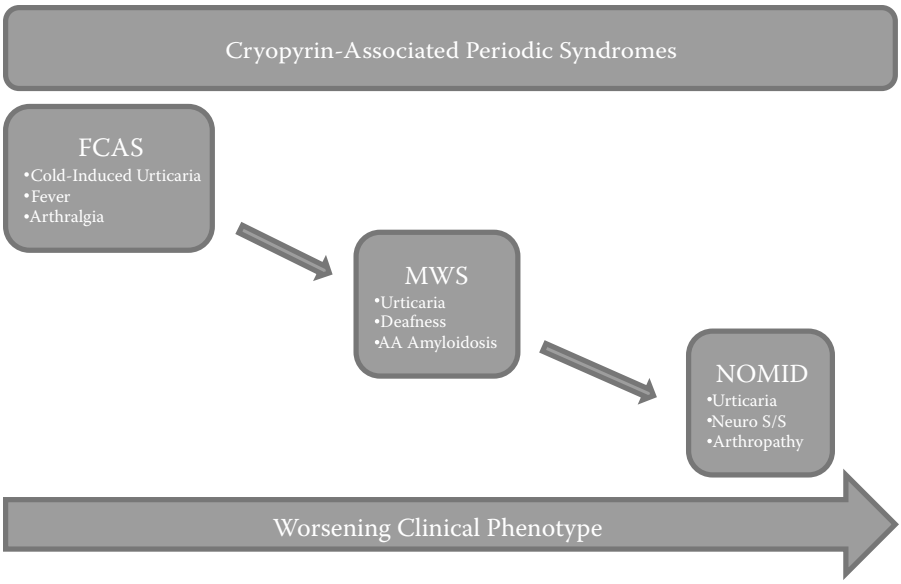

FIGURE 6.4 Spectrum of cryopyrin-associated periodic syndromes (CAPS). (Adapted from Shinkai, K., et al. *Clin Exp Dermatol.* 2008;33(1):1–9.)

and generally subside within 24–72 hours. Treatment includes avoidance of cold exposures, antihistamines to prevent or mitigate urticaria and edema, nonsteroidal anti-inflammatory drugs (NSAIDs) to address joint and muscle pain, and advice to carry an EpiPen in case of anaphylactic shock. Anakinra, a recombinant IL-1R antagonist given as a subcutaneous injection, has shown great efficacy in FCAS with minimal side effects (e.g., site reactions in 50% of patients).[87]

Muckle-Wells syndrome (MWS) is another CAPS, associated with mutations in the cryopyrin gene and inherited in an autosomal dominant fashion. MWS is characterized clinically by the triad of urticaria, sensorineural deafness (70%), and amyloidosis (25%); accompanying fevers, arthralgias, myalgias, and lymphadenopathy are also common. Attacks of inflammation typically begin in adolescence, occur randomly, and typically last 24–48 hours; some patients describe continuous symptoms. Systemic AA amyloidosis typically presents as renal dysfunction and nephritic syndrome. Treatment of MWS is much the same as in FCAS; anakinra has shown similar efficacy, with remission of nephritic syndrome and improvement of hearing loss having been described.[88,89]

Neonatal onset multisystem inflammatory disorder (NOMID) is the third CAPS, and it carries the most severe phenotype. The triad of NOMID consists of urticarial rash, neurologic disturbances, and osteoarticular involvement. Other systemic symptoms of inflammation include fever, lymphadenopathy, conjunctivitis, and uveitis. Neurologic symptoms vary but can include mental retardation, seizures, sterile meningitis, and hearing loss. In contrast to the previous two CAPSs, the joint involvement in NOMID is erosive and destructive, leading to polyarthropathies and joint deformities.[90–91] Also, symptoms tend to be continuous following the typical onset at 6 months of age. Death

before adulthood was common until the introduction of anakinra. Several studies have determined the immediate and sustained efficacy of anakinra in the setting of NOMID, but authors point out that it must be started before the irreversible features of the disease set in if they are to be averted.[92,93]

Recently, a new disorder related to CAPS has been described. Termed deficiency of the IL-1-receptor antagonist (DIRA), it also results in high levels of IL-1, but by deficiency in its naturally occurring antagonist instead of overproduction by an inflammasome. Aksentijevich et al. describe a series of patients and families with mutations in the *IL1RN* gene (chromosome 2q) inherited in an autosomal recessive fashion that resulted in production of a truncated protein that is not secreted. Lack of IL-1RA results in a phenotype similar to that of NOMID; patients present at birth or shortly thereafter with an evolving pustular rash, multifocal osteomyelitis, and periostitis. Osteotic manifestations ranged from widening of ribs to grossly deforming osteolytic lesions. Patients had evidence of sterile inflammation in various locations but lacked fever. Of six patients who were treated with anakinra, all of them showed prompt symptomatic relief and resolution of skin and bone lesions within weeks.[94]

6.2.3.2 Tumor Necrosis Factor Receptor-Associated Periodic Syndrome

As discussed above, TNF-α is a potent proinflammatory cytokine produced by several cells of innate immunity. Tumor necrosis factor receptor-associated periodic syndrome (TRAPS; familial Hibernian fever) is an autosomal dominant disorder associated with missense mutations in the *TNFRSD1A* gene (chromosome 12p13), which codes for the type 1 receptor for TNF-α; most mutations result in misfolding of the receptor's external domain, but the exact mechanism of how this results in disease is still under investigation.

Clinically, these patients present with long-lasting attacks of high fever, severe myalgias and arthralgias, migrating erythematous rash due to monocytic fasciitis, diffuse serositis (peritonitis, pleuritis, scrotal pain), and ocular involvement (conjunctivitis, uveitis, periorbital edema); systemic AA amyloidosis has been also reported. Episodes can begin anywhere from the first years of life to adulthood, typically occur 2–6 times per year, and last 1–3 weeks.[95,96,90] Treatment of TRAPS is challenging; oral corticosteroids are effective but are required for prolonged periods of time. Immunomodulatory medications have been reported as ineffective in TRAPS, and anti-TNF-α biologic therapy is also largely unsuccessful in most patients. In one study of four TRAPS patients, anakinra showed sustained efficacy with no major adverse reactions, but was associated with symptom relapse after drug withdrawal.[97]

6.2.3.3 PAPA Syndrome

The syndrome of pyogenic sterile arthritis, pyoderma gangrenosum, and acne (PAPA syndrome) is another autosomal dominant autoinflammatory disorder associated with mutations in the *PSTPIP1* gene (chromosome 15q24). This gene encodes PSTPIP1, a component of a neutrophil inflammasome that, after interaction with pyrin, causes the secretion of IL-1β. Gain-of-function mutations result in hypersecretion of IL-1β and dysregulation of apoptosis.[98–101]

Signs of PAPA typically begin in childhood and include severe nodulocystic acne pyoderma gangrenosum, and sterile arthritis usually induced by trauma. Fever can be present, and even percutaneous injections can cause the formation of PG lesions or cold abscesses.[90] Treatments of PAPA that have shown utility include pulsed corticosteroids, etanercept (an anti-TNF-α biologic) and anakinra.[78]

6.2.3.4 Acquired Autoinflammatory Syndromes

Two syndromes have so far been described that point to the possibility of acquiring defects in innate immunity: periodic fever, aphthous stomatitis, pharyngitis and adenitis syndrome (PFAPA), and Schnitzler syndrome.

Little is known as to the cause of PFAPA, but so far genetic studies have been unrevealing. As the name implies, PFAPA is characterized by oropharyngeal inflammation and ulceration with recurrent febrile episodes lasting 3–6 days, often preceded by a prodrome of fatigue, headache, abdominal pain, or irritability.[102–104] The syndrome has been recognized since 1987 as a common cause of fever of unknown origin in children.[105] Levels of IL-1, IL-6, TNF-α and IFN-γ are elevated in these patients during episodes of fever, and combined with available data, suggest that PFAPA may be an abnormal, exaggerated immune response to microbial infection.[106] Febrile attacks typically begin before 5 years of age, and spontaneous resolution of all symptoms has been observed in adulthood. Therapies with documented efficacy are limited in PFAPA and center mainly around oral corticosteroids during febrile episodes (very effective) and prophylactic colchicine or cimetidine in attempts to induce remission.[105,107–109] Also, tonsillectomy has shown partial efficacy as well in a number of cases[110–117]; however, there is still some controversy as to the role of surgery as prophylaxis in the treatment of PFAPA.[106]

First described in 1972 by the French dermatologist L. Schnitzler, Schnitzler syndrome is an acquired autoinflammatory syndrome characterized most often by an urticarial rash and a monoclonal IgM gammopathy.[118] Associated features include recurrent febrile episodes, arthralgias, myalgias, lymphadenopathy, hepatosplenomegaly, leukocytosis, and elevated erythrocyte sedimentation rate (ESR).[119] Just as in PFAPA syndrome, diagnostic criteria have been proposed for Schnitzler syndrome, but so far, no definite cause, genetic or otherwise, has been identified, although infectious triggers have been proposed.[120] Various cytokine disturbances have been found in these patients, including elevated levels of IL-6.[121,122] On pathologic examination of the skin, monoclonal bands of IgM along the basement membrane and capillary walls have also been described[123]; the clinical significance of this remains in question. In contrast to PFAPA syndrome, Schnitzler syndrome patients are older, with an average age at onset of 51 years[120]; episodes of urticaria typically last 12–36 hours over a period of 6 weeks and usually accompany fevers over 40°C. Effective treatments for Schnitzler syndrome are various and include oral corticosteroids, antihistamines, colchicine, anti-TNF-α agents, and immunomodulatory agents.[120] The efficacy of anakinra in several patients points to a possible pathogenic role for IL-1β,[124,125] The prognosis in these patients is generally excellent, with a 91% survival rate after 15 years.[120] However, progression to Waldenström's macroglobulinemia and other lymphoproliferative disorders has been described in a minority of patients.[126]

6.3 SECONDARY INFLAMMATORY DERMATOSES

6.3.1 Acne Vulgaris

No dermatosis is perhaps more familiar to dermatologists or any practicing physicians than acne, but surprisingly, only within the last two decades have major discoveries been made into the actual pathogenesis of a condition that affects 85% of the population by 24 years of age.[127] Considered a disorder of the pilosebaceous unit, acne is due to multiple factors, both internal and external to this structure; they include alterations in sebaceous gland biology, hormonal influences, hyperkeratinization, and bacterial pathogens. Discussion of the contributions of each of these is beyond the scope of this chapter, but each of them donates in its own way to the secondary inflammation common to acne and its variants.

The prototypic lesion of acne is the comedo (pl. comedones). The lesion begins to form when corneocytes (enucleated keratinocytes) are retained and begin to collect in the ostium of a follicle. Due to increased cohesiveness and induced hyperproliferation, the corneocyte plug creates an ever-growing impasse behind which cellular debris and sebaceous material begin to accumulate. As the lesion grows, pressure begins to build and eventually rises to the point where debris is extruded into the surrounding tissue. This is recognized as foreign material and inflammation ensues. The type of inflammation that develops determines the clinical lesions. An innate, neutrophil-predominant response results in a pustule, while an adaptive, Th1-predominant response leads to erythematous papules, nodules, and cysts—lesions that are more likely to scar.[127,128]

Apart from forming part of the foreign debris that incites inflammation, there is increasing evidence that keratinocytes and sebocytes may themselves be capable of signaling the innate immune system.[129] For example, sebocytes express Toll-like receptors (TLRs) 2 and 4, CD1, and CD14; they also secrete the AMP human β-defensin-2 (hβD-2) and antibacterial fatty acids (palmitic acid and oleic acid), which act synergistically on a variety of microbes.[130–132] *Propionibacterium acnes*, an aerotolerant, anaerobic, Gram-positive bacterium normal to skin flora, has a proposed pathogenic role in acne and may do so through interactions with TLRs, CD1, and CD14 and subsequent secretion of proinflammatory cytokines.

The definitive role of *P. acnes* in acne is still in question, but several experiments have shown that it is capable of inducing expression of hBD-2 and multiple cytokines from various cell types.[131,133–135] In sebocyte culture, *P. acnes* exposure stimulates secretion of CXCL8 and TNF-α, while exposure to bacterial lipopolysaccharide (LPS) results in the additional secretion of IL-1α; levels of cytokine expression were also much higher when the sebocytes were stressed than under normal states.[133] Ultimately, activation of sebocyte TLRs results in release of IL-α and IL-1β, which go on to stimulate secretion of IL-6 and IL-8 by macrophages. Initiation of inflammation weakens the follicular wall, allowing for extrusion of retained debris and further inflammatory stimulus.

The role of adaptive immunity in acne is even less defined. It is entirely plausible that the chronicity of lesions and the cytokine milieu are appropriate for the evolution of an adaptive response. To this end, some investigations have been done in

efforts to determine the presence and significance of an adaptive immune response. Already, antibodies to several *P. acnes* antigens have been described in the sera of acne patients.[133] Also, a subset of Th1 cells reactive to similar antigens has been documented by three studies.[136–139] A fourth study has also determined the presence of CD4+ T cells reactive to *P. acnes*, but went further, suggesting that the resolution of acne lesions may be dependent on the negative regulation of these T cells.[140] While the exact pathogenic mechanism is yet to be determined, there is undoubtedly an adaptive immune component to the inflammation of acne. As was mentioned above, lesions in which the adaptive response predominates not only have unique morphologies, but also go on to scar more readily; this may have implications for future therapies.

6.3.2 Rosacea

With a prevalence of approximately 10%, rosacea is a common dermatosis affecting mainly fair-skinned people.[141] Arising mainly in the third and fourth decades of life, it has a variety of clinical presentations, but is characterized uniformly by flushing, nontransient erythema, papules and pustules, and telangiectasia. Four main subtypes of rosacea have been described—erythematotelangiectatic (vascular), papulopustular (inflammatory), phymatous, and ocular—along with three variants: granulomatous, periorificial dermatitis, and pyoderma faciale.[142,143]

While the exact cause is yet to be identified, rosacea is essentially a disorder of vascular hyperreactivity. Studies have shown typical lesions exhibit higher rates of blood flow, and that topical application of αa1-adrenergic receptor agonists can resolve erythema.[144–147] Additionally, markers of hemangiogenesis and lymphangiogenesis, including vascular endothelial growth factor (VEGF), CD31, and D2–40, are markedly elevated in the skin of rosacea.[148] What is less clear is the connection between vasodilation, resultant erythema, and the myriad known triggers—emotions, temperature extremes, ultraviolet light exposure, foods, and medications.

Experimentally, ultraviolet light has been shown to promote several factors associated with rosacea. Exposure of mice to UV-B leads to cutaneous angiogenesis that resembles the telengiactesias seen in humans.[150] UV-B irradiation also causes increased secretions of VEGF and fibroblast growth factor 2 (FGF-2; another proangiogenic marker) in human keratinocyes and mouse epidermis.[149–151] Additionally, levels of reactive oxygen species (ROS) rise following exposure to UV light[152,153]; ROS promote inflammation by stimulating secretion of TNF-α and other cytokines, as well as collagen degradation by activation of matrix metalloproteinases (MMPs).[154–157] The exact role of MMPs in the pathology of rosacea remains unclear, but the fact that tetracycline inhibits the activity of several MMPs suggests a possible pathogenic function.[158–160]

Vascular dilation and the resultant leak of serous fluid have also been proposed to promote inflammation, a factor that may be exaggerated with each episode of subsequent vasodilation.[142,161] Dilated blood vessels may also promote leukocyte chemotaxis due to elevated levels of certain AMPs, namely, cathelicidins.[162] Cathelicidin levels are controlled, in turn, by kallikrein 5 (KLK-5), a skin protease that has been shown to be elevated in the skin of rosacea.[163,164]

Levels of AMPs may be elevated because of abnormal innate immune responses to two microbes: *Demodex folliculorum* and *Helicobacter pylori*. In the case of *D. folliculurum*, microbial densities are increased in rosacea patients when compared to normal individuals; serum reactivity to several *Demodex* antigens has also been described, a factor that undoubtedly promotes inflammation. Correlations with levels of *H. pylori* have not been observed, but eradication of gastric colonization improves symptoms of rosacea in certain individuals.[165–172] Either of these organisms may promote inflammation by interactions with TLR2, a Toll-like receptor that is abnormally expressed in the skin of rosacea. [173–175]

Microbes and UV light not only induce inflammation on their own, but are also elements meant to be recognized by the innate immune system.[176,177] Therefore, via interactions with TLRs, ROS, or products of tissue degradation, the immediate situation is made worse with prolonged activation of innate immune mechanisms.[179]

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7 Inflammation and Heart Diseases

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CONTENTS

7.1 INTRODUCTION

The time when people thought that the cardiovascular system was mere pump and pipes is over. The pump function of the heart and the integrity of arteries have been found dynamically affected by a variety of factors—humoral, neuronal, and inflammatory factors. In this chapter, we examine the role of inflammation in heart diseases through the review of clinical and basic scientific literature, examining the relative strengths and weaknesses of individual articles. We will focus on atherosclerosis and heart failure (HF)—the most common heart diseases. After briefly reviewing the history of research on inflammation and these heart diseases, we examine how the literature supports the association of both. We then evaluate whether inflammation results in atherosclerosis and HF. Next, we analyze whether atherosclerosis and HF would cause inflammation. Finally, we review the literature to see if the suppression of inflammation halts the progression of atherosclerosis and HF at a level of either animal research or clinical trials. We found that inflammation facilitates atherosclerogenesis and HF progression, that atherosclerosis and

HF can elicit inflammation, and that the suppression of inflammation in some cases retards the progression of both atherosclerosis and HF. It is possible that further research on inflammation and heart diseases would lead to new strategies to treat them through modulation of specific or general inflammatory processes associated with the diseases.

7.2 INFLAMMATION AND ATHEROSCLEROSIS

7.2.1 Association of Inflammation and Atherosclerosis

More than 150 years ago, a controversy broke out on the issue of inflammation and atherosclerosis between the two most distinguished physician-scientists of the day—Rudolf Virchow and Carl von Rokitansky. In the 1860s, Virchow described inflammatory changes in atheromatous plaques in arterial walls, and he theorized that inflammation played an initiating role in atherosclerogenesis. This concept was fiercely opposed by his contemporary Rokitansky, who viewed the inflammatory changes as a secondary phenomenon following the process of atherosclerosis that, at least initially, is free of inflammation. The debate between the two continued throughout their professional lives and continues to this day (Mayerl et al. 2006).

In the modern era, Minick was the first to show inflammation—caused by immune reaction—could lead to atherosclerosis. In 1966, he injected rabbits with horse serum over a period of 80 days and compared them to a control group of noninjected rabbits. He found in the study group evidence of immune-mediated inflammation and fatty changes in the wall of the aortas (Minick et al. 1966). These data suggested a link between inflammation and atherosclerosis.

Infection and the resultant inflammatory state have been of significant interest to cardiovascular researchers, and a number of studies, starting in the 1970s, evaluated the link between infections, inflammation, and atherosclerosis. Fabricant and others (1978) described virus-induced atherosclerosis in the 1970s. They studied the effect of avian herpes virus infection in 130 chickens. Half of these birds were inoculated with avian herpes virus at 2 days of age. Next, infected and noninfected birds were further divided into two groups, fed a low- or high-cholesterol diet. All surviving birds were sacrificed at 30 weeks of age and autopsies were performed for grossly visible atherosclerotic changes of the vasculature. Gross atherosclerotic lesions were noted in the large coronary arteries, aortas, and major aortic branches of the two groups of infected birds (fed a normal or a cholesterol-rich diet). Strikingly, no grossly visible lesions were detected in noninfected birds on either a normal or a cholesterol-rich diet. On examination of microscopic lesions of atherosclerosis, the greatest numbers of lesions were quantified in the group of birds who were infected and fed a cholesterol-rich diet, followed by the group of infected birds who were fed a normal diet. The fact that noninfected birds fed on either diet did not have significant atherosclerotic changes suggests the facilitative role of viral infection and resultant inflammation in the pathogenesis of atherosclerosis.

Kiechl and others tested whether the presence of infection facilitated atherosclerogenesis by evaluating the cohort of the Bruneck study. The cohort consisted of all inhibitants of Bruneck, Bolzano, Italy—826 men and women 40 to 79 years old.

The subjects underwent high-resolution duplex scanning to assess the status of carotid atherosclerosis in 1990 and 1995, along with various blood tests. The study found that the markers of inflammation were substantially higher in the presence of chronic infection, such as chronic obstructive pulmonary disease with infection-induced exacerbation, chronic bronchitis, recurrent urinary tract infection, periodontitis, and infections from *Helicobacter pylori* and *Chlamydia pneumoniae*. In addition, the presence of chronic infections was associated with the more rapid progression of carotid atherosclerosis, and the subjects with chronic infections faced a several-fold higher risk of atherosclerosis (Kiechl et al. 2001).

Thom and others studied 461 patients with angiographically confirmed coronary artery disease (CAD) and 95 controls with no demonstrable CAD on angiogram. *C. pneumoniae* immunoglobulin G antibody titers were higher for cases than for controls after standardization for age and gender. The estimated risk of CAD was greater among subjects with high antibody titers than those with low titers. Together, these data suggested that *C. pneumoniae* infection and coexistent inflammation were associated with the presence of CAD (Thom et al. 1991).

After the epidemics of influenza swept across Europe and America in the early 1900s, it was noted that approximately half the deaths were attributed to causes other than influenza, most prominently, heart disease (Collins 1932). The first report of an association of influenza vaccination with reduced risk of subsequent myocardial infarction (MI) was noted in 2000 by Naghavi and others. This was a case control study among all patients with a previous history of MI who were seen in an outpatient cardiology clinic during the influenza season of 1997–98. Patients who experienced a new MI during the season were included in the case group (N = 122). After the case group was studied, a control group was selected by systematic random sampling from those who were seen during the same influenza season for their regular follow-up, who had not developed a new MI or exacerbation of their disease. Influenza vaccination status was documented in both groups of patients. Compared with the case group, the control group reported more influenza vaccination in previous years (79% vs. 66%), as well as more vaccination in the current season (71% vs. 47%). Vaccination against influenza was associated with an average reduction of 67% in the risk of subsequent MI (Naghavi et al. 2000).

Mueller et al. prospectively studied the impact of previous cytomegalovirus (CMV) infection on restenosis after aggressive angioplasty with provisional stenting in 78 patients scheduled for 6-month follow-up coronary angiography. Anti-CMV IgG and IgM antibodies were measured on admission. CMV-antibody-positive and -negative patients had similar minimal lumen diameter (MLD) in the target vessel before and directly after the intervention. However, after 6 months, the lumen diameter was significantly narrower in CMV-positive than in CMV-negative patients. The rate of clinically relevant restenosis was significantly higher (31% vs. 7%), and CMV seropositivity was an independent predictor of restenosis (Mueller et al. 2003). Valiegen and others infected apoE knockout mice (a murine model for human atherosclerosis) with CMV. They found that CMV infection increased plasma inflammatory cytokine levels, and aortic atherosclerotic lesions developed as early as 2 weeks postinfection compared to noninfected mice (Vliegen et al. 2004).

Taken together, these reports suggest that CMV infection and resultant inflammation play a facilitative role in atherosclerogenesis.

Urowitz was the first to report the curious bimodal mortality pattern of systemic lupus erythematosus (SLE), a chronic inflammatory disease—deaths early in the course of SLE were due to active SLE or its complications, while deaths in late SLE were associated with atherosclerotic complications (Urowitz et al. 1976). A similar bimodal pattern of mortality has also been noted in other rheumatic diseases, most significantly in rheumatoid arthritis (RA) (Goodson et al. 2002). Subsequent studies also showed that rheumatoid arthritis (Chung et al. 2005) and lupus (Bruce 2005) were associated with premature atherosclerosis. A case control study by Hannawi and others enrolled 80 patients with RA and 40 patient-matched controls without RA disease. They showed that patients with recent-onset RA, when assessed by high-resolution ultrasound, had significantly larger carotid intima-media thickness and plaque burden than controls, suggesting that chronic inflammation causes accelerated atherosclerosis (Hannawi et al. 2007).

Psoriasis is a helper T cell type 1 inflammatory disorder that leads to scaly erythematous plaques in the skin, and in some patients to psoriatic arthritis. Using the General Practice Research Database (the United Kingdom), Gelfand and others identified patients with severe (N = 3837), mild (N = 127,139), and no (N = 556,995) psoriasis, followed these patients for a mean of 5.4 years, and compared the incidence of MI. Patients with psoriasis had a significantly increased adjusted relative risk for MI (1.54 (1.24–1.91) and 7.08 (3.06–16.36) for mild and severe psoriasis, respectively), especially in young ages (Gelfand et al. 2006). Subsequently, Mehta and others followed 3,603 patients with severe psoriasis and 14,330 controls for an average of 3.4 years in a prospective cohort study. They found that patients with severe psoriasis were at an increased risk of cardiovascular (CV) mortality independent of traditional CV risk factors: hazard ratio (HR) = 1.53 (confidence interval (CI) = 1.26–1.96)) (Mehta et al. 2010).

Other nonrheumatologic inflammatory states, including chronic dental infections and their association with coronary artery disease, have been noted. In the Coronary Event and Periodontal Disease (CORODONT) study, investigators studied the association between periodontitis and coronary heart disease (CHD) in a case control fashion. They hypothesized that increased prevalence of periodontal pathogens and pathogen burden in the sublingual biofilms is increased in patients with coronary disease, compared to controls. They enrolled a total of 789 patients, 263 with angiographically confirmed stable CHD defined as at least one stenosis ≥50% of the luminal diameter of a major coronary artery and 526 age- and sex-matched controls without a history of CHD, and followed them for approximately 3 years (Spahr et al. 2006). They measured total periodontal pathogen burden, number of multiple periodontal pathogens in the subgingival biofilm, and periodontal treatment needs. There was a statistically significant association between periodontal pathogen burden (HR = 1.92 (1.34–2.74)) or the number of *Aggregatibacter actinomycetemcomitans* in periodontal pockets (HR = 2.70 (1.79–4.07)) and the presence of CHD (Spahr et al. 2006). Several population-based studies have similarly shown that patients with inflammatory bowel disease are also at increased risk for atherosclerotic disease (Bernstein et al. 2008).

Several pathohistological and immunological studies have also implied the role of inflammation in atherosclerosis. Atherosclerotic plaques contain a number of cellular and acellular components, many of which are implicated in inflammation—endothelial cells (ECs), macrophages, T lymphocytes, vascular smooth muscle cells, oxidized low-density lipoprotein (ox-LDL), and others (Lusis 2000). Although Stratford and others reported that inflammatory cells, including B and T lymphocytes and macrophages, were present in 46% of coronary arteries with atherosclerotic lesions and a documented history of ischemic heart disease (Stratford et al. 1986), the role of inflammation in the adventitia has not been well characterized.

The ox-LDL activates the overlying ECs, which in turn express a number of molecules that mediate the adhesion and recruitment of inflammatory cells—intercellular adhesion molecule (ICAM), P-selectin, E-selectin, vascular cell adhesion molecule (VCAM-1), and CS-1 splicing variant of fibronectin (Lusis 2000). Hwang and others assessed whether adhesion molecules E-selectin and ICAM-1 were expressed in established atherosclerotic disease. Two hundred four patients with coronary artery disease, 272 patients with carotid artery atherosclerosis, and 316 healthy subjects were enrolled from the large, biracial Atherosclerosis Risk In Communities (ARIC) study. Higher levels of E-selectin and ICAM-1, the recruiting and binding adhesion molecules, were observed in patients with CAD and carotid artery atherosclerosis, compared to controls. The odds of CAD and carotid artery atherosclerosis were 5.53 and 2.64, respectively, for those with ICAM-1 levels in the highest quartile, compared with those in the lowest quartile (Hwang et al. 1997).

Li and others investigated the expression of VCAM-1 in the arterial endothelium of rabbits during the early phase of diet-induced atherogenesis (Li et al. 1993). Fifty rabbits were fed either an atherogenic (0.3% cholesterol, 9% coconut oil, and 1% corn oil) or an isocaloric (10% corn oil) diet for 13 weeks. The ascending aorta endothelium of rabbits on the atherogenic diet for 1 week focally expressed VCAM-1 with serum cholesterol levels of 308 ± 57 mg/dl. Rabbits consuming the atherogenic diet for 3 weeks or longer developed lesions in the intima composed of macrophages bearing class II major histocompatibility antigen (MHC-II). Endothelial cells continued to focally express VCAM-1 at sites of MHC-II-positive intimal macrophages for up to 13 weeks. The ascending aortas of control rabbits fed an isocaloric diet lacked VCAM-1- or MHC-II-positive endothelium or macrophages at all times. These observations demonstrated that focal inflammation of arterial endothelium occurred as early as 7 days after initiation of an atherogenic diet (Li et al. 1993).

Hansson and others examined endarterectomy specimens from atherosclerotic carotid arteries of 16 patients who presented with transient ischemic attacks using immunostaining. Approximately 5% of cells were T cells, one-third of which expressed HLA-DR and VLA-1, markers of activated T cells. In addition, interferon could be stained in and around some of the lymphocytes. Taken together, these data suggested that a substantive portion of T cells are activated in atherosclerotic tissue and may contribute to inflammation through an inflammatory lymphokine (Hansson et al. 1989).

Yla-Herttuala and others examined normal and atherosclerotic human and rabbit arteries and showed that expression of MCP-1 was upregulated in macrophage-rich regions of atherosclerotic plaques (Yla-Herttuala et al. 1991). Using Cynomolgus monkeys fed a hyperlipidemic diet for 6–18 months, Yu and others (1992) demonstrated

that MCP-1 is expressed in both macrophage-like and smooth-muscle-cell-like cells in the intima, as well as vascular smooth muscle cells in the media. Employing double immunohistochemical staining using anti-MCP1 and one of the cell type-specific antibodies, Takeya and others (1993) studied human atherosclerotic tissue and found that the predominant source of MCP-1 is subendothelial macrophages.

Lei and Buja (1996) studied the effect of tumor necrosis factor-alpha (TNF-α), a product of activated monocytes, on atherosclerosis, where they measured TNF-α mRNA levels in the aorta of normal and heritably hyperlipidemic rabbits. TNF-α mRNA levels were significantly higher in hyperlipidemic rabbits than in normal rabbits in the later course of atherosclerosis, suggesting that TNF-α in the atherosclerotic intima may accelerate atherosclerosis.

7.2.2 Does Inflammation Cause Atherosclerosis?

One vital question that needs to be answered—the one that Virchow and Rokitansky argued about more than a century ago—is whether inflammatory cells and markers of inflammation precede the development of atherosclerosis, or if they are just markers and nothing more, innocent bystanders in the progression of atherosclerosis.

Interleukin 6 (IL-6) is an acute phase proinflammatory cytokine elevated in the serum of patients with cardiovascular diseases and has been shown to be associated with poor prognostic outcomes in patients with unstable angina (Biasucci et al. 1996). To investigate whether IL-6 directly promotes fatty lesion development, Huber and others injected ApoE$^{-/-}$ mice with recombinant mouse IL-6 (rIL-6, 5,000 U weekly). They found that rIL-6 treatment of ApoE$^{-/-}$ mice increased the atherosclerosis lesion size 1.9- to 2.4-fold over the vehicle treatment without affecting the lipid profiles of these animals (Huber et al. 1999).

Interleukin-18, an endotoxin-induced serum factor from macrophages that stimulates interferon-γ production, has been found overexpressed in human atherosclerotic lesions, but not normal arteries (Mallat et al. 2001). Whitman and others injected recombinant IL-18 (rIL-18) (30 ng/g/day) or saline to male ApoE$^{-/-}$ mice on a chow-normal diet for 30 days. Although serum lipid concentrations did not differ between the two groups, injection of exogenous IL-18 resulted in an approximately twofold increase in atherosclerotic lesion size in the aortas. Compared with mice injected with saline, rIL-18 injections increased the mean number of lesion-associated T lymphocytes by 4.5-fold (Whitman et al. 2002), suggesting that a proinflammatory cytokine can accelerate atherosclerosis.

Interleukin-2 (IL-2), a cytokine produced by activated T lymphocytes, has been found to further activate the T cells and may potentially enhance atherogenesis. Upadhya and others injected ApoE$^{-/-}$ mice on atherogeneic diet with either saline, recombinant murine IL-2 (rIL-2), or anti-IL-2 antibody twice a week for 6 weeks. Injection of rIL-2, but not saline, increased the atherosclerosis burden of the aortas, while injection of anti-IL-2 antibody had a profound antiatherogenic effect (Upadhya et al. 2004).

Endotoxin causes severe immune and inflammatory reactions *in vivo* (Stoll et al. 2006). Lehr and others injected 1.25–2.5 μg of lipopolysaccharide (LPS), an endotoxin, to female New Zealand White rabbits on a high-cholesterol diet

intravenously every week for 8 weeks and quantified the extent of atherosclerotic changes in their aortas. They found that endotoxin injection, but not saline injection, drastically increased the atherosclerotic lesion area (Lehr et al. 2001). In a similar experiment, Ostos and others injected saline or LPS (50 μg intraperitoneally weekly) to ApoE$^{-/-}$ mice fed a normal chow diet for 10 weeks and found that LPS treatment showed a 83% increase in lesion size compared with the saline-treated controls, despite the fact that total cholesterol and triglyceride levels were significantly lower in the LPS group than in the saline group (Lehr et al. 2001).

These studies reviewed above, when taken together, suggest that inflammation results in the acceleration of atherosclerosis.

7.2.3 Does Atherosclerosis Cause Inflammation?

It is difficult, if not impossible, to test whether atherosclerosis in and of itself causes inflammation when evidence is strong that inflammation does accelerate atherosclerosis, as we discussed in Section 7.2.2. The elevation of inflammatory biomarkers at a certain point in time may mean either the presence of inflammation secondary to atherosclerosis or the presence of developing atherosclerosis secondary to inflammation.

The serum amyloid P (SAP) component is a well-characterized, sensitive marker of inflammation in mice (Pepys et al. 1979). Tennent and others compared the SAP levels between ApoE$^{+/+}$ and ApoE$^{-/-}$ mice fed a normal chow diet at 12, 14, 16, and 18 months. There was no statistically significant difference in SAP levels at all four data points between the two groups, despite the fact that ApoE$^{-/-}$ mice exhibited extensive atherosclerosis in their aortas (Tennent et al. 2008), suggesting that atherosclerosis does not cause systemic inflammation.

Extensive histopathological studies on ApoE$^{-/-}$ mice have been performed showing that ApoE$^{-/-}$ mice exhibit all the phases of human atherosclerosis, including lipid accumulation in the subendothelial matrix (Tamminen et al. 1999), macrophage recruitment via adhesion molecules on ECs (Dong et al. 2000; Patel et al. 1998), and the formation of fatty streak, foam cells, and fibrous plaque (Nakashima et al. 1994). Furthermore, a gene expression analysis revealed that ApoE$^{-/-}$ mice, in comparison with ApoE$^{+/+}$ (wild-type) mice, overexpress a number of inflammatory genes, such as CD18, VCAM1, macrophage inflammatory protein (MIP), CD14, and monocyte chemoattractant protein-1 receptor (MCP-IRA) (Wuttge et al. 2001). Atherosclerosis may directly cause inflammation locally. It is entirely possible that a highly sensitive test to detect local inflammation, such as hs-CRP, is elevated in patients with no systemic inflammation, but with ongoing inflammation due to atherosclerogenesis in vascular walls.

7.2.4 Does the Modulation of Inflammation Halt Atherosclerogenesis?

We discussed that there is a substantive body of evidence showing the involvement of inflammatory cells and cytokines in the process of atherosclerosis and their causative role in atherosclerosis. A next question is: Does the suppression of inflammation halt the progression of atherosclerosis?

Under the low-density lipoprotein receptor (LDLr)-deficient genetic background ($LDLr^{-/-}$), P- and E-selectin double-deficient mice ($P^{-/-}E^{-/-}$) exhibited fatty streaks that were five times smaller than those in the wild-type counterpart ($P^{+/+}E^{+/+}$) (Dong et al. 1998). Under the ApoE-deficient genetic background ($ApoE^{-/-}$), ICAM-1 double-deficient mice ($ICAM1^{-/-}$) had significantly less lesion area than $ICAM1^{+/+}$ mice (Collins et al. 2000). These data suggest that the blockage of adhesion molecule (P- and E-selectin) pathways retards the progression of atherosclerosis.

Using monocyte chemotactic protein-1 (MCP-1) and LDL receptor double-deficient mice ($MCP1^{-/-}LDLr^{-/-}$) and the control ($MCP1^{+/+}LDLr^{-/-}$), Gu and others (1998) showed that $MCP1^{-/-}LDLr^{-/-}$ mice had significantly fewer macrophages in the aortic walls and 83% fewer lipid depositions, suggesting that MCP-1 plays a facilitative role in atherosclerogenesis, and that the inhibition of the MCP-1 pathway halts the progression of atherosclerosis.

In order to test whether proinflammatory cytokine interferon-γ promotes atherosclerogenesis, Gupta and others generated interferon-γ and ApoE double-deficient mice ($IFN\gamma^{-/-}ApoE^{-/-}$) and compared the atherosclerotic lesion size of these animals with that of control mice ($INF\gamma^{+/+}ApoE^{-/-}$). They found that the lack of interferon-γ was associated with a 60% reduction in lesion lipid accumulation, a decrease in lesion cellularity, and a marked increase in potentially atheroprotective phospholipid/apoA-IV-rich particles, suggesting that interferon-γ facilitates atherosclerogenesis through its proinflammatory activities as well as its proatherogenic effect on plasma lipoproteins, and that the pharmacological inhibition of interferon-γ improves the prognosis of atherosclerosis in humans (Gupta et al. 1997).

Emeson and others fed C57BL/6J mice an atherogenic diet for 20 weeks. There were four groups in the study: the first group received anti-CD4 monoclonal antibody (0.25 mg, weekly, intraperitoneally), the second group anti-CD8 monoclonal antibody (0.1 mg), the third group both, and the fourth group saline. CD4 cells normally represent helper T cells and monocyte-macrophages, while CD8 cells represent cytotoxic T cells. Flow cytometric analyses showed that anti-CD4 and anti-CD8 treatment drastically reduced the number of CD4- and CD8-positive cells (down to 1% of the control group). In this system, the mice treated with anti-CD4, but not the mice treated with anti-CD8 or saline, exhibited 70% reduction in the aortic lesions. The treatment with both anti-CD4 and anti-CD8 antibodies did not show any additional benefits than the treatment with anti-CD4 antibody alone. These data suggest that selective depletion of T lymphocytes retards the progression of atherosclerosis (Emeson et al. 1996).

Yamashita and others treated $ApoE^{-/-}$ mice on an atherosclerotic diet with propagermanium, an inhibitor of C-C chemokine receptor 2 (CCR2) function, for 12 weeks and evaluated the extent of atherosclerotic lesion. MCP-1 is a ligand of the CCR2—the interaction between MCP-1 and CCR2 is crucial for monocyte-macrophage migration to the area of inflammation. They found that the atherosclerotic lesion area was reduced by 50% in the propagermanium-treated mice, compared with the control mice. The accumulation of macrophages in the lesion was also markedly reduced in the study group (Yamashita et al. 2002). Meanwhile, Boring and others generated mice lacking CCR2 in the $ApoE^{-/-}$ genetic background and fed them a high-cholesterol diet for 13 weeks. In comparison with $CCR^{+/-}ApoE^{-/-}$ mice, $CCR^{-/-}$ $ApoE^{-/-}$ mice had significantly smaller atherosclerotic lesion areas. There was no

difference in lipid profiles between the two groups (Boring et al. 1998). These data, taken together with Yamashita's study, suggest that suppression of inflammation by blocking macrophage recruitment to the atherosclerotic intima through the disruption of the MCP1-CCR2 interaction decreases the progression of atherosclerosis.

Xiao and others generated TNF-$\alpha^{-/-}$ApoE$^{-/-}$ and TNF-$\alpha^{+/+}$ApoE$^{-/-}$ mice and fed them a normal chow diet for 3 weeks. While there was no difference in lipid profiles in these groups, TNF-$\alpha^{-/-}$ApoE$^{-/-}$ mice had significantly smaller early fatty-streak lesions than did TNF-$\alpha^{+/+}$ApoE$^{-/-}$ mice. Transcription levels of proatherogenic cytokines, including IL-1, IL-6, and adhesion molecules, were downregulated in TNF-d$^{-/-}$ApoE$^{-/-}$ mice. TNF-e deficiency seems to retard early fatty-streak lesions by influencing the expression of inflammatory markers (Xiao et al. 2009).

Osteopetrotic (op) mice are mice with a frame-shift mutation in the macrophage colony-stimulating factor (M-CSF) gene, leading to the complete absence of M-CSF and the dramatically decreased number of total leukocytes, lymphocytes, monocytes, and peritoneal macrophages. Qiao and others generated Op$^{-/-}$ApoE$^{-/-}$ and Op$^{+/+}$ApoE$^{-/-}$ mice and fed them an atherogenic diet for 15 weeks. Total cholesterol levels were significantly higher in Op$^{-/-}$ApoE$^{-/-}$ mice than in Op$^{+/+}$ApoE$^{-/-}$ mice (1,100 vs. 388 mg/dl). However, the atherosclerotic lesion area was dramatically less (1/6) in Op$^{-/-}$ApoE$^{-/-}$ mice than in Op$^{+/+}$ApoE$^{-/-}$ mice, suggesting the lack of inflammatory cells halts the progression of atherosclerosis despite the fact that Op$^{-/-}$ApoE$^{-/-}$ mice had more severe hyperlipidemia than did Op$^{+/+}$ApoE$^{-/-}$ mice (Qiao et al. 1997).

Chronic infection is always accompanied by chronic inflammation that may contribute to atherosclerogenesis. Muhlestein and others maintained three groups of New Zealand White rabbits on an atherogenic diet for 12 weeks and evaluated their aortas for the extent of atherosclerotic lesions: (1) rabbits infected with *Chlamydia pneumoniae* but treated with azithromycin, (2) rabbits infected with *C. pneumoniae* but not treated, and (3) rabbits not infected with *C. pneumoniae*. They found that treatment of *C. pneumoniae*-infected rabbits with azithromycin decreased the atherosclerotic lesion area to that of the noninfected animals, suggesting that eradication of active *C. pneumoniae* infection by antibiotics treatment decreased the progression of atherosclerosis (Muhlestein et al. 1998). Encouraged by the animal studies by Muhlestein and others, the WIZARD (Weekly Intervention with Zithromax for Atherosclerosis and Its Related Disorders) study investigators randomized patients with previous myocardial infarction (>6 weeks) and a *C. pneumoniae* IgG titer > 1:16 (N = 7747) to a 3-month course of either azithromycin or placebo and followed them for 48 months. There were no significant risk reductions for any of the components of the primary endpoint, including death, recurrent MI, revascularization procedures, or hospitalization for angina. It was possible that the negative results were due to either (1) the insufficient duration of azithromycin treatment or (2) the failure to detect real *C. pneumoniae* infection by the IgG titer (O'Connor et al. 2003). In the ACES (Azithromycin and Coronary Events Study), patients with stable coronary artery disease (N = 4,012) were randomized to receive either azithromycin or placebo weekly for 1 year. The median length of observation was ~3.9 years. There was no significant risk reduction in the azithromycin group compared with the placebo group with regard to the primary endpoint, consisting of death due to CAD, nonfatal MI, coronary revascularization, or hospitalization for unstable angina

(Grayston et al. 2005). The titers of inflammation-related biomarkers were not determined in either study. These data suggest that the eradication of *C. pneumoniae* or azithromycin-responsive infection, in either the short or long term, does not improve the outcome of coronary artery disease.

The association of influenza with coronary heart disease has been well documented, and clinical studies were designed to test the hypothesis that influenza vaccination might be an important defense against atherosclerotic disease. The FLU Vaccination in Acute Coronary Syndromes (FLUVACS) was a randomized controlled trial in which 301 patients hospitalized for either myocardial infarction or angioplasty were randomly assigned to receive influenza vaccination or remain unvaccinated. At 6 months, the primary outcome of cardiovascular death occurred in 2% of the patients in the vaccine group versus 8% in controls. The triple composite endpoint rates (a composite of cardiovascular death, nonfatal myocardial infarction, or severe ischemia) occurred in 11% of the patients in the vaccine group vs. 23% in unvaccinated group. The incidence of cardiovascular death at 1 year was lower in the vaccination group than with controls (6 vs. 17%, $p = 0.002$). The incidence of the composite triple endpoint at 1 year was also statistically significantly lower in the vaccination arm than with controls (22 vs. 37%). At 12 months after randomization, the need for coronary revascularization was significantly less frequent among patients assigned to vaccine (5%) than among those assigned to control (9%) (Gurfinkel et al. 2004). It is possible that the prevention of influenza infection led to the freedom from actual influenza infection, inflammation, and atherosclerosis progression. The American Heart Association and American College of Cardiology now recommend influenza immunization with inactivated vaccine as part of the comprehensive secondary prevention in persons with coronary and other atherosclerotic disease (class I, level B indication) (Davis et al. 2006).

C-reactive protein (CRP) is an acute phase protein, a member of the pentraxins family, and synthesized exclusively in the liver, and its circulating concentration rises rapidly and extensively in a cytokine-mediated response to tissue injury, infection, and inflammation. Several large epidemiological studies have shown that elevated levels of CRP were predictors of future cardiovascular events (Ridker et al. 1998, 1999, 2000). Taking this a step further, Ridker and others hypothesized that statin treatment is beneficial in patients with elevated hs-CRP and without hyperlipidemia. In the JUPITER trial, they randomly assigned apparently healthy men and women with LDL < 130 mg/dl and hs-CRP > 2 mg/L to either 20 mg of rosuvastatin daily or placebo and followed them for 1.9 years (N = 17,802). Patients were excluded from the study if they had evidence of cardiovascular disease, hepatic or renal dysfunction, diabetes, uncontrolled hypertension, or inflammatory conditions. Rosuvastatin treatment was associated with a drastic reduction in LDL cholesterol levels (rosuvastatin vs. placebo = 55 vs. 109 mg/dl) and with a decrease in hs-CRP levels (1.8 vs. 3.3 mg/L). The trial was stopped at 1.9 years by its independent data monitoring board, due to 44% reduction of all vascular events, a 54% reduction in myocardial infarction, a 48% reduction in stroke, a 46% reduction in the need for arterial revascularization, and a 20% reduction in all-cause mortality (Ridker et al. 2008), suggesting that rosuvastatin was capable of reducing both LDL and hs-CRP levels and of improving cardiovascular outcomes of apparently healthy persons

without severe hyperlipidemia but with elevated hs-CRP. However, the trial did not evaluate the group of patients without hyperlipidemia or elevated hs-CRP. Would rosuvastatin have been beneficial to these patients? Would the degree of the reduction of cardiovascular events conferred by rosuvastatin have been equal between the group of patients without hyperlipidemia or elevated hs-CRP and the group of patients without hyperlipidemia but with elevated hs-CRP? In order to speculate on these questions, we will discuss several basic and epidemiological research studies recently performed on human CRP (hCRP).

Hirshfield and others generated $ApoE^{-/-}$ mice with transgene-mediated human CRP overexpression ($ApoE^{-/-}hCRP^{+}$). The circulating hCRP levels in $ApoE^{-/-}hCRP^{+}$ mice were markedly elevated. They kept both $ApoE^{-/-}hCRP^{+}$ (hCRP concentration = 27.2 mg/L) and $ApoE^{-/-}hCRP^{-}$ (hCRP = not detectable) mice on a normal chow diet for 56 weeks for the quantification of atherosclerotic lesions. There were no differences in weight, plasma cholesterol or triglyceride concentration, or survival rate between the two groups. There was also no difference in aortic atherosclerotic plaque size between the two groups (Hirschfield et al. 2005).

In order to further study the role of CRP in atherosclerogenesis, Koike and others generated a line of transgenic rabbits (line 2) expressing a drastically high (57.8 mg/L, N = 12) hCRP and compared the atherosclerotic phenotype of the animals with that of nontransgenic control rabbits (hCRP = not detectable). They fed these rabbits a high-fat diet for 16 weeks, at which time plasma cholesterol levels were equally elevated at ~1,000 mg/dl. Expression of hCRP in transgenic rabbits did not lead to an increase in atherosclerotic plaque size, suggesting that hCRP does not have any atherosclerogenic activities (Koike et al. 2009).

Finally, Ortiz and others treated high-fat-diet-fed $ApoE^{-/-}$ mice with either azide- and endotoxin-free hCRP or vehicle continuously via osmotic pumps for 4 weeks. hCRP-treated and control mice developed the same size of atherosclerotic lesions in their aortas. No differences were observed in macrophage or T lymphocyte infiltrates, and there were no changes in circulating levels of proinflammatory cytokines, such as IL-6 or TNF-α (Ortiz et al. 2009).

Taken together, these three studies strongly suggest that hCRP in and of itself has no atherosclerogenic effects. The previous studies (Labarrere and Zaloga 2004) showing the facilitative role of hCRP in atherosclerogenesis are now considered by many to be plagued by the contamination of hCRP by azide or lipopolysaccharides (LPSs) (Taylor et al. 2005).

That hCRP possesses no atherosclergenic activities is further supported by the recent study by Zacho and others, who showed that certain hCRP polymorphisms that result in markedly increased hCRP levels are not associated with an increased risk of ischemic vascular disease (Zacho et al. 2008). Meanwhile, Elliott and others showed that five genetic loci whose polymorphisms were strongly associated with CRP levels had no correlation with coronary artery disease, further suggesting that there is no causal association between hCRP and coronary heart disease (Elliott et al. 2009).

In summary, these data suggest that hCRP is simply a marker of systemic (and possibly local—when highly sensitive assays are employed) inflammation without any atherosclerogenic biological activity.

Based on the studies discussed above, it is possible that the hs-CRP levels are the function of both hCRP polymorphisms and the degree of "inflammation burden" from either ongoing atherosclerosis or other conditions.

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2)—expressed abundantly in the necrotic core of coronary lesions—is produced by macrophages and binds to various lipoproteins. It catalyzes the hydrolysis of phospholipids and induces inflammation and cell death, potentially rendering plaque vulnerable to rupture (Zalewski et al. 2005). The Lp-PLA_2 level has been shown to be an independent predictor of CAD (Packard et al. 2000; Koenig et al. 2006). Mohler and others showed that darapladib is a potent Lp-PLA_2 inhibitor, decreasing Lp-PLA_2 activity in humans up to 66% (160 mg once daily). Darapladib also significantly reduced serum IL-6 levels by 12.3% (Mohler et al. 2008). The Integrated Biomarkers and Imaging Study-2 trial randomized 330 patients with angiographically documented coronary heart disease to receive either 160 mg darapladib or placebo once daily and followed the patients for 12 months. All patients received angiography and intravascular ultrasound (IVUS) to characterize the composition of atheroma both at the time of randomization and after 12 months. While darapladib treatment decreased Lp-PLA_2 activity by 59%, hs-CRP levels were not affected by the treatment. At 12 months, total atheroma volume was identical between placebo and treatment groups. However, the necrotic core volume was significantly greater in the placebo group than in the treatment group, suggesting that darapladib treatment halted necrotic core expansion, a key determinant of plaque vulnerability (Serruys et al. 2008).

Interleukin-1 (IL-1) is an inflammatory cytokine involved in the atherosclerotic process. The MRC-ILA-HEART study will randomize patients with non-ST-segment elevation myocardial infarction (NSTEMI) to receive either anakinra (IL-1 receptor antagonist, currently used to treat rheumatoid arthritis and Still's disease) or placebo for 14 days. The primary outcome measure is the area under the curve of serum hs-CRP over the first 7 days. It will test the hypothesis that the inhibition of IL-1 by anakinra will decrease hs-CRP levels (thus inflammation) in patients with NSTEMI. The study has been recently completed, and the results will be reported in the near future (Crossman et al. 2008).

One of the most definitive ways to evaluate the role of inflammation in atherosclerogenesis is to evaluate whether an anti-inflammatory agent with no effects on lipid profiles (unlike statins) or on thrombogenicity (unlike cyclooxygenase II inhibitors) would improve the prognosis of atherosclerosis. Two approaches are possible: (1) to attack a single inflammatory pathway that is most crucial to atherosclerogenesis and (2) to attack as many inflammatory pathways as possible using a global anti-inflammatory agent such as methotrexate. Ridker and others are using very low-dose methotrexate (VLDM; 10 mg orally weekly) in the cardiovascular inflammation reduction trial (CIRT), randomizing 7,000 stable coronary artery disease patients with persistent elevations of hs-CRP to placebo or VLDM, and will follow them for 3–4 years. VDLM has been extensively used in and well tolerated by patients with rheumatoid arthritis and psoriasis. It is shown to reduce TNF-α, IL-6, and hs-CRP levels without affecting LDL or HDL levels. If successful, CIRT would both confirm the inflammatory hypothesis of atherosclerosis and open novel approaches to the treatment and prevention of cardiovascular disorders (Ridker 2009).

As we reflect on the impassioned discussion between Rudolf Virchow (a proponent of inflammation as the initiator of atherosclerosis) and Carl von Rokitansky (a proponent of inflammation as the secondary response mechanism to injury) (Mayerl et al. 2006), it appears fair to say today that both were equally right—the process of atherosclerosis begins when LDL particles accumulate in the intima (Tamminen et al. 1999). Within minutes, these LDL particles are oxidized to form ox-LDL (Goldstein et al. 1979), a potent chemoattractant to macrophages (Gimbrone 1999), and these macrophages in turn produce a number of inflammatory cytokines and take up ox-LDL to form foam cells. While LDL diffusion into the intima in and of itself does not represent inflammation, LDL is immediately oxidized in the intima to serve as a chemoattractant—the initiation of inflammation (Lusis 2000). However, the final verdict may not be in yet—further research may well reveal something that is completely unexpected and drastic, as it has always done. Truly, as Hippocrates said. "Art is long, life is short [*Ars longa, vita brevis*]."

7.3 INFLAMMATION AND HEART FAILURE

7.3.1 Association of Inflammation and Heart Failure

Almost 2,500 years ago, Hippocrates and his Greek physician colleagues described the symptoms and signs of heart failure (HF) in a collection of books called the Hippocratic Collection. They discovered that signs of advanced HF—such as anasarca and swelling—were often associated with wasting and cachexia, signs of inflammation. In the *Sacred Disease XI*—one of the books of the Hippocratic Collection—they wrote, "The flesh is consumed and becomes water … as a result of the impurities, the feet and legs swell and the shoulders, clavicles, chest and thigh melt away" (Katz and Katz 1962).

Although the association of advanced HF and profound anorexia and wasting had been noted since the time of Hippocrates, the cause of cachexia seen in end-stage HF had been unclear. In 1990, Levine and others noted that cachexia seen in end-stage HF was similar to that in cancers, chronic infections, and connective tissue diseases. Around that time, it was shown that the long-term administration of TNF-α—an inflammatory cytokine produced by macrophages and others—induced cachexia seen in the chronic illnesses described above (Tracey et al. 1988). In addition, elevated levels of TNF-α were reported in various neoplastic (Balkwill et al. 1987) and chronic inflammatory states (Maury and Teppo 1989). Based on these findings, Levine and others investigated the role of TNF in the pathogenesis of cardiac cachexia by measuring serum levels of TNF-α in 33 patients with chronic HF and in age-matched healthy controls. Patients with HF had a significantly higher mean serum levels of TNF-α than healthy controls (115 vs. 9 U/ml, $p < 0.001$). In addition, the HF patients with higher levels of TNF were more cachectic and had more advanced HF. This was the first reported association of inflammation and HF (Levine et al. 1990). Subsequently, TNF-α has been shown to be an independent predictor of mortality in patients with advanced HF (Deswal et al. 2001), and TNF-α levels have been shown to be elevated not only in patients with severe HF, but also in community patients with HF with both preserved and reduced ejection fraction (EF) (Dunlay et al. 2008).

At around the same time that the association between TNF-α and HF was first established, CRP levels were noted to be elevated in patients with HF (Pye et al. 1990). Pye and others prospectively assessed the serum concentration of CRP in 37 patients with various degrees of HF and found that CRP levels were abnormally elevated in 70% of the HF patients (Pye et al. 1990). Like TNF-α, elevated levels of CRP have been found to be associated with the advanced HF classes (Pye et al. 1990), more frequent hospitalizations (Alonso-Martínez et al. 2002), and increased mortality (Kaneko et al. 1999).

Munger and others first reported that plasma IL-6 levels were significantly elevated in patients with moderate to severe HF (New York Heart Association [NYHA] class II to IV) (N = 75) (Munger et al. 1996; MacGowan et al. 1997). IL-6 levels correlated with the severity of left ventricular (LV) dysfunction (Raymond et al. 2001). In addition, high plasma IL-6 levels provided prognostic information in patients with CHF, independent of LV ejection fraction (Tsutamoto et al. 1998), suggesting the presence of a link between IL-6, an inflammatory cytokine, and HF.

Vasan and others followed 732 elderly patients without previous myocardial infarction or HF, a subgroup of Framingham study subjects, for 5.2 years. At the beginning of the follow-up, the levels of IL-6, CRP, and TNF-α, and peripheral blood mononuclear cell (PBMC) counts, were determined. Subjects with elevated IL-6 levels, PBMC counts, TNF-α levels greater than the median values, and CRP ≥5 mg/dl had a 4.1-fold risk of developing HF over the follow-up period, suggesting that inflammation can precede and, possibly lead to, HF (Vasan et al. 2003).

7.3.2 Does Inflammation Cause Heart Failure?

It is clearly established that the inflammation of myocardium manifesting itself in an inflammatory cellular infiltrate with or without associated myocyte necrosis—myocarditis—can lead to HF (Cooper 2009). Myocarditis results most commonly from viral and other infections, such as HIV, coxsackievirus B, parvovirus B19, hepatitis C virus, Epstein-Barr virus, cytomegalovirus, human herpesvirus 6, *Borrelia burgdorferi* (Lyme disease), and *Trypanosoma cruzi* (Chagas disease) (Cooper 2009). However, it can result less commonly from toxic or hypersensitivity drug reactions, giant-cell myocarditis, or sarcoidosis (Cooper 2009). We will discuss below whether systemic inflammation results in the progression of HF.

Several studies now show that acute and chronic administration or overexpression of proinflammatory cytokines can lead to the progression of HF. Eichenholz and others (1992) injected a single dose of TNF-α in dogs and found that TNF-α injection led to LV cavity dilatation and a decrease in LV systolic function. Bozkurt and others continuously infused TNF-α or vehicle into the peritoneal cavity of rats using an osmotic infusion pump and found that the continuous infusion of TNF-α, but not vehicle, led to severe LV dysfunction, manifesting itself in a 15–20% decrease in LV fractional shortening and an increase in LV end-diastolic volume. At a cellular level, cardiomyocytes isolated from TNF-α-treated rats exhibited a significant 25–35% decrease in the extent of cell shortening, compared to cardiomyocytes from vehicle-treated rats. Intriguingly, the negative inotropic effects of TNF-α were fully

reversible when its infusion was terminated. Furthermore, the deleterious effects of TNF-α on LV function were completely reversed by TNFR:Fc, a specific TNF-α antagonist (Bozkurt et al. 1998). These data suggest that circulating TNF-α, at the levels seen in HF patients, is capable of inducing reversible and profound LV dysfunction in experimental animals.

In order to evaluate the role of TNF-α in LV function, Kubota and others generated a strain of mice where TNF-α was selectively overexpressed in the heart. All transgenic mice, expressing a large amount of TNF-α in the heart, died before weaning. Histological examination of the heart showed a substantial, diffuse lymphohistiocytic inflammatory infiltrate, associated with interstitial edema, suggesting that higher-than-physiological levels of TNF-α induced fulminant myocarditis (Kubota et al. 1997a). The same group of investigators then generated another line of transgenic mice, expressing a lesser amount of TNF-α in the heart. TNF-α levels of the heart (both atria and ventricles) were several-fold higher in the transgenic mice than in control mice. The LV of transgenic mice was dilated with significantly increased LV end-diastolic and end-systolic volumes as determined by cardiac MRI. LV ejection fractions of transgenic and wild-type mice were 51 and 71%, respectively. Myocardium of the transgenic mice revealed interstitial cellular infiltrates and fibrosis. The mortality of these transgenic mice was calculated at 23% over the 6-month follow-up period (Kubota et al. 1997b). These data suggest that the heart-specific overexpression of TNF-α can result in the phenotype typical for myocarditis and dilated cardiomyopathy.

In order to evaluate the role of endothelian-1 (ET-1) in the progression of HF, Yang and others generated transgenic mice exhibiting conditional cardiac-restricted ET-1 overexpression, using the α-myosin heavy-chain (α-MHC) promoter-dependent cardiac-specific tetracycline-regulated (tet-off) gene expression system (ET^+/tTA^+). The tetracycline-regulated ET-1 gene expression was necessary to avoid the lethality of transgenic embryos constitutionally overexpressing ET-1. Total ET-1 expression of ET^+/tTA^+ mice increased ~10 times when the administration of doxycycline was stopped. Over 50% of the ET^+/tTA^+ mice released from doxycycline were dead within 8 weeks due to severe HF, where the heart was dilated and myocardium showed extensive inflammatory cell infiltration and overexpression of inflammatory cytokines, including IL-1, IL-6, TNF-α, and INF-γ. These data suggest that ET-1-induced inflammation, manifesting itself in upregulation of inflammatory cytokines, contributed to the development of severe HF in this model (Yang et al. 2004).

7.3.3 Does Heart Failure Cause Inflammation?

Kapadia and others used an *ex vivo* Langendorff perfusion system to test the effect of pressure overload on myocardial TNF-α production. When the hearts excised from adult cats were perfused at a constant and normal pressure of 80 mmHg, there was no detectable TNF-α gene expression. However, when the hearts were perfused at 200 mmHg of pressure, TNF-α gene expression nearly doubled as assessed by the Northern blot analysis. In addition, they placed an elastic band on the ascending aorta of cats for 30 min to generate a mean gradient of ~45 mmHg and a pressure overload condition *in vivo*. They found a robust elevation of

TNF-α mRNA levels in pressure-overloaded animals, but not in sham-operated animals (Kapadia et al. 1997). In a similar study, Palmieri and others subjected an excised rat heart on the Langendorff perfusion system to different end-diastolic pressures—5 mmHg (unstretched control myocardium), 15 mmHg (moderate stretch), and 35 mmHg (severe stretch)—and evaluated the status of IL-6 and TNF-α expression. Moderate and severe mechanical stretch resulted in a modest and a large increase in IL-6 and TNF-α expression, respectively, at both message and protein levels, as determined by Northern and Western blot analyses (Palmieri et al. 2002). These data suggest that simple mechanical stress on the heart can elicit the production of inflammatory cytokines.

7.3.4 Does the Modulation of Inflammation Halt the Progression of Heart Failure?

In the Myocarditis Treatment Trial, Mason and others randomized 111 patients with a histopathological diagnosis of myocarditis and LVEF < 45% to receive conventional therapy alone or combined with a 24-week regimen of immunosuppressive therapy (prednisone with either cyclosporine or azathioprine) and evaluated a change in the LVEF at 28 weeks. There was no difference among the two groups in the mean LVEF at baseline, week 28, or week 52, and LVEF in both groups equally improved during the course of the trial. There was no significant difference in survival between the two groups, suggesting that the routine treatment of myocarditis with immunosuppressive drugs does not improve clinical outcome of these patients. Intriguingly, clinical manifestations of disease at the time of enrollment in the trial were less severe in patients with a more robust inflammatory response—such as higher levels of cardiac IgG antibodies, general IgG antibodies, anti-skeletal muscle IgG antibodies, white cell count, natural killer cells, and macrophages—suggesting that a prominent immunological and resultant inflammatory response may be a benefit rather than a principal cause of the disease (Mason et al. 1995). Thus, in the Myocarditis Treatment Trial, the suppression of inflammation by immunosuppressive therapy did not halt the progression of HF.

As we discussed above, it was shown that the negative effects of TNF-α on LV function were completely reversed by TNFR:Fc (etanercept), a specific TNF-α antagonist (Bozkurt et al. 1998). More specifically, TNFR:Fc is a chimeric fusion protein consisting of the extracellular domain of the human type 2 TNF receptor (p53) fused in duplicate to the Fc portion of the human IgG1 molecule (MW = 150 kDa) (Mohler et al. 1993). Attempts were made to translate these promising findings to humans through multiple well-designed, randomized, placebo-controlled clinical trials utilizing anti-TNF-α therapies. RENAISSANCE and RECOVER tested two different doses of etanercept (Mann et al. 2004), whereas the ATTACH trial used infliximab (Chung et al. 2003). Infliximab is a humanized monoclonal antibody against TNF-α. Both etanercept and infliximab are potent inhibitors of the TNF-α pathway.

RENAISSANCE and RECOVER enrolled patients with NYHA class II to IV chronic HF and a left ventricular ejection fraction of ≤30%. These two clinical trials differed only in the doses of etanercept used. In RECOVER, patients received

placebo or subcutaneous etanercept in doses of 25 mg every week or 25 mg twice per week, and a total of 1,123 patients were enrolled. In RENAISSANCE, patients received placebo, 25 mg of etanercept twice per week, or 25 mg of etanercept three times per week, and a total of 925 patients were enrolled. The primary endpoint in RENAISSANCE and RECOVER was a change in clinical status from baseline to 24 weeks. This endpoint was based on a composite score, wherein patients were considered improved, worsened, or unchanged on the basis of death, CHF hospitalization, NYHA class, and patient global assessment. On the basis of prespecified stopping rules, both trials were terminated prematurely owing to lack of benefit. The median time from randomization to the last visit was 5.7 months in RECOVER and 12.9 months in RENAISSANCE. At the time the studies were closed, 37 and 73% of the patients in RECOVER and RENAISSANCE, respectively, had completed the 24-week evaluation. Etanercept had no significant effect on clinical status in RENAISSANCE or RECOVER. The authors concluded that these results were sufficiently unfavorable as to rule out a clinically relevant benefit of targeted anticytokine therapy with the soluble TNF-α antagonist etanercept on the rate of death of HF hospitalization (Mann et al. 2004).

In the ATTACH trial, 150 patients with stable NYHA class III or IV HF and LV EF ≤35% were randomly assigned to receive placebo (N = 49), 5 mg/kg infliximab (N = 50), or 10 mg/kg infliximab (N = 51) at 0, 2, and 6 weeks after randomization and were followed up prospectively for 28 weeks. The primary endpoint of the study was the change in clinical status at 14 weeks. Clinical status was assessed by the clinical composite score, which categorized each patient as improved, worse, or unchanged using prespecified criteria. Neither dose of infliximab significantly improved clinical status at 14 weeks. In response to the infliximab treatment, serum levels of CRP and IL-6 showed a robust reduction within the first week, followed by a modest reduction up to 14 weeks, gradually returning toward baseline levels by 24 weeks. After 28 weeks, 13, 10, and 20 patients were hospitalized for any reason in the placebo, 5 mg/kg infliximab, and 10 mg/kg infliximab groups, respectively. The combined risk of death from any cause or hospitalization for HF through 28 weeks was increased in the patients randomized to 10 mg/kg infliximab (Chung et al. 2003).

Several speculations have been made to explain the tendency for TNF-α antagonism to adversely affect the outcomes of patients with HF. One possibility proposed by Mann and others (2004) is that proinflammatory cytokines do not play a deleterious role in the progression of HF. A second possibility is that the doses of etanercept and infliximab used in the trials were not sufficient to completely block the TNF-α signaling pathway (Mann et al. 2004). A third possibility is that the blockage of TNF-α pathway alone was not sufficient in the presence of many other inflammatory cytokines (such as IL-6 and IL-1β) shown to be activated in patients with HF (Mann et al. 2004). A fourth possibility is that soluble TNF-α by complexing with circulating etanercept, may remain in the circulation longer, prolonging the exposure of cardiac tissue to TNF-α leading to cardiomyocyte toxicity (Chung et al. 2003). A fifth possibility is that infliximab, by binding to and oligomerizing TNF-α attached to the surface of cardiomyocytes (i.e., transmembrane TNF-α may induce pardoxical apoptosis in these cells (Chung et al. 2003). A sixth possibility is that low physiological levels of TNF-α may be cytoprotective under

stress, and the complete neutralization of TNF-α by either infliximab or etanercept may lead to the failure of cardiomyocytes to adapt and survive under stress (Chung et al. 2003). Nevertheless, the RECOVER, RENAISSANCE, and ATTACH trials failed to show that the use of etanercept or infliximab as an anticytokine therapy targeting TNF-α and its pathway is clinically beneficial.

In contrast to the highly selective intervention on a certain inflammation pathway used in the clinical trials above (Chung et al. 2003; Mann et al. 2004), there have been different approaches using less selective intervention to simultaneously block multiple inflammatory pathways. Circulating autoantibodies of the IgG class against various cardiac antigens have been identified and are thought to play a causative role in the pathogenesis and progression of dilated cardiomyopathy. Müller and others performed extracorporeal immunoglobulin adsorption on 17 patients with autoantibodies against β1-adrenoceptors (β1-AABs) using adsorption columns that contained polyclonal anti-human immunoglobulin antibodies produced in sheep. Immunoadsorption was performed on 5 consecutive days with a goal of reducing total IgG to 120 mg/dl and eliminating β1-AABs. β1-AABs have been shown to induce dilated cardiomyopathy in rats (Jahns et al. 2004). The matched control group consisted of 17 patients who received maximum medical therapy. Within 1 year, the immunoadsorption group, but not the control group, showed a significant increase in LVEF (from 22% to 38%), a significant decrease in LV end-diastolic diameter (from 74.5 mm to 63.7 mm), and a significant improvement in NYHA class (Müller et al. 2000).

Staudt and others treated patients with severe HF (LVEF < 30%) with protein-A immunoadsorption (high affinity to IgG1, 2, and 4; low affinity to IgG3; N = 9) or anti-IgG immunoadsorption (high affinity to all IgG subclasses; N = 9) and found that anti-IgG immunoadsorption, but not protein-A immunoadsorption, significantly improved cardiac index and LVEF (Staudt et al. 2002), suggesting that autoantibodies belonging to the IgG3 subclass can play a facilitative role in the progression of HF. Immunoadsorption therapy is a new and promising treatment option for patients with dilated cardiomyopathy and HF, but further investigations and larger studies are needed to elucidate the mechanism of action of the particular therapy.

Felix and others performed immunoadsorption on patients with dilated cardiomyopathy (N = 11, LVEF < 30%, cardiac index < 2.5 L/min/m^2) for 3 consecutive days. Immunoadsorption was associated with a reduction of IgG plasma levels (10.7 to 2.4 g/L, $p < 0.01$) and an increase in cardiac index (2.2 to 2.7 L/min/m^2, $p < 0.01$). The eluents from the immunoadsorption columns of patients with dilated cardiomyopathy, but not healthy volunteers, inhibited rat cardiomyocyte contraction *in vitro*. The eluents were found to be mainly antibodies that were capable of interacting with various myocardial proteins (Felix et al. 2002).

These data suggest that immunoadsorption therapy removes circulating IgGs that bind to myocardial proteins and exert deleterious effects on the LV function, and that it represents a promising new therapy that can immediately improve the cardiac function of HF patients. However, there are several issues associated with the immunoadsorption strategy. First, the immunoadsorption procedure removes IgGs from circulation, not inflammatory cytokines (such as TNF-α, IL-1β, IL-6) (Felix et al. 2002). Second, more basic science research is needed to evaluate the role of individual

circulating factors—both antibodies and cytokines—in the progression of HF. This may involve the characterization of the eluents (mainly IgGs) from the immunoadsorption column using molecular approaches. Third, patients with active myocarditis should be excluded based on the observation in the Myocarditis Treatment Trial that the patients with acute myocarditis and high IgG titers had milder clinical manifestations (Mason et al. 1995). Fourth, a larger-size, randomized clinical trial is needed to definitively assess the clinical benefit of the immunoadsorption therapy in HF.

Our interpretation and understanding of the dynamics of HF has, over the decades, shifted from perceiving it as a local and static condition originating from the imbalance between the pump function of the heart and the amount of fluid it is forced to handle, to what is now essentially recognized as a systemic and dynamic condition of inflammation—both local (myocardium) and systemic—initiating and contributing to the progression of HF. Though the earlier clinical trials to suppress inflammation through TNF-α antagonism and immune modulation have been disappointing, new anti-inflammatory strategies have already shown great promise and may evolve into a mainstay in HF therapeutics as more basic scientific research elucidates the exact mechanism by which these new approaches benefit HF patients.

7.4 EPILOGUE

We have reviewed the relationship between inflammation and heart diseases (atherosclerosis and HF). We find that there is a large body of evidence supporting the association between atherosclerosis and inflammation and between HF and inflammation. Evidence is also strong that inflammation (either local or systemic) would lead to the progression of atherosclerosis and HF. The suppression of inflammation targeting a certain inflammatory pathway would retard the progression of atherosclerosis, while it is still not clear whether such targeting strategies would benefit HF patients. The question as to whether atherosclerosis or HF in and of itself can cause systemic inflammation is difficult to answer, although data exist to show that the LDL diffusion and oxidation to the intimal space—the very early step of atherosclerosis—definitely induce inflammation, and that mechanical LV stress locally induces inflammatory cytokines such as TNF-α and IL-6 in the heart. Extensive basic and clinical research to further address the role of inflammation in atherosclerosis and HF will certainly lead to exciting and unexpected strategies to favorably modify the clinical outcome of patients with atherosclerosis and heart failure.

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8 Wound Inflammation
From Initiation to Resolution

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CONTENTS

8.1 CHRONIC WOUNDS

Chronic wounds represent a silent epidemic that affects a large fraction of the world population and poses a major and gathering threat to public health and the economy (Sen et al. 2009). In the United States alone, chronic wounds affect 6.5 million patients (Crovetti et al. 2004; Singer and Clark 1999). The immense economic and social impact of wounds in our society calls for enhancing our understanding of the biological mechanisms underlying cutaneous wound complications (Sen et al. 2009). Chronic wounds fail to progress through the normal phases of healing, and therefore enter a state of prolonged pathologic inflammation (Menke et al. 2007).

8.2 PHASES OF WOUND HEALING

Wound healing represents the outcome of a large number of interrelated biological events that are orchestrated over a temporal sequence in response to injury and its microenvironment (Falanga 2004; Martin 1997; Singer and Clark 1999). Wounds can be clinically classified into two categories based on the duration to closure. Acute wounds are those that progress through the process of healing in a stepwise manner and achieve closure within days. Chronic wounds are those that are open for over 4 weeks because they have been derailed from the physiological healing cascade. These wounds are frequently characterized by persistent inflammation that complicates restoration of the barrier function of the skin (Menke et al. 2007; Schreml et al. 2010). The process of wound healing is well regulated and, for ease of understanding, is divided into specific functional phases: hemostasis, inflammation, proliferation, and remodeling (Figure 8.1). All these phases of healing take place in an overlapping series of programmed events to promptly reestablish barrier function of the skin.

8.2.1 Hemostasis

Following injury, hemostasis begins with the formation of fibrin plug. This process also lays the foundation for subsequent inflammation and healing processes (Broughton et al. 2006b). The fibrin plug and the surrounding wound tissue release pro-inflammatory cytokines and growth factors, such as transforming growth factor (TGF-β), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF). Fibrin also provides the structural support for the cellular constituents of inflammation.

8.2.2 Inflammation

The initial phase after cutaneous injury is dominated by inflammatory reactions mediated by cytokines, chemokines, growth factors, and their actions through cellular receptors (Schreml et al. 2010). The inflammatory phase begins with the organized influx of specific populations of leukocytes to the site of injury. This response is self-limiting and resolves through the release of endogenous anti-inflammatory mediators and clearance of inflammatory cells. Polymorphonuclear leukocytes (PMNs) are the first leukocytes to arrive at the site of injury. The ability of PMNs to remove

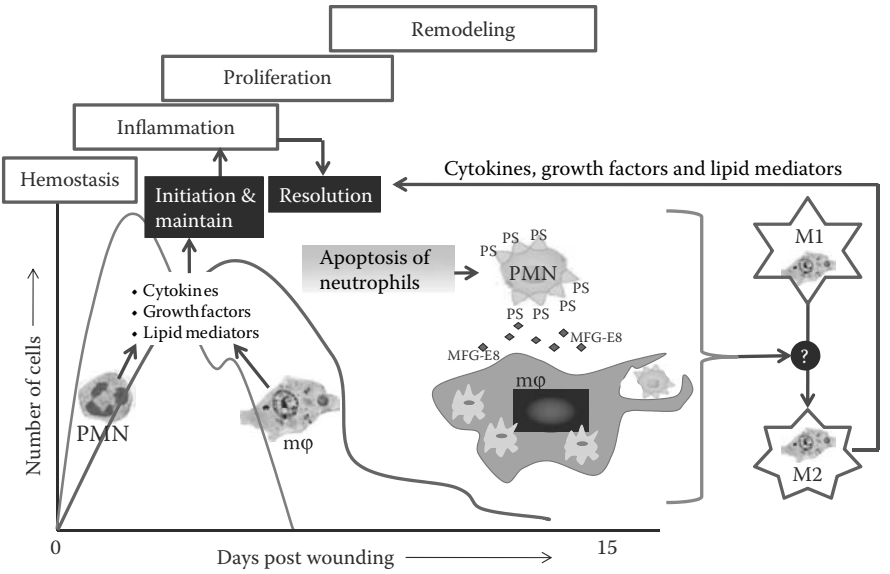

FIGURE 8.1 (See color insert.) Wound healing phases: initiation, maintenance, and resolution of the inflammatory response. The process of wound healing is well regulated and divided into different phases, taking place in an overlapping series of programmed events: hemostasis, inflammation, proliferation, and remodeling. During the early inflammatory phase in wounds, PMNs are the first leukocytes to arrive at the site of injury (orange line). The PMNs are eliminated from the site via apoptotic cell death. As the level of neutrophils falls, there is an influx of monocytes/macrophages to the site (red line). One of the primary functions is to accomplish clearance of apoptotic PMNs. A number of molecules have been implicated in the recognition and engulfment of apoptotic cells by macrophages. Such engulfment of apoptotic cells results in attenuation of pro-inflammatory and an increase in anti-inflammatory molecule production, thus facilitating resolution of inflammation. Macrophages are assigned to two groups: type I macrophages (M1, proinflammatory) and type II macrophages (M2, anti-inflammatory). The uptake of apoptotic cells by macrophages has been proposed as one of the cues that drive the switching of macrophages toward an anti-inflammatory state. Once inflammation is resolved, the healing of a wound progresses through proliferative and remodeling phases. MFG-E8, milk fat globulin E8; mϕ, macrophages; PMN, polymorphonuclear leukocyte; PS, phosphatidyl serine.

bacteria, amplify inflammation, and release an arsenal of bactericidal agents establishes them as the primary effector cells in host defense. Macrophages represent the second wave of leukocyte effectors at the injured tissue. Thorough release of an array of growth, angiogenic, and inflammatory factor macrophages marks the transition of the inflammatory phase to the proliferative phase of wound healing (Gronert 2008).

The inflammatory response in the wound is tightly regulated by signals that initiate, maintain, or resolve inflammation (Eming et al. 2007). An imbalance between these signals may cause chronic inflammation, derailing the healing cascade (Figure 8.1). While the signals that initiate and maintain wound inflammation have been extensively studied (Eming et al. 2007; Oberyszyn 2007; Werner and Grose 2003), the signals that resolve wound inflammation remain poorly understood (Khanna et al. 2010; Roy 2010).

8.2.3 Proliferation

Temporally, the proliferative phase usually closely follows and overlaps the inflammatory phase. This phase is generally characterized by epithelial proliferation and migration over the provisional matrix within the wound (reepithelialization). Chemotactic signals and growth factors released at the wound site stimulate the migration and activation of wound fibroblasts and deposition of a newly synthesized extracellular matrix. These components include fibroblasts, collagen, and blood vessels. Once in the wound, fibroblasts proliferate profusely and produce matrix proteins, which further support the cell migration and are essential for the repair process. In the early proliferative phase, fibroblasts are limited to cellular replication and migration. Collagen synthesis occurs in the later stages, followed by cross-linking of collagen, which is responsible for vascular integrity and mechanical strength of new capillary beds. At this stage, fibroblasts start attaching to the fibronectin and collagen in the extracellular matrix (ECM). Wound contraction is a crucial step in the reparative process that helps to draw the wound edges together and promote rapid closure of the wound (Velnar et al. 2009).

8.2.4 Remodeling

Remodeling represents the final phase of wound healing. This phase is well controlled by the balance between the synthesis and breakdown of the extracellular matrix components. During the course of normal wound healing, collagen deposition reaches a peak by the third week after wounding. Collagen acts as a framework on which new tissues are laid. As this framework becomes more organized and hydroxylated, it increases the tensile strength of the wound tissue. Contraction of the wound is an ongoing process contributed in part by differentiated fibroblasts called myofibroblasts, which resemble contractile smooth muscle cells (Velnar et al. 2009). Remodeling continues even months after wound closure and influences the scar outcomes of the healed wound.

8.3 INITIATION AND MAINTENANCE OF THE INFLAMMATORY RESPONSE

The inflammation response in adult injured tissue is substantial and often referred to as exaggerated. The primary aim of such response is to fight possible infection. In such form inflammation is viewed to be in conflict with regenerative healing and a major contributor to scar outcomes. During the first and second trimesters of gestation, both human and animal feti heal skin wounds without scarring (Colwell et al. 2003; Oberyszyn 2007). One key difference between the healing of fetal and adult skin is the magnitude of inflammation mounted in response to wounding. Unlike adult cutaneous wounds, fetal wounds heal in a scarless manner with an attenuated inflammatory response (Colwell et al. 2003).

In an acute inflammatory phase, chemotaxis is the primary mechanism by which movements of inflammatory cells are directed in response to wounding. Chemotaxis involves a complex cascade of events, including formation of signaling complexes

via receptor–cytokine interactions (Heit and Kubes 2003). The high-density GeneChips™ study examining acute changes in the wound-edge tissue during the inflammatory phase revealed that chemotaxis and cytokine–receptor interaction pathways represent the early upregulated genes in response to wounding (Roy et al. 2008). Interactions of leukocytes with endothelial cells represent early-intermediate events in acute inflammation wound repair (McIntyre et al. 2003). Components of the leukocyte–endothelial cell interaction pathways were upregulated during the intermediary phase (12–96 h) after wounding. Genes upregulated in response to wounding in the late phase (48–96 h) of inflammatory response belonged to the cell cycle pathways, which are known to be implicated in the supply of additional cells necessary for closing wounds (D'Souza et al. 2002; Seah et al. 2005).

Infiltrating leukocytes represent the key cellular components of the inflammatory response in wound healing. They not only are combat invading pathogens, but also are involved in tissue degradation as well as tissue formation (Eming et al. 2007). In this chapter we review the major players that regulate wound inflammation.

8.3.1 Inflammatory Cells

A small number of the cells of leukocyte lineage are present as resident cells in resting tissues. These numbers are increased multifold by recruitment of these cells from the circulation in response to inflammatory cues at the injury site (Oberyszyn 2007). *Platelets* are the first cells visiting the site of injury as a result of direct spill from injured vessels to initiate the coagulation process. Platelets aggregate at the ends of damaged blood vessels, convert fibrinogen to fibrin, and prevent loss of blood from damaged vessels. *Neutrophils* begin arriving at the wound site within minutes of injury. Peak recruitments takes hours and a lower level of recruitment may continue for several days. Although the primary role of neutrophils is to cleanse microbes invading the open wound, neutrophils are also a source of pro-inflammatory cytokines, including interleukins (IL-1α and β) and tumor necrosis factor α (TNF-α), which provide some of the earliest signals activating local fibroblasts and keratinocytes. During phagocytosis, neutrophils generate copious amounts of reactive oxygen species (ROS) by way of respiratory burst to kill pathogens. Apart from killing pathogens, ROS in the wound fluid help drive redox signaling. Excessive ROS are deleterious for the regenerating host tissue. This is particularly apparent in chronic wound situations, and might well underlie the persistent tissue-destroying nature of such wounds. Neutrophils generally populate skin wounds in high numbers within hours of the injury, while macrophage numbers at the wound site peak a day or two later (Martin and Leibovich 2005). It is therefore suggested that neutrophils help recruit macrophages into the wound site. In 2003, a study by Dovi et al. contradicted this contention, demonstrating that depleting neutrophils during wound healing did not alter the number of macrophages recruited to the wound site (Dovi et al. 2003). Studies using antibody-based approaches to deplete neutrophils in mice suggest that neutrophils may retard the healing process, as depletion of neutrophils enhances the rate of reepithelialization (Dovi et al. 2003). A novel role of wound neutrophils in the regulation of innate immune responses through modulation of macrophage phenotype has been elucidated (Daley et al. 2005). *Macrophages* are predominant

cell types in healing a wound 3–5 days after injury. The primary function of wound macrophages, which arrive at an injury site a little later than neutrophils, is to operate as voracious phagocytes cleansing the wound of all matrix and cell debris, including fibrin and apoptotic neutrophils. Macrophages also produce a range of cytokines, and growth and angiogenic factors that play key roles in the regulation of fibroblast proliferation and angiogenesis (Eming et al. 2007; Martin and Leibovich 2005; Rappolee et al. 1988). In a classic study, Leibovich and Ross (1975) demonstrated that antimacrophage serum combined with hydrocortisone diminished the accumulation of macrophages in healing skin wounds of adult guinea pigs. Such depletion resulted in impaired disposal of damaged tissue and provisional matrix, compromised fibroblast count, and delayed healing (Leibovich and Ross 1975). A number of recent studies using macrophage-specific gene knockout have also provided additional information about the overall significance of macrophages in healing and inflammation (Goren et al. 2009; Mirza et al. 2009). These studies elucidated a pivotal function of wound macrophages in the integration of inflammation and cellular movements at the wound site to enable efficient skin repair (Goren et al. 2009; Mirza et al. 2009). The role of macrophages in the resolution of inflammation has been reviewed elsewhere (Roy 2010). *Mast cells* also accumulate at sites of injury during the inflammatory response (Egozi et al. 2003). Although these cells are most well known for their role in allergic reactions, they degranulate and release a variety of prestored mediators from their granules after injury (Puxeddu et al. 2003). Studies have specifically looked at the acute phase of healing using the $Kit^{W}/Kit^{W\text{-}v}$ mouse strain (Egozi et al. 2003; Weller et al. 2006). These studies demonstrated that wounds from mast cell-deficient ($Kit^{W}/Kit^{W\text{-}v}$) mice contained fewer wound site neutrophils than wild-type mice, suggesting that mast cells may be critical regulators of neutrophil infiltration into the wound. How exactly mast cells may modify wound-induced inflammatory response remains an open area of inquiry.

8.3.2 Cytokines

Cytokines directly control cell immune responses. Pro-inflammatory cytokines, e.g., IL-1α, IL-1β, IL-6, and TNF-α, are prominently upregulated during the repair process (Werner and Grose 2003). IL-6 knockout animals take up to three times longer to heal than wild-type controls (Gallucci et al. 2000), suggesting a key role of this cytokine in driving the wound repair process. Persistent expression of the inflammatory cytokines IL-1α and TNF-α was observed in an excisional wound healing model in diabetic (db/db) mice (Khanna et al. 2010; Wetzler et al. 2000). TNF-α is a pleiotropic cytokine that supports the inflammatory response to wounding. The overall significance of TNF-α in wound healing is somewhat unclear at present (Sander et al. 2009). Depending on the concentration, length of exposure, and presence of other cytokines, TNF-α can be beneficial or deleterious (Sander et al. 2009). *In vitro*, cytotoxic and growth inhibitory effects of TNF-α have been demonstrated in endothelial cells and fibroblasts (Frater-Schroder et al. 1987; Mauviel et al. 1988). Subcutaneous injection of TNF-α increases collagen deposition and enhances wound disruption strength (WDS) in adriamycin-treated animals (Mooney et al. 1990). Lowering of the functionally available levels of the pro-inflammatory cytokine TNF-α using

anti-TNF-α therapy directed at managing activated macrophages restores diabetic wound healing in ob/ob mice (Goren et al. 2007). Studies using TNF-α null mice demonstrated that lack of TNF-α potentiates Smad-mediated fibrogenic reaction in the healing dermis, potentially leading to fibrosis, abnormal contraction, and eventually organ dysfunction (Shinozaki et al. 2009). IL-10 is recognized as a major suppressor of the wound-induced inflammatory response (Moore et al. 2001). An important role of this anti-inflammatory cytokine in attenuating the expression of pro-inflammatory cytokines in fetal wounds, resulting in minimized matrix deposition and scar-free healing, has been demonstrated (Liechty et al. 2000). Increased levels of the pro-inflammatory cytokines TNF-α and IL-6 and a decreased level of IL-10, an anti-inflammatory cytokine, were reported in diabetic wound tissue, compared to nondiabetic healing wound tissue.

8.3.3 Lipid Mediators

Lipid mediators such as eicosanoids, derived from oxygenation of arachidonic acid (i.e., ω-6 C20:4), are released from membrane phospholipids by phospholipases A2 in response to inflammatory stimuli (Gronert 2008). Eicosanoids consist of a family of biologically active metabolites, including prostaglandins (PGs), prostacyclin (PC), thromboxanes (TXs), leukotrienes (LTs), and lipoxins (LXs). Free arachidonic acid may be metabolized through the cyclooxygenase (COX) pathway, involving COX-1 and COX-2, along with terminal synthases, to generate PGs, PC, and TXs. These metabolites may act via a series of G-protein-coupled receptors. Alternatively, arachidonic acid may be oxidized by the lipoxygenase pathway, i.e., 5-lipoxygenase, 12/15-lipoxygenase, LTA4 hydrolase, and LTC4 synthase, to produce several classes of leukotrienes and lipoxins (Haeggstrom et al. 2010). Eicosanoids thus generated are well known to initiate, amplify, and perpetuate inflammation in both acute and chronic wounds (Broughton et al. 2006a). Induction of COX-2 represents one of the earliest responses following cutaneous injury. Consequent deployment of pro- and anti-inflammatory prostaglandin signaling mechanisms drives progression of the healing response (Oberyszyn 2007). COX-2 is the primary enzyme responsible for increased production of the pro-inflammatory mediator PGE2 in wounded skin (Abd-El-Aleem et al. 2001). Elevated COX-2 protein expression and PG production in chronic venous leg ulcers may contribute to the failure of these wounds to properly resolve inflammation and close in a timely manner (Abd-El-Aleem et al. 2001).

8.3.4 Oxygen and Reactive Oxygen Species in Wound Inflammation

In its molecular form, oxygen is required for oxidative metabolism-derived energy synthesis, protein synthesis, and the maturation (hydroxylation) of extracellular matrices such as collagen (Sen 2009). Molecular oxygen is also required for NO synthesis, which in turn plays a key role in the regulation of vascular tone, as well as in angiogenesis. In a wound setting, large amounts of molecular oxygen are partially reduced to form reactive oxygen species (ROS). ROS includes oxygen free radicals such as superoxide anion, as well its nonradical derivative hydrogen peroxide (H_2O_2)

(Sen 2009). Superoxide anion radical is the one electron reduction product of oxygen. NADPH oxidases represent a major source of superoxide anion radicals at the wound site. NADPH oxidases in phagocytic cells help fight infection. Superoxide anion also drives endothelial cell signaling, such as required during angiogenesis. ROS generated at low concentrations during the acute inflammatory phase are known to be required for wound healing (Sen 2009). Excessive ROS, however, may complicate the healing process.

8.4 RESOLUTION OF WOUND INFLAMMATION

8.4.1 Macrophage Phenotype

Macrophages are dynamic and heterogeneous cells broadly categorized into two groups: classically activated or type I macrophages, which are pro-inflammatory effectors, and alternatively activated or type II macrophages (Martinez et al. 2009). In the inflamed tissue, it is unclear whether the type II macrophages that appear during the healing phase originate from newly attracted monocytes or from a switch in the activation state of previously pro-inflammatory macrophages. A study by Porcheray et al. (2005) clearly demonstrated that a macrophage population first taking part in inflammation may change its phenotype and assume the role to resolve inflammation. A recent study demonstrated that PLCβ2 plays a novel and key role in regulating the switch of macrophages from an inflammatory (M1) phenotype to an angiogenic (M2-like) phenotype, suggesting that regulation of this pathway might provide an additional target for the regulation of inflammation, wound healing, and fibroproliferative processes (Grinberg et al. 2009). Macrophages from diabetic wounds display dysfunctional inflammatory responses (Khanna et al. 2010). A persistent inflammatory state of diabetic wound macrophages may be due to impairment in the ability of these cells to phagocytose apoptotic cells, which in turn prevents the switch from M1 to M2 phenotype (Khanna et al. 2010). A better understanding of the mechanisms and cues that are responsible for changing macrophage phenotype will be critical in designing strategies that can restore macrophage function and promote resolution of inflammation and healing in chronic wounds.

8.4.2 Dead Cell Clearance

Impaired phagocytosis of apoptotic cells has been implicated in the pathogenesis of a number of diseases, such as diabetes and atherosclerosis (Khanna et al. 2010; Tabas 2010). Because the phagocytosis of apoptotic cells has distinctive morphologic features and unique downstream consequences, deCathelineau and Henson (2003) coined the term *efferocytosis* (taken from the Latin *effero*, meaning "to take to the grave" or "to bury"). Phagocytic clearance of apoptotic cells, or efferocytosis, consists of at least four distinct steps: (1) secretion of "find me" signals that help recruit phagocytes at the site rich in apoptotic cells, (2) recognition ("eat me") of dying cells by phagocytes through a number of bridge molecules and receptors, (3) engulfment of apoptotic cells by a unique uptake process, and (4) processing of engulfed cells within phagocytes (Erwig and Henson 2007).

8.4.2.1 Find Me

Apoptotic cells release attraction signals that direct phagocytes to their location (Grimsley and Ravichandran 2003). The supernatants of several types of apoptotic cells exhibit chemoattractant activity for monocytes and primary human macrophages (Grimsley and Ravichandran 2003). Caspase-3-mediated activation of Ca^{2+}-independent phospholipase A2 (iPLA2) in apoptotic cells leads to release of chemoattractant signals such as lysophosphatidylcholine (LPC) (Grimsley and Ravichandran 2003).

8.4.2.2 Recognition (Eat Me)

Cells undergoing apoptosis flag themselves for removal by presenting eat-me signals at the cell surface (Grimsley and Ravichandran 2003). These flags on the dying or dead cells differentiate them from viable cells and tag them for recognition by macrophages at the wound site. Phosphatidylserine (PS) externalization is a general feature and hallmark of apoptosis (Bratton et al. 1997; Fadok et al. 2001). Selective NADPH oxidase-dependent oxidation of PS and subsequent externalization have been demonstrated in a wide range of apoptotic models (Kagan et al. 2003). Externalization of oxidized PS, in conjunction with its nonoxidized counterpart, serves as an eat-me signal (Grimsley and Ravichandran 2003). The significance of PS oxidation and externalization in apoptotic cell clearance during wound healing remains to be understood. NADPH oxidase plays a critical and specific role in PS oxidation and externalization (Borisenko et al. 2003). Patients with chronic granulomatous disease (CGD), an inherited condition in humans characterized by an impairment in NADPH oxidase activity (Roos and Curnutte 1999), frequently develop disorders of autoimmunity against inadequately cleared dead tissue (Gaipl et al. 2004).

8.4.2.3 Bridging Molecules and Phagocyte Receptors

Most receptors on phagocytes do not bind to phospholipids directly, but rather via soluble bridging proteins (Zullig and Hengartner 2004). An increasing number of these soluble factors are emerging whose role is to opsonize apoptotic cells and develop molecular bridges between specific components of the apoptotic cells and phagocyte surface. This family of specialized proteins includes annexin I (Anx I or lipocortin), thrombospondin (TSP), milk-fat-globule-EGF-factor 8 (MFG-E8), Del-1, β2-glycoprotein I, protein S, and growth arrest specific gene 6 (Gas6) (Wu et al. 2006; Zullig and Hengartner 2004). MerTK has been extensively studied and has been proposed to facilitate phagocytosis of apoptotic cells and downregulate activation in macrophages (Scott et al. 2001). MerTK (also known as Eyk, Nyk, and Tyro-12) belongs to a family of receptor tyrosine kinases (RTKs) that include Axl and Tyro3. Mice lacking expression of all three RTKs exhibit hyperactivated macrophages, which in turn drive systemic autoimmunity (Scott et al. 2001). GAS6 is a ligand for MerTK, which binds to PS expressed on the inverted plasma membrane of apoptotic cells. Recognition of a GAS6-PS complex facilitates binding of apoptotic cells and subsequent phagocytosis by macrophages (Tibrewal et al. 2008). A direct link between Tyr-867 of MerTK and activation of cytoskeletal assemblages leading to phagocytosis has been identified (Tibrewal et al. 2008). Phosphorylation of Tyr-867

results in the activation of both Akt and PLC2, which in turn leads to the downstream activation of PKC, and affects the actin cytoskeleton, resulting in tyrosine phosphorylation of FAK and p130cas (Tibrewal et al. 2008). MerTK plays a central role in regulating the engulfment of apoptotic cells by macrophages (Tibrewal et al. 2008).

Complements may be involved in the uptake of apoptotic cells via direct binding of bridging factors in some physiological circumstances involving opsonization and engagement of the complement receptors (Amarilyo et al. 2010). IC3b-opsonized apoptotic cells are characterized by macrophage secretion of IL-10 and lack of TGF-β secretion (Amarilyo et al. 2010). In cells with IC3b receptors, opsonized apoptotic cells mediate a distinct anti-inflammatory response and blockage of the pro-inflammatory transcription factor NF–κB (Amarilyo et al. 2010). MFG-E8 (also known as lactadherin), one of the key bridging molecule proteins, was identified as protein secreted by activated macrophages (Hanayama et al. 2004). *In vivo* studies have recognized the significance of MFG-E8 in apoptotic cell clearance (Hanayama et al. 2004). MFG-E8 is capable of binding to PS on apoptotic cells as well as to integrins on macrophages (Hanayama et al. 2004). Mice lacking MFG-E8 suffer late-onset autoimmune disorder, a disease often associated with impaired clearance of apoptotic cells (Gaipl et al. 2004). MFG-E8 binds to either αvβ3 or αvβ5 integrin receptors on the macrophage surface via a typical tripeptide Arg-Gly-Asp (RGD) motif located within its two EGF repeats (Hanayama et al. 2004).

8.4.2.4 Engulfment and Processing of Engulfed Cells

Information on the final step of apoptotic cell clearance is limited. We look forward to learn more about how processing of apoptotic bodies is regulated and how it differs from the processing of classically opsonized or microbial cells that employ a route of degradation from phagosomes to lysosomes. Candidate genes identified in screening studies on *Caenorhabditi elegans* that is defective in apoptotic cell clearance point toward cell adhesion, and migration pathways are being associated with apoptotic cell clearance. The genes in *C. elegans* (ced-2, ced-5, ced-10, and ced-12) and their mammalian counterparts (Crk II, DOCK180, ELMO, and Rac1) encode proteins that comprise an evolutionarily conserved Rho-GTPase signaling module that functions in a wide variety of cells (Wu et al. 2006). Crk, DOCK180, and Rac1 are downstream of adhesion receptors (i.e., integrin) and RTKs (Wu et al. 2006). In a general model of MFG-E8-mediated binding and internalization of apoptotic cells by phagocytes, a central role of αvβ5 integrin was noted. β5 cytoplasmic tail, and engagement of the αvβ5 heterodimer, results in recruitment of the p130cas-CrkII-Dock180 molecular complex, which in turn triggers Rac1 activation and phagosome formation (Albert et al. 2000).

8.4.2.5 Interactions with Apoptotic Cells: Functional Consequences for Immunity

Phagocytosis of apoptotic cells is actively anti-inflammatory and anti-immunogenic with generation of anti-inflammatory mediators such as transforming growth factor-beta (TGF-β) and anti-inflammatory eicosanoids (Freire-de-Lima et al. 2006). Evidence from murine studies demonstrates a central role of Mer receptor tyrosine

kinase in suppressing pro-inflammatory signals from TLRs (Wu et al. 2006). Khanna et al. (2010) demonstrated that elevation of apoptotic cell load at the wound site results in increased inflammatory response, and that impaired dead cell clearance activity in diabetic wound macrophages compromises resolution of inflammation in diabetic wounds. Cytokines and chemokines are centrally involved in the inflammatory responses elicited in a chronic wound. The expression of these factors, in many cases, is regulated directly at the level of transcription following the activation of relevant transcriptional activators, including NF-κB. Apoptotic cells actively suppress an inflammatory response (Voll et al. 1997). In the presence of apoptotic peripheral blood lymphocytes, monocytes produced substantially more of the anti-inflammatory cytokine IL-10 and less of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-12 (Voll et al. 1997). In 1998 Fadok et al. demonstrated that clearance of apoptotic cells by macrophages inhibits the production of pro-inflammatory cytokines such as IL-8 and IL-1β, and induces the secretion of TGF-β, platelet-activating factor, and prostaglandin E2 (Fadok et al. 1998).

While numerous studies have explored a variety of death-associated markers displayed by apoptotic cells, functional characterizations of these molecules have focused on engulfment and not immunosuppression as an experimental endpoint (Birge and Ucker 2008). Understanding the defining molecular signatures of apoptotic cell death, and the signaling pathways and mechanisms by which they affect immunosuppression, remains a challenging biological problem (Birge and Ucker 2008). Current evidence supports that engulfment of apoptotic cells by macrophages induces transcriptional repression via an immediate-early response mechanism that occurs in the absence of *de novo* protein synthesis (Birge and Ucker 2008).

8.4.3 Lipid Mediators

Polyunsaturated fatty acids (PUFAs) are rapidly released by cells in response to stress, injury, or inflammatory stimuli. Several independent laboratories have demonstrated that the ω-3 PUFAs eicosapentaenoic (EPA; i.e., ω-3, C20:5) and docosahexaenoic acid (DHA; i.e., ω-3, C22:6) are transformed, in a manner equivalent to arachidonic acid metabolism, by COX-2 and LOX enzymes (Figure 8.2), to generate novel classes of endogenous lipid autacoids with anti-inflammatory and protective activities (Gronert 2008). Lipidomic analyses indicate that DHA is a relatively abundant endogenous fatty acid existing in murine skin (in full thickness, 300–3,000 ng/g skin tissue). Gel-controlled release of DHA and three other essential fatty acids to wounds significantly promoted wound healing, unveiling a therapeutic potential for DHA derivatives in wound healing (Tian et al. 2010). A brief review of the major lipid autacoids reported to contain anti-inflammatory activity is presented below.

8.4.3.1 Lipoxins

First isolated in 1984, lipoxins are generated through transcellular biosynthesis. Lipoxins A4 and B4 (LXA4 and LXB4) are generated by the action of platelet 12-lipoxygenase on neutrophil leukotrienes A4 (LTA4s) (Kilfeather 2002). Lipoxins are shown to inhibit PMN chemotaxis (Levy et al. 2001), PMN adhesion to and transmigration through endothelial cells, as well as PMN-mediated increases in vascular

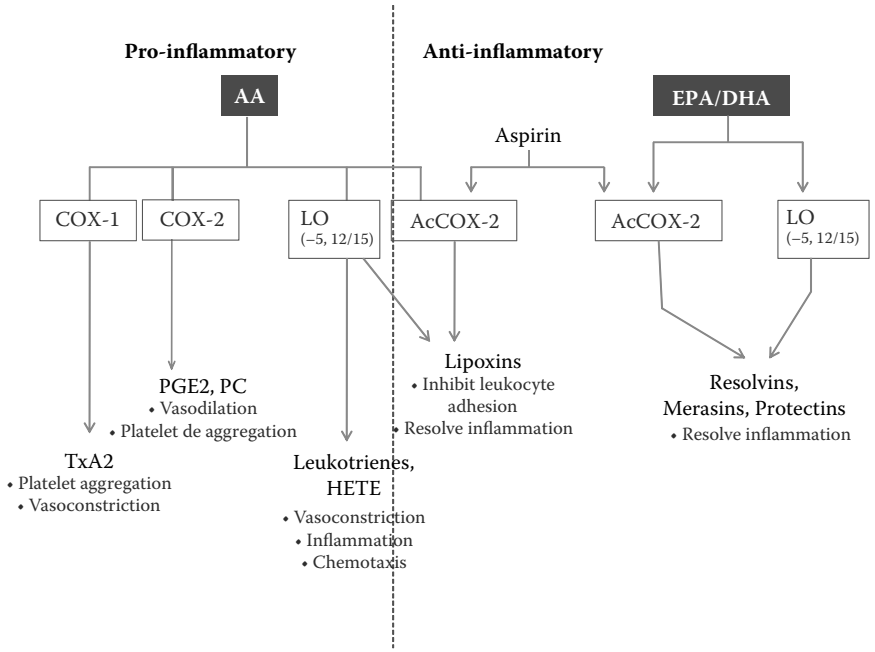

FIGURE 8.2 Inflammatory/anti-inflammatory lipid biosynthesis pathways and functions. Pro-inflammatory (left) and anti-inflammatory (right) lipid mediators regulate the induction and resolution of wound inflammation. These lipids are synthesized from polyunsaturated fatty acids, such as arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). AA is converted to pro-inflammatory thromboxanes (TXs), prostacyclin (PCs), and prostaglandins via the cyclooxygenase (COX) pathway. AA can also be converted to pro-inflammatory leukotrienes (LTs) and prostaglandins (PGs) or hydroperoxyeicosatetraenoic acids (HPETEs), which can be further enzymatically reduced to the hydroxylated form (HETE). LO enzymes are also involved in the production of anti-inflammatory lipoxins (LXs), resolvins (RvEs and RvDs), and protectins (PDs) from AA, EPA, and DHA. These anti-inflammatory lipids can also be generated via COX-2 in the presence of aspirin. Aspirin induces acetylation of COX-2. The acetylated COX-2 (AcCOX-2) can also catalyze the formation of lipoxins and resolvins from AA and EPA/DHA.

permeability (Serhan et al. 1999). Lipoxins also attract monocytes and stimulate monocyte adherence to vascular endothelium (Maddox and Serhan 1996) without releasing reactive oxygen species (Jozsef et al. 2002).

8.4.3.2 Cyclopentenone Prostaglandins

Cyclopentenone prostaglandin (15dPGJ2) is formed by the *in vivo* and *in vitro* dehydration of PGD2 by COX-2. 15dPGJ2 inhibits TNF-α stimulated expression of vascular cell adhesion molecule 1 (VCAM1) and intercellular adhesion molecule 1 (ICAM1) by human endothelial cells. Furthermore, this prostaglandin also inhibits monocyte binding to human aortic endothelial cells. However, 15dPGJ2 does not influence neutrophil adhesion, but does block adhesion-dependent oxidative bursts in neutrophils (Lawrence et al. 2002).

8.4.3.3 Resolvins and Protectins

In addition to arachidonic acid, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) serve as precursors to potent bioactive mediators that possess anti-inflammatory properties. The term *resolvins* or *resolution phase interaction products* was coined by Professor Charles N. Serhan and colleagues because these compounds were first encountered by this group in resolving inflammatory exudates (Serhan et al. 2000). Compounds derived from EPA are designated as resolvins of the E series, while those formed from the precursor DHA are denoted as either resolvins of the D series or protectins (neuroprotectins) (Serhan et al. 2002). E series member resolvin E1 blocks human neutrophil transendothelial migration, thus reducing inflammation *in vivo* (Serhan et al. 2000). Resolvin E1 levels are increased in the plasma of individuals taking aspirin or EPA (Arita et al. 2005). In endothelial cells COX-2 is acetylated in response to aspirin treatment. Acetylated COX-2 converts EPA to 15R HEPE and 18R HEPE; both are known to potently inhibit transendothelial migration of PMN (Serhan et al. 2000). Bioactive members from DHA-containing conjugated triene structures or docosatrienes that possess immunoregulatory and neuroprotective actions are collectively known as neuroprotectins. In studies addressing resolvin formation in brain tissue in response to aspirin treatment, it was shown that new docosatrienes, initially termed neuroprotectins, are produced. In recognition of the fact that the protective actions of these docosanoids are not restricted to neural tissue, it has been suggested that the more generic term *protectins* be used instead (Serhan et al. 2004).

8.4.3.4 Maresins

Maresins (14S-HDHA) are anti-inflammatory lipid mediators primarily generated by macrophages and are novel metabolites of the 14-lipoxygenase pathway (Serhan et al. 2009). Addition of either DHA or 14S-hydroperoxydocosa-4Z,7Z,10Z,12E,16Z,19Z-hexaenoic acid (14S-HpDHA) to either human or murine macrophages converts these substrates to dihydroxy-containing products that possess potent anti-inflammatory and pro-resolving activity. The potency of these anti-inflammatory mediators is in the range of RvE1 and PD1 (Serhan et al. 2009).

8.5 STRATEGIES FOR THE MANAGEMENT OF WOUND INFLAMMATION

Because excessive inflammation is one of the leading causes of complicated healing of chronic wounds, treatments aimed at resolving inflammation related to chronic wounds are of outstanding interest. A range of therapies exist for the treatment of inflammation-driven diseases, such as asthma, rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosus, Crohn's disease, multiple sclerosis, and systemic vasculitis. Unfortunately, in their current form, such therapeutics come with the potential of undesirable side effects. For instance, steroids can cause osteoporosis and impair wound healing. In the clinical treatment of reducing wound inflammation, debridement is commonly practiced and primarily involves surgical removal of dead, damaged, or infected tissue to improve the healing potential of the remaining healthy tissue.

By decreasing the necrotic and dead tissue burden, debridement resets the chronic to the acute healing phase. Diabetic human wounds are stalled at the inflammatory phase because of insufficiencies in the inflammation and phagocytosis processes (Loots et al. 1998). In diabetic wounds, impaired efferocytosis leads to increased inflammatory response (Khanna et al. 2010), suggesting that at the wound site successful debridement at the cellular level is a prerequisite to resolution of inflammation and successful healing (Roy 2010).

Other methods aimed at altering the inflammatory cascade involve:

1. Use of exogenous cytokines and growth factors to shift the degradative disequilibrium found in a chronic wound toward a more synthetic mode. In theory, alteration of the molecular environment of the wound may disrupt the inflammatory phase and allow normal progression of the wound healing process.
2. Use of anti-inflammatory drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs). Due to the availability of the broad class of NSAIDs, our understanding of the role of prostaglandins (PGs) in inflammation is reasonably well developed. NSAIDs inhibit the conversion of AA to PG by targeting cyclooxygenase (COX) enzymes, and are effective in managing PG-associated pain and inflammation (Khanapure et al. 2007).
3. Nutritional interventions like using PUFA supplementation. While dietary PUFA supplementation may change circulating or tissue levels of bioactive lipids and alter inflammation or healing, information about their clinical effects on acute and chronic wound healing is highly limited.

At present, the treatment of inflammatory diseases is primarily based on interrupting the synthesis or action of mediators that drive the host's response to injury. Developing therapeutic agents or approaches that drive resolution of inflammation will be productive to treat diseases caused by chronic inflammation. Such therapeutics may target improving the phagocytotic activity of macrophages in a chronic inflammatory setting. A better understanding of the mediators and mechanisms that are central to the initiation and resolution of wound inflammation will help design improved strategies to manage persistent nonresolving inflammation commonly associated with chronic wounds.

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9 Role of Inflammation in Infectious Disease

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CONTENTS

9.1 INTRODUCTION

Following Pasteur's one organism–one illness model, each infectious disease was, for many decades, assumed to have its own mechanism. Yet while groups of closely related organisms, such as viruses, plausibly caused similar systemic syndromes, this approach eventually was seen to pose more questions than it answered. It could not, for example, explain how experienced clinicians inevitably confuse a viral disease with one caused by a spirochete[1] or a protozoan.[2] Similarly, typhoid is well known to be readily diagnosed as malaria,[3] and falciparum malaria in travelers returning to a temperate country is very commonly mistaken for influenza, with laboratory tests required to differentiate them. These complexities implied there was every possibility for the earlier rationales for these diseases, such as viruses causing illness and death by killing the cells they invade, and falciparum malaria by obstructing vascular flow, being far off the mark. Instead, some common theme seemed to be required.

There is little doubt that the present cytokine concept of the diseases caused by infectious agents, be they viruses, protozoa, or something intermediate in size, has rewritten the field. It rationalizes the above clinical confusions and, as will be discussed, much else besides. We must, of course, expect clinical and pathological dissimilarities in systemic diseases that have this common fundamental origin. Different triggers for cytokines (Gram-negative bacterial wall lipopolysaccharide (LPS), Gram-positive toxins, fungal toxins, malaria toxins, or modulation of retinoic acid-inducible gene-I (RIG-1) gene expression) can be expected to generate different ranges, profiles, concentrations, and kinetics of cytokine and chemokine generation and release. Different sites (e.g., restricted to one or more tissues, or circulating) of release are obvious additional causes for these variations. Nevertheless, the principles remain the same.

9.2 SYSTEMIC INFLAMMATION BEFORE THE DAYS OF CYTOKINES

These days, the term *inflammation* automatically brings to mind the harmful effects of pro-inflammatory cytokines, and the effects of these mediators, of which TNF is the prototype, will fill most of the pages of this chapter. To understand the wider picture that gave these chapters a context, the reader must be aware of how the word *inflammation* came to be used in a systemic sense, and then became shorthand for excessive effects of this class of cytokine.

In the late nineteenth century the term *inflammation* was already in use, and the local reddening, heat, and swelling it described were primarily studied to understand the vascular dilation thought to drive these changes.[4] Decades later this type of lesion was still a focus of research, with the role of bacteria by then also under

the spotlight.[5] So far as we are aware, the term *systemic inflammation* was first used in print in 1945 in order to convey that rheumatoid arthritis, formerly thought of as purely a focal inflammatory state, was, as evidenced by freely distributed nodular perineuritis and nodular polymyositis, in fact a systemic condition.[6] These nodules appear to have been referred to as inflammatory purely because when sectioned, they proved to be formed mostly of lymphocytes. A few years later a malaria researcher with sepsis research experience suggested that inflammation was the most likely explanation for the mechanism of this disease.[7] This being decades before the concept of cytokines, it was presumably the vascular events of inflammation he largely had in mind, in that his group later demonstrated kallikrein, a serine protease that liberates kinins from kininogens and generates plasmin from plasminogens, to be a pathogenic agent in a monkey malaria.[8,9] This approach was subsequently extended into posttrauma sepsis,[10] an infection caused by a spirochete,[11] typhoid,[12] and Rocky Mountain spotted fever, a rickettsial infection.[13] Meanwhile, Lewis Thomas, a scientist with a genius for popularizing advances in biology, suggested the possibility of the bacterially infected host harming itself through activating complement and releasing lysozymal enzymes,[14] and others had been addressing, in general terms, the importance of understanding disease processes through systemic inflammation.[15,16]

9.3 HOW CYTOKINES BECAME INVOLVED IN SYSTEMIC INFLAMMATION

The advent of cytokines as the mechanism of disease pathogenesis made a fundamental difference to what had gone before. For the first time we had, in tumor necrosis factor (TNF), the notion of a mediator of host origin that could have several functions that, on the surface at least, appeared quite unrelated. Described first as a tumor killer,[17] TNF was then shown to be a nonspecific killer of malaria parasites,[18,19] and argued, when excessively released, to initiate a range of malarial and sepsis pathology through its effects on host cells and metabolism.[18,20] As this chapter relates, this aspect of its function has expanded greatly, and continues to do so. Once cloned, and available as a recombinant protein, TNF was soon appreciated to have many physiological functions at low concentrations. This area of the literature also continues to expand, and TNF now has over 80,000 hits on PubMed. Its functional importance among the cytokines is implied by it having a much larger literature than any other cytokine.

9.3.1 Cytokines—A Synopsis

Cytokines have primary roles in physiology, innate and acquired immunity against pathogens, and the inflammatory response that is central to most disease. In brief, they are peptides, and most are classified under the general term *interleukins*,[21] numbered from interleukin-1 (IL-1) to (currently) IL-34. Others, such as the interferons (IFNs),[22] the lymphotoxins (LTs),[23] TNF,[17] and the transforming growth factors (TGFs)[24] and chemokines,[25] have unique terminologies. They are classified into groups, termed families and superfamilies, with similar structures and functions. For instance, the TNF superfamily has at least 19 members signaling through 29 receptors.[26]

Some cytokines, such as TNF, LTs, and IL-1, are pro-inflammatory, whereas others, such as IL-4, IL-10, and the TGF family, are usually anti-inflammatory, being subsequently induced to inhibit inflammation.

Cytokines are also characterized by their capacity to cooperate to form cascades, and operate through autocrine and paracrine pathways. Both IL-17 (reviewed by Onishi and Gaffen[27]) and, two decades earlier, TNF (reviewed by Nathan[28]) are good examples of cytokines known not for what they do directly, but for the wide array of other mediators, mostly other cytokines, that they induce. For example, IL-17 induces TNF, and IL-1 is induced by TNF, and shares many of its activities, including the capacity to induce IL-6, a cytokine often used as a marker for systemic inflammatory reactions because it typically appears in the circulation later, when illness is advanced, and stays high for longer than do TNF and IL-1. These two, particularly TNF, are correspondingly more difficult to detect in plasma when possibilities for collecting blood in individual patients are restricted by practical considerations. As another example of the complexities, both TNF and IL-1 have been known for over 20 years to induce IL-8, the prototype chemotactic cytokine, or chemokine, terms given to cytokines that attract cells up their concentration gradients. Another example is the role of interferon-γ (IFN-γ), which is predominantly secreted by natural killer (NK) cells and, upon instruction by IL-12 and IL-18, T cells. These two cytokines are primarily produced by dendritic cells and macrophages in response to Toll-like receptor (TLR) signaling.

It is appropriate here to summarize two sources of confusion that were allowed to creep into the nomenclature. The first was in 1985, when the rules of precedence were ignored and LT, described[23] and cloned[29] before TNF,[17,30] was permitted to be rebadged as TNF-β.[31] This meant that TNF became TNF-α. Unfortunately, it is still TNF-α in the minds of many, even though the α, added in 1985, is now meaningless because the original LT is no longer TNF-β, but became LT-α, to make a place for LT-β. The original term, *TNF*,[17] which was currency for 10 years, has now officially been returned to its former status by a number of standard textbooks, but new publications containing the now redundant term *TNF*-α still appear. The second source of confusion, seen less in current literature, is for TNF to be termed cachectin. In brief, an LPS-induced macrophage product came to light in 1982, as yet in an unnamed form,[32] through researchers trying to understand weight loss in trypanosomiasis. Its defining characteristic was an ability to inhibit the enzyme lipoprotein lipase, and 3 years later (a decade after TNF) it was purified[33] and termed cachectin. Like many other soluble mediators, it proved, upon sequencing,[34] to be identical to a previously sequenced[30] molecule that had been termed TNF 10 years earlier.[17] Unfortunately, in some circles precedence was again ignored, and the term *cachectin* was often used alone to designate TNF, but in due course it dropped out of currency.

Some cytokines, the prime example being TNF, are highly pleiotropic, whereas others have a single function only. Another multifunctional cytokine is IL-17, which, as well as its pro-inflammatory functions, regulates normal growth of intestinal epithelial cells.[35] TNF also controls much normal physiology, being, for example, a homeostatic agent in cell proliferation throughout the body, with low levels enhancing proliferation of a cell type that higher levels destroy.[36] Examples of this control are seen in thymocytes,[37,38] hepatocytes,[39,40] and hematogenesis.[41,42] TNF is also a key mediator

of innate immunity,[18] and the master cytokine that precipitates the inflammatory response,[43] albeit itself induced by members of the IL-17 family.[44–46] Several negative feedback loops of cytokines and their products have been shown to limit TNF generation. An example is hemoxygenase-1 (HO-1). This generates carbon monoxide (CO) from hemoglobin, and in small amounts is anti-inflammatory[47] because it inhibits TNF. We note here that a hugely successful biotechnology industry based on antagonizing TNF in patients (some $20 billion in sales in 2010) serves to generate excellent experimental tools to explore its role in diseases further.

9.4 INFLAMMATION AND SYSTEMIC ASPECTS OF DISEASE

9.4.1 Clinical Evidence of Shared Mediators of Innate Immunity and Pathology

The clearest evidence that the same cytokines that cause pathology also control innate immunity[18] is that pathogens can be reactivated in patients receiving chronic anticytokine therapy in order to treat a disease that this cytokine, but not this pathogen, causes. In other words, a treatment directed against the systemic inflammation that causes illness in one disease also inhibits the innate immune response against another. In particular, it has been extremely instructive for researchers to learn that dormant infections with certain organisms can reactivate in patients receiving chronically administered anti-TNF agents. Most cases involve tuberculosis,[48–51] but salmonella,[52,53] listeriosis,[54,55] brucellosis,[56] staphylococcal sepsis,[57] and leishmaniasis[58] are recorded. Since so many millions of patients are now chronically treated with these agents, one must carefully balance single case reports against larger studies, some of which[49,59] clearly carry considerable weight. The latter reference which appears to be the official 2009 consensus document for the rheumatology field, makes the case for keeping the acknowledged risk in perspective, in that the risk rate for all bacterial diseases for TNF neutralizing agents was 0.07–0.09 per patient year, compared to 0.06 for all other disease-modifying antirheumatic drugs.[59] Nevertheless, the presence of this phenomenon is extremely telling.

Viral diseases have not been investigated in this context as closely as those caused by bacteria. As reviewed in 2008[60] and 2010,[61] the influence of anti-TNF therapy on concomitant viral diseases seems to have been surprisingly mild. This is a reasonable observation, since TNF has been shown to exert, for example, a powerful *in vitro* effect against influenza virus in human epithelial cells.[62] Nevertheless, neutralization of TNF *in vivo* has been reported to not increase viral titers in mouse lung.[63] There are as yet very few guidelines on whether or not to screen, and for what conditions. Nonetheless, with close to a million patients having received long-term TNF-neutralizing drugs for rheumatoid arthritis or Crohn's disease by 2004,[64] and a call being made in that year for alertness to the possibility of hepatitis or HIV exacerbation,[65] there as yet appear to be few reports of enhancement of viral disease, apart from single cases of cytomegalovirus[66] and H1N1 influenza.[67] In contrast, reports of exacerbation of tuberculosis, and various bacteria, as above, while uncommon, emerge regularly. The reason for this difference has not yet been formally ascertained. Conceivably anti-TNF drugs remove the excess TNF, leaving enough to

suppress viruses. Nevertheless, a central role for TNF in host defense against pathogens, as well as its well-known capacity to harm the host in infectious disease, has been demonstrated. It will be informative to learn, in due course, whether clinical use of agents such as anti-IL-17 generates similar evidence for that cytokine.

9.4.2 How BCG Pointed to Cytokines in Host Defense and Disease in Malaria and Sepsis

As has often been reviewed,[68–71] the disease pathogenesis field turned to mediators, subsequently termed cytokines, when researchers trying to understand, in the mid-1970s, how pretreatment with the Bacillus Calmette-Guérin (BCG) strain of *Mycobacterium tuberculosis* controlled a subsequent infection with several species of hemoprotozoa (*Babesia* spp. and *Plasmodium* spp.) in mice. No antibody was induced, yet parasites were dying in circulating red cells, not after phagocytosis, as BCG protection might predict, so a previously unsuspected soluble factor, presumably a product of macrophages, was probably responsible. Moreover, these hemoprotozoa greatly sensitized mice to bacterial LPS, as did BCG,[72] producing the same pathology, metabolic changes, and increased levels of the same cytokines in the circulation, and every macrophage-activating organism known to protect against tumors *in vivo* acted the same as BCG when tested against hemoprotozoa.[69]

The timely publication of the first paper on a post-BCG circulating serum factor released from LPS-triggered macrophages, termed tumor necrosis factor (TNF) by cancer researchers in New York,[17] allowed an interpretation of the above hemoprotozoa data. Accordingly, in 1981 roles for TNF and functionally related cytokines in both host defense and disease pathogenesis in malaria and sepsis were collaboratively proposed.[18,20] Some 40 key background papers that demonstrate the development of the idea of TNF doing much more than killing tumors are shown in Table 1 of a 2003 review.[69] While this was years before access to the tools made possible by rTNF, key predictions of the model stood the test once these became available. Specifically, rTNF reproduced malaria[73] and sepsis[74] pathology, TNF levels peaked at a time consistent with its induction by parasite products released at the time of synchronized schizont rupture,[75] and in patients serum levels of this and some similar cytokines correlated with disease severity.[76–78] Likewise, the inducible form of nitric oxide synthase (iNOS), which can be induced by TNF, IL-1, or LT,[79] proved to be widespread in many tissues from African children who had died of either malaria or sepsis.[80] This was also true of hemoxygenase-1 (HO-1),[81] a cytokine-inducible enzyme that triggers the protective generation of carbon monoxide, which in small amounts is anti-inflammatory because it provides a negative feedback that helps limit TNF production.[47] In malaria studies based in Papua New Guinea,[82] others are currently extending the concept of TNF and associated cytokines involving host defense as well as disease pathogenesis. Our group has recently published several reviews on the roles of inflammatory cytokines on the development of the noncerebral aspects of malarial disease,[69,83–86] and the reader is referred to these. Malarial encephalopathy, or cerebral malaria, is discussed in Section 9.1.

9.5 INFLAMMATION AND SYSTEMIC ASPECTS OF DISEASES OTHER THAN MALARIA

The concept of TNF (and functionally related cytokines) causing pathology in infectious disease generally had a smooth transition from babesiosis and malaria into being implicated in the diseases caused by various other infectious agents. The initial examples of this were *Mycobacterium* spp.,[87] *Leishmania* spp.,[88,89] *Toxoplasma gondii*,[90] *Salmonella* spp.,[91] *Brucella abortus*,[92] and *Listeria monocytogenes*.[93] These fields of research have since expanded to provide insights consistent with the earlier studies. For example, in leishmaniasis interaction between TNF and IL-10 is now better understood,[94] in terms of the relative roles of soluble and membrane TNF.[95] TNF is also reported to control the chemokines that attract the cells that form tubercular granulomas.[96]

The complexity of the number and interactions of cytokines in the host's reaction to these pathogens continues to emerge, and the pattern of lower levels helping the host by limiting parasite survival and larger doses causing host disease still appears to hold. This can be illustrated with IL-17, originally identified as cytotoxic T lymphocyte-associated antigen-8 (CTLA-8), and IL-22, originally termed IL-10-related T cell-inducible factor (IL-TIF). These two mediators are produced by lineages that include CD4+T cells, γδT cells, and T helper 17 (Th17) cells. This Th17 pathway is thought to have evolved as part of innate immunity against pathogens, and has been reviewed extensively[97] from this perspective. Specific examples are *Pneumocystis carinii*,[98] *Listeria monocytogenes*,[99] *Citrobacter rodentium*,[100] *Candida albicans*,[100] and *Shigella flexneri*.[101] These cytokines can also, in oversupply, mediate tissue damage and pathophysiology that is recognized as disease. For instance, virally induced IL-17 is reported to cause, through activating neutrophils, fatal liver necrosis in systemic herpes infections.[102] Likewise, IL-17 produced during *Trypanosoma cruzi* infection has been shown to play a central role in regulating parasite-induced myocarditis. IL-22, a member of the IL-10 cytokine family, has recently[103] been demonstrated to have a strong pathogenic role in toxoplasmosis established by the natural oral route. Mice treated with anti-IL-22 antibody developed significantly less intestinal pathology than controls, even though both groups displayed similar parasite burdens. There is little doubt that these advances will soon be tested to see how generally they extend within infectious disease as a whole.

9.5.1 Viral Diseases

Like others, we often wondered in the past why malaria and influenza are so clinically confusable. Indeed, diagnostic mistakes with serious consequences are still not uncommon in returning travelers. Fortunately, we knew this, and in 1987 visited tumor specialists in Boston who inadvertently discovered dramatic side effects, which they described as influenza-like, in patients treated with their new experimental drug, rTNF.[104] To us they were clearly malaria-like, in every way. Witnessing their experience led us, in 1989, to combine their observations with our work on TNF in malaria, and summarize the logic of influenza, hepatitis B, dengue, and yellow fever having, like malaria, an inflammatory cytokine origin.[105] In 1993 the main Centers

for Disease Control and Prevention (CDC) filovirus work[106] was still based on the assumption that increased endothelial cell permeability in filovirus infections was caused directly by viral invasion of these cells, leading to cytopathic multiplication within them. Nevertheless, within a few years this same group had demonstrated that the observed increased permeability in fact arose from the virally induced TNF that monocytes and macrophages released.[107] Since then, the literature on the pathogenesis of serious viral infections, including those caused by hantavirus,[108] Marburg[109] and Ebola[110] viruses, Lassa[111] and Junin[112] viruses, dengue viruses,[113] influenza, and pox viruses, has become largely focused on arguments for the central roles of inflammatory cytokines. These last two examples are expanded upon below, and in Section 9.3 influenza and dengue are the viral infections in which the associated encephalopathies are discussed.

9.5.1.1 Influenza

TNF generation and circulating levels are increased in influenza,[63] the more pathogenic strains generating more of it. For example, influenza A virus stimulates the release of TNF from macrophages,[114,115] and the avian influenza virus, H5N1, which is particularly virulent in humans, generates more TNF in human macrophages than do a range of less virulent strains of human influenza.[116] The same is true of the ability of H5N1 to induce inflammatory cytokine responses in primary cultures of human alveolar and bronchial epithelial cells.[117] In addition, TNF-related apoptosis-inducing ligand (TRAIL) and TNF mRNA are upregulated in human monocyte-derived macrophages infected with H5N1/97 virus,[118] and higher levels of inflammatory cytokines and chemokines are associated with a fatal outcome.[119] Moreover, a reconstructed version of the strain of influenza virus responsible for massive human mortality in 1918–1919, but not nonvirulent constructs or strains, induces a strong and prolonged pro-inflammatory cytokine response during the fatal infections it causes in mice[120] and macaque monkeys.[121] This approach to understanding influenza disease has continued with the topical nvH1N1,[122] which not surprisingly provides data incorporating the newer Th17 contribution to innate immunity (see Section 9.3.1).

Several years ago, using iNOS immunohistochemistry as a marker for induction of the inflammatory cytokines, such as TNF and IL-1, that induce this enzyme, we compared the degree of microvasculature staining from sections of fatal human cases of falciparum malaria, *Escherichia coli* sepsis, and H5N1/97 influenza.[84] As shown in Figure 9.1, all three were equally stained for iNOS protein, while the control was not.

9.5.1.2 Pox Viruses

The nature of the illness caused by pox viruses provides an interesting example of the competing theories for how pathology can develop. Traditionally these infections were regarded as a race between CD8+T cells killing infected cells and a cytopathic virus killing host cells. By the mid-1990s it was appreciated that both a strong killer T cell response and a range of pro-inflammatory cytokines were generated in severe mousepox (caused by the natural mouse pathogen, ectromelia virus) in certain strains of mice. When, in 2000, mice were infected with ectromelia expressing mouse IL-4, they developed a fulminant infection[123]; this was interpreted as acting through CD8+ killer T cells, being downregulated through suppression of IL-12 and IFN-γ, and

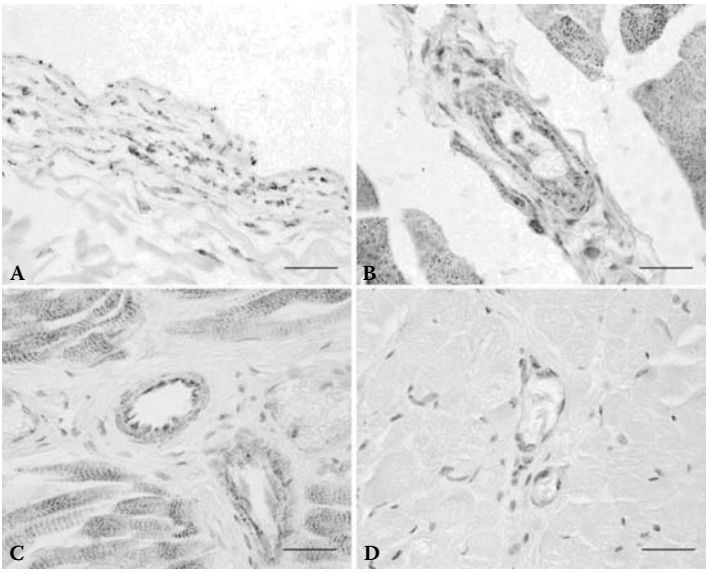

FIGURE 9.1 (See color insert.) Small blood vessel walls from fatal cases of (a) influenza H5N1/97), (b) falciparum malaria, (c) *E. coli* sepsis, and (d) control stained 1:1000 for iNOS. Scale bar, 100μm. (Reproduced from *Travel Medicine and Infectious Disease* 6, 68–81 (2008). With permission.)

death arising from virus-infected cells surviving, so that uncontrolled replication led to organ failure. This has been seen as evidence in favor of the cytopathic argument of disease and death. However, it must be borne in mind that this artificially excessive IL-4 could have been expected to have severely inhibited TNF,[124] thus removing a potential cause of both virus and host death, a pro-inflammatory cytokine excess, or "storm." In 2007 others[125] pointed out that this mechanism has yet to be investigated in mousepox. This approach had been proposed on indirect evidence 3 years earlier in smallpox-infected monkeys,[126] but when cytokine production from peripheral blood mononuclear cells was assayed, it was found that, in contrast to cells from Ebola-infected monkeys, TNF protein was essentially absent.[127] It is unknown whether smallpox generates a strong anti-TNF activity, as does the closely related tanapox virus.[128] Nevertheless, high levels of IFN-γ, known to cause severe general illness in excess,[129] were present in the smallpox infections.

9.6 INFLAMMATION AND CEREBRAL MANIFESTATIONS OF SYSTEMIC DISEASE

It has been general practice, when describing the role of inflammation in infectious disease, to restrict the narrative to the usual systemic manifestations of the condition, with any cerebral aspects discussed elsewhere in specialized articles. In our view the growth of knowledge in the roles of the cytokines that, in excess, mediate inflammation, but also form much of the normal physiological traffic in neurophysiology

as well as systemic physiology, has reached the stage where an appreciable part of a chapter such as this should be devoted to the effects of infectious disease on the brain. As we discuss, much can be learned from the effects of excessive production of these cytokines in apparently noninfectious conditions, mainly Alzheimer's disease, which is increasingly generating a large literature about inflammatory cytokines, particularly TNF.

9.6.1 TNF in Neurophysiology

The best introduction to the pathological effects of these cytokines on brain function comes from an appreciation of the neurophysiological roles, which are briefly summarized here, using TNF as an example. For some time TNF has been realized to be generated not just by microglia, as their macrophage-like character would predict, but also by neurons and astrocytes.[130] Its origins in the tumor literature[17] meant that, in the brain, as elsewhere, TNF was initially regarded only as causing cell death, but nowadays it is evidently a physiological gliotransmitter concerned with normal communication. This particularly occurs between astrocytes and synapses,[131] but also between microglia and synapses,[132] and is therefore evidently central to synapse regulation. TNF thus appears to be central to the tripartite view of synaptic physiology,[133] in which astrocytes have been given a more central role than previously. As an example of growing awareness of its functional capacity, in 2005 the capacity of TNF to rapidly upregulate calcium-permeable amino methyl propionic (AMPA)/kainate channels on neurons, with obvious implications for its capacity to influence synaptic transmission, was reported.[134] In the next year the phenomenon of the strength of all synapses on a cell adjusting in response to prolonged changes in the cell's electrical activity, known as synaptic scaling, was linked to TNF.[132] Subsequently, TNF has been shown to control regulation of the type 1 inositol-1,4,5-trisphosphate receptor (IP_3R), which is central to calcium homeostasis, and calcium-dependent functions, of neurons.[135] More recently, this cytokine has been reported to modulate synaptic plasticity through inducing sphingomyelinase-2, and thus controlling the insertion of N-methyl-D-aspartate (NMDA) receptors into neuron membranes.[136] Additionally, TNF can be expected to alter synaptic function through its striking ability to regulate the morphology of synapses.[137]

Key physiological roles of TNF in astrocytes are also being more appreciated. These predominant glial cells of the central nervous system are now being realized to have the potential to orchestrate the brain as an entity, with processes of one astrocyte contacting tens of thousands of synapses, and controlling discrete large territories.[138] While astrocytes do not generate action potentials, they nevertheless have the capacity to communicate well with neurons.[139] As has been recently reviewed,[140,141] astrocyte origin TNF normally stimulates the release of glutamate from these cells into the synapse microenvironment through selective activation of purinergic P2Y1 receptors on their cellular membranes.[142] From these examples, it is clear that TNF has to be within homeostatic limits for the brain to function normally.

Systemic infectious diseases dominate thinking about excess production of proinflammatory cytokines in disease pathogenesis, but the CNS provides two important conditions, brain trauma and Alzheimer's disease, that demonstrate how helpful

it is, when trying to understand inflammation and disease, not to draw a line between organ pathology obviously caused by pathogens and that which is not infectious. Given the physiological roles of cytokines in normal brain (TNF is the most studied, and probably as important as any, since it initiates cascades of other potentially harmful cytokines), all that is required to generate recognizably similar pathology is a source of excess levels of this cytokine in the brain, whether the TNF is triggered by products of infectious agents or something endogenous. Whenever TNF is known to be present in more than physiological concentrations, there will be fundamental similarities in clinical outcomes, although it is clear that they are not part of an infectious disease. Despite their acknowledged broad links with infectious disease, inflammatory cytokines are probably most studied in the brain in Alzheimer's disease (considered noninfectious) and local traumatic injury (certainly noninfectious). The most useful way to think of all of these cerebral conditions is as encephalopathies induced by an excessive concentration of cytokines that are normally present at that site, fulfilling physiological roles.

9.6.2 Inflammatory Cytokines and the Pathogenesis of Alzheimer's Disease

As has been reviewed,[143] the concept of a role for inflammation in Alzheimer's disease has been proposed for many years. The point has often been raised that it might be a secondary response to another primary mechanism, such as amyloid β (Aβ) formation,[144] although the evidence is now firmly in favor of it having a very early, and most likely primary, role. For instance, when TNF levels were assayed in cerebral spinal fluid (CSF) from 56 individuals who had mild cognitive impairment, a condition that can develop into frank Alzheimer's disease, and 25 age-matched controls, those with higher TNF levels proved much more likely to develop Alzheimer's disease.[145] Another group, taking advantage of the increased sensitivity of assaying for soluble TNF receptors rather than TNF itself, found that their levels in serum and CSF predicted, over a 4- to 6-year period, conversion to clinical Alzheimer's disease.[146] Others have studied plasma levels of C-reactive protein (CRP) and α1-antichymotrypsin (ACT),[147] or CRP alone,[148,149] and found that these markers of inflammation were present in serum and CSF before any indications of increased Aβ or tau. ACT and CRP are examples of positive acute phase proteins, which are upregulated by TNF or interleukin-1.

These relatively limited acute phase protein studies have now been extended and confirmed by an impressive report of plasma levels of another acute phase protein, clusterin (apolipoprotein J), being intimately associated with onset, progression, and severity of this disease.[150] A novel and impressive proteomic neuroimaging paradigm was employed. Unfortunately, the authors speak only of the amyloid chaperone function of clusterin, and seem to have been unaware of its role as an acute phase protein,[151] and therefore a marker, as surely as are CRP and ACT, of inflammation. One of their more telling findings was that clusterin was raised 10 years earlier than fibrillar Aβ deposition. Taken together, these arguments are consistent with inflammation being the primary instigator of Alzheimer's disease.

As discussed below, this approach is quite compatible with the past dominance of the Alzheimer's literature by Aβ, since TNF is intimately associated with its production and downstream effects. In brief, the bulk of the literature on amyloid precursor protein (APP; one of a family of highly conserved transmembrane proteins) is on developmental embryology. It is present throughout fetal development, peaking in the last few weeks of gestation and disappearing a week or so later.[152–154] It is essential for normal brain development, including the neuronal and axonal pruning that normally occurs during the weeks when its levels are highest. Recent work has demonstrated that this pruning is a consequence of a cleaved fragment of APP occupying a previously orphan receptor, termed DR6, in the brain.[155] What is absent in these reports is an appreciation that TNF is normally high in the mammalian brain at this time,[156,157] and that induction of APP by inflammatory cytokines, including IL-1, is a widespread phenomenon, having been reported in endothelial cells,[158] skeletal muscle,[159] and 3T3 L1 adipocytes[160] as well as brain.[161,162] Increased APP in the brain in AIDS dementia[163] (Section 9.1) and cerebral malaria[164] (Section 9.1), as well as Alzheimer's disease, is a further indication of the involvement of the same cytokine-induced pathways in both infectious and noninfectious encephalopathies. This effect of TNF is consistent with its high levels in the brain at this time being the ultimate cause of the neuronal and axonal trimming during development, with implications for the same process occurring in adults during Alzheimer's disease and the postcerebral malaria syndrome, which exhibits various neurological deficits.[165]

Normal metabolism of APP generates Aβ. Much present research into the pathogenesis of Alzheimer's disease has the goal of removing or deactivating this molecule, although it will be interesting to see how much this focus changes with recent evidence that physiological levels are not harmful, but a useful part of innate immunity.[166] In this it follows the pattern of LL37, a cathelicidin with a similar structure.[167] In brief, the generation of Aβ arises through the proteolysis of APP by the sequential actions of β- and γ-secretases. In 2004 it was shown that IFN-γ, IL-1β, and TNF specifically stimulate γ-secretase activity, with an accompanying increased production of Aβ.[168] Several years later IFN-γ and TNF were shown to enhance Aβ production from APP-expressing astrocytes and cortical neurons, and the numbers of astrocytes expressing IFN-γ had increased.[169] In addition, this group showed that TNF directly stimulated β-site APP-cleaving enzyme (BACE-1, or β-secretase) expression, and thus enhanced β-site processing of APP in astrocytes, and TNFR1 depletion reduced BACE-1 activity.[170] Taken together, these data imply that anti-TNF agents should be effective APP cleavage inhibitors. Results obtained in a mouse Alzheimer's model after long-term inhibition of TNF are functionally consistent with this.[171] The studies summarized here are consistent with physiological levels of TNF in the brain maintaining APP and Aβ homeostasis, and excessive TNF generation, from whatever origin, upsetting this to the detriment of synaptic function because it generates Aβ too rapidly.

Generation of Aβ has traditionally been considered the key to understanding Alzheimer's disease, since it has been taken, and still is in many quarters, to cause neuronal pathology directly. However, it is now realized that inflammatory cytokines such as TNF mediate events downstream of Aβ, as well as its formation (see above). Nearly a decade ago TNF was reported to alter synaptic transmission

in hippocampal slices.[172] Several years later[173,174] it was shown that this earlier observation explained the ability of Aβ, through TNF, to do the same. Other laboratories expanded the roles of TNF in this context.[175,176] The capacity of Aβ to act as a ligand for CD14 and TLR2 (Toll-like receptor 2)[177–179] makes these findings on Aβ[173,174] consistent with basic immunology, since for about a decade it has been accepted that occupying CD14 and TLRs is how the usual bacterial and protozoal origin inducers of TNF operate.[180]

Thus, TNF appears to have three interlocking pathogenic roles in encephalopathies in which its brain levels are raised. The first upregulates APP levels to what they were during early neonatal life, fooling physiological reflexes to cause pathology. The second participates in driving APP to Aβ, and the third, induced by Aβ, apparently mediates its key harmful effects. An obvious implication of this chain of events is that TNF induced by Aβ might be expected to provide a positive feedback for generating additional Aβ, thus giving Alzheimer's disease its characteristic slow downward spiral. In contrast, the explanation for encephalopathies associated with infectious diseases not having an inevitable downward trajectory can be explained by the supply of pathogen-induced TNF (and functionally similar cytokines) drying up once the organism is beaten by either the immune response or specific therapy. Figure 9.2 summarizes this newer inflammation-based Alzheimer's literature, for which there is every reason to assume, unless proved otherwise, that parallels exist in brain trauma (below) and the infectious disease encephalopathies.

9.6.3 Inflammatory Cytokines and the Pathogenesis of Brain Trauma

Another noninfectious encephalopathy useful to consider before discussing those caused by pathogens is brain trauma. Tissue levels of TNF are well recognized to be high in trauma,[181] including that affecting the brain. The trigger for posttrauma TNF has been uncertain until recently. Intriguingly, the most plausible explanation seems not to be completely removed from infectious organisms as a by-product of the successful revival of the notion that organelles are microbial in origin.[182] This is consistent with the recent observation that mitochondrial DNA (mtDNA), unlike mammalian DNA but like bacterial DNA, is high in unmethylated CpG repeats, and therefore, with the assistance of the formyl peptides, also released from mitochondria as cells are damaged during trauma, induces TNF.[183] Thus, what might be termed a misguided innate immune response is initiated and, as in other circumstances, causes pathology if overexuberant. This is consistent with the protective action of intracerebral TNF inhibition[184] and IL-10, an anti-inflammatory cytokine[185] in brain trauma.

9.7 THE DYNAMICS OF SYSTEMIC AND CEREBRAL TNF

Before discussing individual diseases, literature that addresses what can happen to the brain when systemic TNF levels (doubtlessly other similar cytokines as well) become too high is summarized here. In 1997 it was reported that experimental noncerebral inflammation influences the hypothalamo–pituitary–adrenal axis via TNF signaling within the brain.[186] Evidence that i.p. injection of LPS generates transcripts of inflammatory cytokines within the brain also appeared in that year.[187] In 2006

FIGURE 9.2 Summary of interactions between TNF, amyloid β, microbial growth and neuronal function in physiology and disease, with reference numbers from text.

a mouse model of liver failure subsequent to cholestasis was used to demonstrate that TNF-secreting monocytes migrate from the periphery to the brain, and microglia are triggered to release this same mediator soon afterwards.[188]

Observations on interactivity of systemic and cerebral inflammation led Perry and coworkers[189] to propose in 2007 that successive systemic inflammatory events might cause and exacerbate neurological malfunction that is associated with an excessive cerebral innate immune response. Soon afterwards, serum levels of IL-6, a stable cytokine induced by both TNF and IL-1,[190] were reported to be inversely related to hippocampal grey matter size in middle-aged human volunteers.[191] Perry's group has since published mouse[192] and human[193] data consistent with the original proposal. A recent experimental example is colonic inflammation in the rat inducing cells in the hippocampus to generate TNF.[194] This TNF level was functionally significant, since infusion of TNF antibody into the cerebral ventricles prevented the seizures associated with gut inflammation, but not the degree of gut inflammation itself. Likewise, the anxiety state present in recent studies in mice with a chronic gut inflammation caused by *Trichuris muris* infection (used as a model to understand the human equivalent that is common and can be severe in any chronic gut inflammation[195]) has been demonstrated to be ablated by etanercept, a commercial anti-TNF antibody-based construct.[196]

At least four pathways have been studied to understand the link between systemic and cerebral cytokine production. The first is the demonstration of saturable specific transporters for TNF across the blood–brain barrier, detected in the mouse some time ago.[197] The second concerns the difference in duration, brain production of these host origin mediators being much more persistent than systemic production when LPS is injected intraperitoneally. Remarkably, mouse brain TNF production remains high for at least 10 months after a single systemic LPS injection, whereas serum levels peaked at 9 hours.[198] This outcome is consistent with earlier work in which the TNF switch-off that occurs systemically after a second LPS injection (the basis of LPS tolerance) proved to be absent in the CSF.[199] Thus, TNF production evidently persists in the brain for much longer than in the periphery, probably because the pathways that control it in the periphery are absent within the CNS, in which innate immunity dominates. A possible mechanism comes from work on IL-1 antagonists, which would be relevant for TNF as well, being weaker in brain than systemically.[200]

The third line of inquiry concerns migrating monocytes being attracted across to the brain by a chemokine originating from the microglia.[201] Brain pericyte detachment from the basal lamina after i.p. LPS[202] suggests a likely route. A literature also exists on monocyte migration to the brain in Alzheimer's models, under the influence of the same chemokine.[203] Clearly, this could seed the brain for excess TNF production[188] and have it persist there in any circumstance where TNF is generated, infectious or noninfectious, and affect brain function accordingly. Malaria is the most common example of an infectious equivalent, as discussed in Section 9.1. A fourth approach is based on the site of induction of matrix metalloproteinase-9 (MMP-9), one of a family of zinc-dependent proteolytic enzymes that can degrade the extracellular matrix. A recent publication[204] has reported rapidly increased brain and CSF levels of MMP-9 after intravenous injection of TNF into mice. Since serum concentrations of MMP-9 are much greater in influenza with encephalopathy than without it,[205] the authors[204] explained their data through the known ability of

high-serum MMP-9 to increase blood–brain permeability, allowing TNF to enter the brain and, among other actions, increase local MMP-9. Clearly, this argument is directed at explaining the severe end of the spectrum, such as acute influenza or malaria encephalopathy, characterized by sudden onset and cerebral edema or increased intracerebral pressure.

9.8 EXAMPLES OF SYSTEMIC INFECTIOUS DISEASES WITH AN ENCEPHALOPATHY COMPONENT

9.8.1 Inflammatory Cytokines in the Pathogenesis of AIDS Dementia

At one level, AIDS dementia is an important practical clinical challenge needing an answer. In this context it can be viewed as another encephalopathy that is part of a systemic infectious disease, with much data on inflammatory cytokines, particularly TNF, connecting the two and explaining the syndrome. Thus, at this level its logic sits alongside the acute encephalopathies, such as those caused by other viruses, bacteria, and hemoprotozoa, discussed in this section. At another level the chronicity of AIDS dementia gives it an affinity with Section 9.6, since it is an extremely instructive model for how chronic systemic inflammation, defined in terms of increases in cytokines that mediate normal functions as well as inflammation, can extend to the brain, in either infectious or noninfectious states, and alter its function.

Since 1992, a number of researchers have reported higher TNF[206] and TNF receptor levels[207] in CSF of patients with AIDS dementia than in controls. In that year TNF generation was noted to be intrathecal[208] as well as systemic. At about this time it was reported that the gp120 envelope protein of HIV induces cells to generate TNF.[209] Curiously, production has been noted in microglia neighboring HIV-infected cells, rarely those microglia themselves.[210] Although levels can be higher in patients with secondary CNS infections,[206,207] they are also increased when the only infection is with HIV.[207] It has also been noted that the presence of the A allele at the TNF-α-308 site associated with overproduction of TNF is overrepresented among adults with HIV dementia compared to those without dementia.[211,212] One example of the interesting ramifications of the presence of excess cerebral TNF in AIDS dementia is that the capacity of this cytokine to arrest neurogenesis[213] provides a plausible explanation for its presence in this disease.[214]

It should also be noted that IL-1β, a fellow traveler with TNF in many circumstances, is also present in CSF of AIDS dementia patients,[215] and from these data might be the more sensitive indicator. Of particular interest is the association between the epsilon 4 variant of apolipoprotein E and AIDS dementia,[216] at least in older patients.[217] The wider ramifications of this association for this and the other disease states, infectious and noninfectious, are summarized in Section 9.9.

9.8.2 Inflammatory Cytokines in the Pathogenesis of Malaria Encephalopathy

Malaria is probably the most conspicuous and historic of these conditions. The systemic aspects of this disease were discussed earlier (Section 9.4.2). Its

encephalopathy component, or cerebral malaria, has a mouse model seen, in some mouse strains only, during infections with a particular strain of *Plasmodium berghei*, termed *P. berghei* ANKA. While this parasite was instrumental in the TNF disease model[218] being extended to the cerebral aspects of the disease, the arguments it engendered became less secure when LT knockout mice, but not TNF knockout mice, were protected from the disease.[219] In addition, the traditional basis for human cerebral malaria has been sequestration of parasitized erythrocytes blocking blood flow, whereas in the *P. berghei* ANKA mouse model it is, instead, monocytes that sequester. For these reasons we will restrict our discussion to the human disease.

Five species of malaria are recorded as causing human disease, and we shall concentrate on the two that are by far the most common, those caused by *Plasmodium falciparum* and *Plasmodium vivax*, and termed falciparum malaria and vivax malaria, respectively. Although regarded as clinically indistinguishable in their early and middle stages,[220] the general impression over the decades has been that vivax malaria is rarely fatal and does not lead to the range of complications and fatal outcome that occur in naive falciparum-infected individuals not treated early enough. The cornerstone with which to explain this difference has been that red cells containing later stage *P. falciparum* sequester on, i.e., adhere to, the walls of small blood vessels, thus restricting oxygen and nutrient supply. The symmetry of the argument included the observation that *P. vivax*, which did not cause the red cells it inhabits to adhere appreciably, caused negligible fatal outcome. Hence, the literature on malarial fatality is always about adherence and blood flow obstruction. Any malaria textbook worth its salt from the last 100 years has depicted this difference with a drawing of the erythrocytic stages of the various human malaria parasites in peripheral blood smears. Later stages of the erythrocytic cycle are always illustrated in smears from cases of vivax, but not of falciparum malaria, because in infection with this parasite they are sequestered in the "deep" vasculature, hindering blood flow and thus causing death.

This traditional explanation of events has been questioned in many Indian clinical reports of the nonsequestering species, vivax, causing complications that included cerebral malaria (i.e., encephalopathy).[221,222] These cases seem to have been largely dismissed as somehow misdiagnosed falciparum malaria, even in recent papers where vivax monoinfection was confirmed by polymerase chain reaction (PCR) diagnostics. The same technology was again applied in a recent publication of an additional 40 cases of severe vivax malaria,[223] which reports a range of organ dysfunctions, often simultaneously, including coma, all of which are traditionally regarded as consequences of sequestration. In 2007 a study in Indonesian New Guinea that admitted nearly 6,000 patients found no difference in mortality between falciparum and vivax malaria.[224] Since it was a retrospective study, clinical aspects, such as coma, were little discussed. What seems to have really swayed the field was presented in two large independent studies, one from each side of the border down the middle of the island of New Guinea.[225,226] The former, over an 8-year period, involved about 9,500 confirmed cases of severe malaria in a rural health center setting, and the latter about 12,000 confirmed cases admitted to hospital over a 4-year period. The proportion of patients exhibiting signs of encephalopathy (impaired consciousness specified in one study[226]) were essentially the same in each

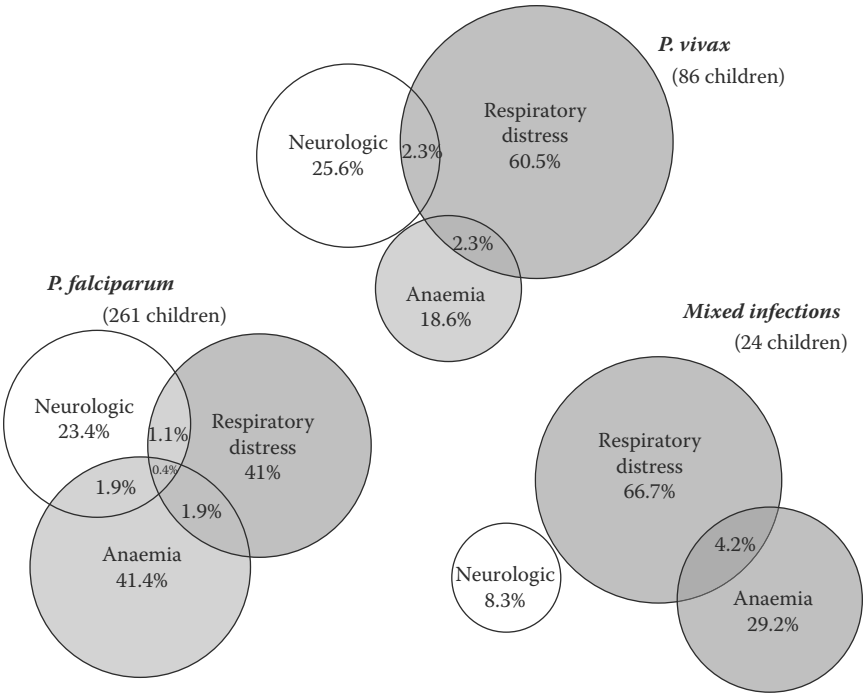

FIGURE 9.3 Comparisons of the incidence of neurological signs in children in Papua New Guinea infected with *P. fakciparum* and *P. vivax*, two species of malaria parasites with very different capacities to adhere to endothelium, and thus block cerebral blood flow. (Reproduced from *PLoS Medicine* 5, e127 (2008).)

study (23.4% for falciparum and 25.6% for vivax[225] (Figure 9.3), 30% for falciparum, and 39% for vivax[226]), and mortality was very similar at both sites.

It is likely that the severe drug resistance of *P. vivax* in New Guinea might account for the differences in mortality between this country and Thailand.[227] It seems impossible, other than through the unlikely revelation that *P. vivax* malaria sequesters as densely as does *P. falciparum*, to interpret the same incidence of encephalopathy in both parasites as a consequence of vascular blockage. The newest *in vitro* data suggest one-tenth the concentration.[228] It therefore seems that, as for the encephalopathies in other systemic infectious diseases (Sections 9.2 and 9.3), its origin in malaria requires something more subtle than variants of the traditional vascular blockage theme. As discussed in the rest of this section, and expanded in a number of reviews, we see malarial encephalopathy as essentially no different, in terms of its pathogenesis, from septic or viral encephalopathy. We have previously laid out the arguments that a cerebral focus of sequestration, when it occurs, is a secondary phenomenon of high levels of inflammatory cytokines.[71,229] It may then hasten death through hypoxia, but cerebral sequestration is not always present,[80] and evidently human malaria can be just as lethal when sequestration is negligible.[225,226]

The literature on assaying TNF in falciparum malaria patients has some interesting complexities, arising from the severe form of this disease in African children,

who are the most studied group, typically having severe systemic disease as well as encephalopathy. This combination has usually been referred to as cerebral malaria, and its severity has typically correlated with circulating TNF levels.[77,230] However, when the encephalopathy is considered in isolation, TNF levels in the CSF, not serum, correlate with it, and also with the residual neurological deficit that often occurs.[231] Thus, examining if anti-TNF agents, such as those used in rheumatoid arthritis, counter the encephalopathy of malaria and the other diseases in these sections seems warranted. Furthermore, it seems logical to administer these agents to ensure they reach the CSF, as has so far been done only in an open trial on Alzheimer's disease.[232]

9.8.3 Inflammatory Cytokines in the Pathogenesis of Septic Encephalopathies

Septic encephalopathy is a syndrome commonly seen in intensive care.[233] It has been characterized as an encephalopathy showing a diffuse disturbance of cerebral function (typically impairment of consciousness) that occurs in the context of a systemic response to bacterial infection without direct neuroinvasion, with the depth of coma linked with mortality.[234] Mild cases often recover completely, while survivors of severe cases may have persistent neurological deficit.[235] In line with adult CM, the severity of encephalopathy parallels the severity of systemic organ failure.[235] Clearly, it has many features that resemble cerebral malaria.

A parallel entity, much less investigated, accompanies noninfectious conditions in which inflammatory cytokines are raised, such as traumatic surgery,[236,237] pancreatitis,[238,239] and burn injury.[240,241] When studying LPS-injected rats, a standard sepsis model, it had been found[242] that the transcription of several pro- and anti-inflammatory cytokines and chemokines, including TNF and IL-1β, was raised in the cerebrum. A pancreatitis model in which prior administration of a neutralizing antibody to TNF prevented encephalopathy[243] is consistent with these cytokines being central to its pathogenesis. This principle is confirmed for all these brain dysfunctions through observations that TNFR1 knockout mice are protected from LPS-induced encephalopathy.[345] Below, neurobrucellosis is given as an example of how the right approach to a rare problem can sometimes provide data consistent across a wide range of circumstances.

9.8.3.1 Neurobrucellosis

This particular septic encephalopathy has been singled out because informative studies have been reported. Central nervous system involvement is a serious, rare but instructive, complication of brucellosis, a highly contagious zoonosis typically caught by ingesting *Brucella abortus* along with unsterilized meat or milk from ruminants. It is now rare outside less developed countries that have not eradicated this pathogen from their cattle populations. Termed neurobrucellosis, this disease state can exhibit highly significant impairment in some cognitive function measures (mental control, logical memory, visual reproduction) and higher scores on depressive symptoms,[244] all of which are characteristic of Alzheimer's disease. Depressed scores can be

readily documented by the Mini-Mental State Examination (MMSE) and Hamilton Depression Rating Scale (HDRS) tests used in Alzheimer's and depression, and killing the pathogen with antibiotics returns the test values to normal.[245]

The organism is a small Gram-negative rod that lives intracellularly, contains lipoproteins that function like LPS,[246] and therefore generates much TNF. The syndrome it generates (headache, fever, sweating, weight loss, and back pain[247]) is consistent with this, and for the same reasons characteristically observed in influenza or malaria. It warrants mentioning in this section on encephalopathies caused by inflammatory cytokines because of the cerebral syndrome it produces should the organism itself enter the brain, and generate TNF there in microglia and astrocytes.[248] Mechanisms such as passage of TNF across the blood–brain barrier by a saturable carrier[197] or entry of TNF-producing monocytes,[201] could instead, or in addition, increase brain TNF to pathological levels, producing this Alzheimer's-like syndrome.

9.8.4 Inflammatory Cytokines in the Pathogenesis of Viral Encephalopathies

9.8.4.1 Influenza Encephalopathy

The encephalopathy associated with influenza is a serious complication of this disease. It mostly affects young children, although it has been reported in adults,[249] and is for unexplained reasons more common in Asia, particularly Japan.[250,251] Changes range from aphasia and altered consciousness to coma, and neurological deficits,[252] yet there is no evidence of neuroinvasion by influenza virus.[253] The cerebral malaria-like condition was extensively reviewed in 2008.[254]

Many data are consistent with it being a cytokine-generated disease, with, as discussed elsewhere in this chapter, the same logic behind it as the encephalopathies seen in bacterial and protozoal diseases, as well as those caused by other viruses. Serum TNF and soluble TNF receptor 1 (sTNFR1) correlate statistically with severity.[255] However, serum levels of the TNF-induced cytokine, IL-6 (which remains detectable much longer than does TNF), evidently have an excellent predictor value—levels were >6,000 pg/ml in children with brain dysfunction, about 150 pg/ml in children without brain dysfunction, and <80 pg/ml in controls. Likewise, once the serum IL-6 level was increased to >15,000 pg/ml, none of the children survived, and the lower the maximal serum IL-6 level, the milder the CNS sequelae.[255] Subsequently, another group focused on the CSF, and reported that levels of TNF, sTNFR1, and IL-6 in that compartment all correlated well, and negatively, with clinical outcome.[256] The same group later examined NF-κB activity in circulating monocytes and found it to be a useful indicator of severity.[257] This is consistent with the evidence of TNF-generating monocytes gaining access to the CSF.[201] Other mechanisms of plasma cytokine transfer that would upset cerebral TNF homeostasis[197] (Section 9.7) are also plausible, although not yet studied in patients so far as we are aware. A single case of acute necrotizing encephalopathy, a severe subtype of encephalopathy with extensive bilateral necrotic lesions, has recently been reported[258] in a 2-year-old child infected with nvH1N1 influenza. This condition has previously been associated with abnormally high TNF levels.[259]

9.8.4.2 Dengue Encephalopathy

Dengue is a disease caused by four closely related virus serotypes belonging to the genus *Flavivirus* and spread by the mosquito *Aedes aegypti*. Some 200 million people are at risk in tropical and subtropical countries. It has a mild form termed dengue fever, and a more severe form, dengue hemorrhagic fever (DHF). Death is not uncommon. Plasma levels of TNF have been known for some time to be increased in both forms.[113,260,261] In the early 1990s the term *dengue encephalopathy* began to be employed for cases of DHF with cerebral involvement, and studies on the condition are regularly published.[262–264] For some time the encephalopathy has been thought to be associated with dengue-induced liver failure.[262,265] Since liver failure of whatever cause can be associated with an encephalopathy (hepatic encephalopathy)[266] and with increased TNF (but not IL-1α or IL-1β),[267,268] it seems that this liver origin TNF would add to the virus-induced TNF. The functional importance of this is demonstrated by the report that anti-TNF antibody treatment reduces mortality in experimental dengue infection.[269] Strong circumstantial evidence suggests that this excess TNF is the basis of brain dysfunction, or encephalopathy, seen in dengue, and does so through placing the essential physiological brain functions of this cytokine (see Section 9.6.1) in disarray. There is no evidence at present for TNF being produced in the brain in dengue, but the evidence within Section 9.7 on systemic TNF entering the brain and inducing more to be produced there makes it a reasonable prediction. The same principles would apply, as discussed, to the other conditions in this section.

9.9 THE IMPLICATIONS OF APOE ASSOCIATIONS FOR UNDERSTANDING INFLAMMATORY DISEASES

Apolipoprotein E protein (apoE) is expressed in humans as three common isoforms, E2, E3, and E4, and in 1993 it was argued that *apoE4* is a susceptibility gene with a highly significant linkage with late-onset and familial Alzheimer's disease.[270] Many studies have shown that the APOE4 allele also plays an important role in much other human disease pathophysiology. In 1995 it was reported that apoE4-positive patients who had experienced head trauma were 10 times more likely to develop Alzheimer's disease, whereas apoE4 positives with no head trauma history have double the risk.[271] As noted above,[216,217] apoE4 positivity is also associated with excess dementia in HIV infection. It has been noted to be associated with earlier onset of Parkinson's disease.[272] Furthermore, it is also associated with the dementia seen in high-exposure professional boxers[273] and doubles the duration of delirium in septic encephalopathy patients in intensive care units.[274] These examples argue that whatever is going on affects both infectious and noninfectious disease.

Because apolipoproteins regulate lipid metabolism, APOE4 was initially assumed to cause pathology by disturbing these pathways. However, in 1997 Laskowitz and coworkers[275] unexpectedly reported that either APOE3 or APOE4 would inhibit the *in vitro* ability of astrocytes to produce TNF, with APOE4 being marginally, but not significantly, less active. The importance of this phenomenon *in vivo* was established over the next 2 years when APOE (–/–) mice were shown to be extremely sensitive to a fatal

outcome when infected with *Listeria monocytogenes*[276] or *Klebsiella pneumoniae*,[277] and make much more TNF than infected controls. In 2001 Lynch et al.[278] extended this, both to the CNS and further cytokines, by demonstrating that apoE downregulates CNS production of TNF, IL-1β, and IL-6 mRNA following stimulation with LPS.

The difference between APOE3 and APOE4 began to be understood in 1995 when Ophir et al.[279] studied the effects of apoE genotype on hippocampal gene expression in LPS-treated mice, transgenic for either apoE4 or the benign allele, apoE3. These authors found that the expression of inflammation-related genes following intracerebroventricular injection of LPS was significantly higher and more prolonged in apoE4 than in apoE3 transgenic mice. Moreover, gene clusters that responded differently in apoE4 and apoE3 mice and were significantly enriched in NF-kappaB response elements, and measurement of NF-kappaB-regulated genes revealed that their activation was greater in the apoE4 mice. An additional essential insight was obtained when Riddell and coworkers[280] used transgenic mice to demonstrate that astrocytes preferentially degrade APOE4, leading to reduced APOE4 secretion, and thus reduced brain levels of total APOE. As clearly presented in a recent review,[281] which also argues for innate differences in the activity of APOE3 and APOE4, the neuropathology of diseases that associate with APOE4 can therefore be expected to have been caused by excessive production of inflammatory cytokines. In terms of this chapter, this includes all the conditions in Section 9.8.

9.10 RATIONAL ANTIDISEASE TREATMENTS BASED ON THESE PRINCIPLES

Since it has become evident that inflammatory cytokines are responsible for much disease pathology, agents with a history of reducing the production of these mediators, or neutralizing their effects in other ways, have come to the fore as treatments. A rationale for neutralizing TNF and similar cytokines indirectly, through inhibiting the pathway that generates them, depends on potential advantages based on either cost, administration by a more convenient route, or both. Disadvantages include having other actions as well, and not being as potent as agents that are expensive, but specifically neutralize TNF itself. These will be discussed below. Possible alternative approaches, cheaper and simpler to administer, may nevertheless have their place, and are summarized briefly here. Alzheimer's models have often been used to test them, no doubt because of the scale of the problem this disease presents. Nevertheless, they are relevant, in principle, to all the other cytokine-driven pathologies, systemic as well as cerebral, infectious and noninfectious, discussed in this review.

9.10.1 Thalidomide, Resveratrol, and Curcumin

Notwithstanding its harmful effects on the developing fetus, the anti-inflammatory properties of thalidomide and its potential use in patients other than women of child-bearing age have been widely explored. In summary, it reduces TNF levels[282,283] and protects mice against LPS shock,[284] largely a TNF-mediated phenomenon. There is considerable research on generating thalidomide derivatives to focus and

refine its anti-TNF properties.[285–287] In addition, mouse Alzheimer's models have been explored.[288,289]

A polyphenol of plant origin, termed resveratrol, is noted to inhibit TNF production in microglia[290] and monocytes.[291] There are two recent reports of its efficacy in brain trauma.[292,293] Curcumin, an extract of the Indian spice turmeric, has a considerable history as an anti-inflammatory agent. In terms of Alzheimer's disease, proposed mechanisms of action have been as an antioxidant[294] and to suppress NF-kappaB,[295] and therefore produce pro-inflammatory cytokines. We note that a 12-month, randomized, placebo-controlled study of oral curcumin for mild to moderate Alzheimer's disease was reported to have failed to show benefit (http://www.clinicaltrials.gov/ct2/show/NCT00099710). Nevertheless, its positivity in so many types of laboratory trials implies that this anti-inflammatory approach will continue.

9.10.2 Tacrolimus, Siromilus, and Imipramine

Tacrolimus (FK506) is widely used clinically as an immunosuppressive agent to prevent rejection of organ transplants. It is thought to function through inhibiting the activity of calcineurin (protein phosphatase 2B), a widespread enzyme that, apart from involvement in T cell activation, is the most abundant phosphatase in the central nervous system.[296] In addition, inflammatory cytokines, including TNF, induce various calcineurin-dependent activities,[297] so inhibiting calcineurin can be expected to inhibit these downstream functions of TNF. Thus, tacrolimus has been employed against inflammatory bowel disease[298] and rheumatoid arthritis,[299] as have anti-TNF agents. Tacrolimus has a long history of patient use, but it inhibits a number of important cytokines in addition to TNF.[300] This has implications that would require further exploration before considering long-term use. It could be counterproductive, for instance, to downregulate the anti-inflammatory cytokine IL-4. Likewise, long-term use of tacrolimus carries a risk of nephrotoxicity[300,301] during a chronic inflammatory disease, but it seems reasonable to consider its usefulness in more acute events, such as brain trauma or infectious disease encephalopathies.

Siromilus (rapamycin), a macrolide antibiotic generated by *Streptomyces hygroscopicus*, was first identified as an immunosuppressant about a decade ago,[302] and more recently has been shown to inhibit TNF production.[303–308] Again, like tacrolimus, sirolimus has recently been reported to abolish cognitive deficits in mouse models of Alzheimer's disease.[309,310] Thus, tacrolimus and sirolimus, two agents presently in use for their immunosuppressive activity, join imipramine,[311] an antidepressant, as drugs already in clinical use for other purposes that, through their TNF-suppressing qualities, serve as plausible cases for testing against the encephalopathies discussed in this review. Toxicity[307] might well preclude the use of siromilus in states requiring long-term treatment, but short-term treatment in brain trauma, and certain encephalopathies induced by infectious agents shows brighter prospects.

9.10.3 Rosiglitazone and Gemfibrozil

The peroxisome proliferator-activated receptor-gamma (PPAR-γ) is a member of the nuclear receptor superfamily of ligand-dependent transcription factors. They have

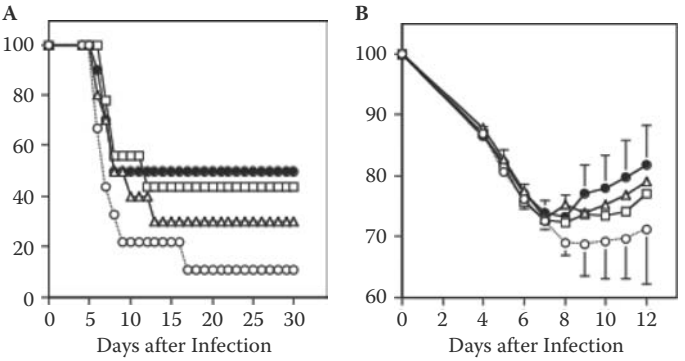

FIGURE 9.4 Dose-dependent effect of gemfibrozil on survival (A) and body weight (B) of BALB/c mice with severe influenza. Mice were infected with influenza virus A/Japan/305/57 and treated with vehicle (○) or 20 mg/kg (△), 40 mg/kg (□), or 60 mg/kg (■) gemfibrozil on days 4 to 10 after infection. (Reproduced from *Antimicrobial Agents and Chemotherapy* 51, 2965–2968 (2007). With permission.)

been shown to inhibit TNF release and downregulate macrophage activation,[312] as well as impair the production of inflammatory cytokines, including TNF, from monocytes.[313] These agents are also active in the brain.[314] Glitazones, a class of drugs that are agonists of PPAR-γ, reduce the ability of both LPS and Aβ to activate monocytes and microglia, and inhibit TNF production from cells of this lineage.[315] Thus, glitazones are possible therapies for the pathologies described in this chapter through their anti-inflammatory properties.[316]

Others[317] have recently reported that rosiglitazone rescued memory impairment in a transgenic mouse model of Alzheimer's disease, and, like Landreth and coworkers,[316,318] have argued the outcome in terms of inhibiting expression of pro-inflammatory cytokines, such as TNF, which they assayed. Likewise, recent data on roziglitazone improving survival in malaria[319] are based entirely on the rationale of the observed reduction in TNF levels. We have similar data with the same rationale, but a different pathogen, in which mice have been protected against highly virulent mouse influenza by gemfibrozil, a functionally similar PPAR-α agonist[320] (Figure 9.4) or rosiglitazone (Figure 9.5; L. M. Alleva, unpublished). A phase III trial of rosiglitazone is presently in progress in Alzheimer's patients (see http://www.alzforum.org/drg/drc/detail.asp?id=116), whereas imipramine, tacrolimus, and sirolimus (Section 9.10.2) have not yet reached this stage of testing in any disease.

9.10.4 Neutralizing TNF Once It Is Produced

In view of the importance of inflammatory cytokines in infectious disease, neutralizing excess TNF with agents specifically designed for the purpose is an obvious treatment. The products presently available are infliximab (Remicade), a monoclonal murine-human chimeric antibody to TNF; etanercept (Enbrel), a fusion protein of two p75 chains of the TNF receptor II and the Fc portion of IgG1; and adalimumab (Humira), a fully human anti-TNF monoclonal antibody. So far as we

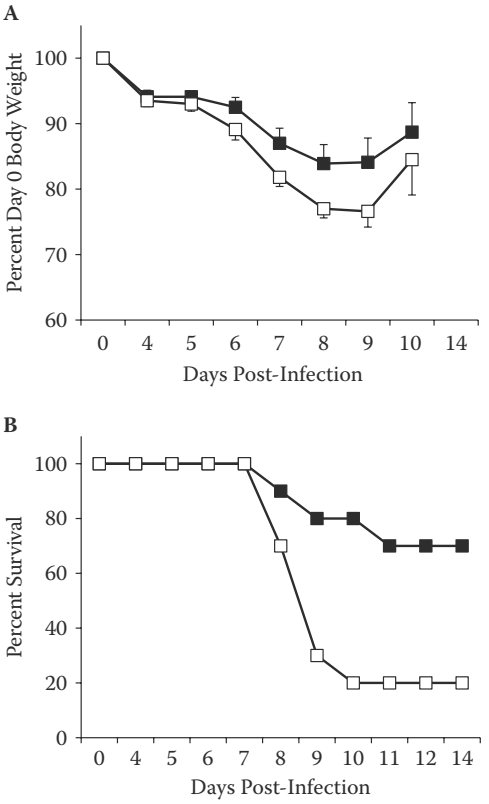

FIGURE 9.5 The effect of rosiglitazone on the morbidity and mortality of mice infected with A/PR/8/34 (PR8) influenza virus. Female BALB/c mice, 6-7 weeks old, were treated by oral dosing with either 150 mg/kg rosiglitazone (closed squares) or PBS vehicle (open squares) in a 20 µl volume (n = 10 mice per group). Treatment began before infection on day -4, then mice were dosed again on day -2 and day -1. Mice were anesthetized with isoflurane on day 0 and infected with 50 pfu per mouse via intranasal instillation. All mice were treated daily with rosiglitazone or vehicle alone until day 10 post-infection. Mice treated with rosiglitazone lost less weight than control mice (A) and fewer mice treated with rosiglitazone died (B). The cut-off for euthanasia was 25% weight loss, as approved by the Animal Experimentation Committee at the Australian National University. We thank Dr. Edward Bertram for supplying the influenza virus stocks.

are aware, they have never been efficacious in an acutely ill patient suffering from an infectious disease. Specifically, their use has failed to significantly protect against falciparum malaria[321–323] or human sepsis.[324,325] This should not be surprising, and does not negate TNF being central to the disease process. Many early studies in mice demonstrated that serum TNF peaks at 90 minutes after intravenous LPS, when the animals are only slightly sick. These anti-TNF agents are very specific drugs, and excess TNF comes before appreciable illness, and triggers harmful cascades[43] that carry on, causing disease once TNF is removed. In other words, TNF rises before onset of appreciable illness,[326] too early for the physician to realize something serious

is occurring. Indeed, an argument could be made for NF-κB inhibitors and PPAR ligands being better in treating illness that is in full flight because many cytokines inhibited by these treatments are farther down the cascade than TNF, and therefore more amenable to downregulation for effective amelioration of disease.

In contrast, when injected before LPS, anti-TNF antibody prevents death in mice.[34] Likewise, baboons needed to be treated no later than 15 minutes into a lethal 2-hour infusion of *Escherichia coli* (before illness onset) if they were to survive.[327] As noted in Section 9.3.2, anti-TNF reduced mortality in experimental dengue virus infections, but the daily treatment regimen began before the mice were ill.[269] Hence, the best time to administer an anti-TNF agent has passed once severe illness is present. Indeed, the best way to ensure that an anti-TNF agent works against an acute excess TNF syndrome, or "cytokine storm," is to administer it prophylactically, before the TNF trigger is present. Practical examples in patients include before OKT3 therapy, which minimizes graft rejection,[328,329] and before bone marrow transplantation in order to prevent graft vs. host disease.[330,331] Similarly, anti-TNF prevents the human Jarisch-Herxheimer reaction caused by penicillin administered to kill *Borrelia recurrentis*, an organism high in LPS, and the cause of louse-borne relapsing fever.[332] Without pretreatment, the reaction to OKT3 therapy, acute graft vs. host disease, and the Jarisch-Herxheimer reaction all generate acute sepsis syndromes.

Anti-TNF agents have been very successful treatments for chronic inflammatory diseases once they are clinically evident, and indeed have been for years. The first success was with rheumatoid arthritis,[333] the second with Crohn's disease,[334] and the third with psoriasis.[335] All three are recognized treatments, and millions of people are treated, typically at weekly intervals. In 2006, in an open trial with etanercept injected by a route that drains into Batson's plexus, 15 Alzheimer's patients were treated for 6 months.[232] Case reports[336] and reviews of the method and rationale[337] have also been published. This review presents evidence that this route is much less invasive but functionally equivalent to the intracerebroventricular route used for anti-TNF biological agents of this size to access the CSF in basic animal studies on roles of TNF in brain function.[194,338–340] Nevertheless, calls for a double-blind human Alzheimer's trial of administering etanercept by this route[232] have not yet attracted industry or government funding.

Might subcutaneous administration of anti-TNF agents achieve the same end? This is a reasonable possibility, in that the anxiety and depression often seen in rheumatoid arthritis and psoriasis have been reported to be suppressed by this treatment.[341,342] As discussed in Section 9.7, the same has been accomplished in an experimental model of the anxiety experienced by patients with inflammatory bowel disease.[196] The model used is actually an infectious disease, in that the gut inflammation is a consequence of infection with the gut nematode, *Trichuris muris*. Nevertheless, these are cerebral origin states in which the TNF that reaches and influences the brain (see Section 9.7 for mechanisms) can be presumed to have arisen systemically. In contrast, the TNF in Alzheimer's appears to be of cerebral origin, albeit topped up from time to time by systemic infections.[343] This could explain a report[344] of patients not responding to 24 weeks of subcutaneous treatment of Alzheimer's disease with etanercept.

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10 Implications of Inflammation for Neuropsychiatric Disease *Contributions and Consequences*

Thaddeus W. W. Pace, Charles L. Raison, and Andrew H. Miller

CONTENTS

10.1 INFLAMMATION: NOT JUST A PLAYER IN SOMATIC DISORDERS

10.1.1 Overview of Inflammatory Phenomena in Major Somatic Diseases

The very existence of the book you now hold in your hands testifies to the increasingly central place inflammatory processes play in our understanding of pathological processes important to the development of medical morbidity and mortality in the modern world. Indeed, the last decade has seen many modern maladies reconceptualized as conditions in which inflammation either is a primary causative factor or plays a role somewhat akin to the classic Mafia hit man who does the dirty work for others. In this case "the others" are not Mafia dons, but a variety of genetic or environmental factors linked to the development of specific disease states. Examples of the former conditions include cardiovascular disease (CVD) and diabetes, in which inflammation is now recognized as a primary pathological mechanism. Examples of the latter include cancer and dementia. In cancer, inflammatory processes have been linked to tumor survival, metastatic spread, and resistance to chemotherapeutic agents. In dementia, inflammation is thought to promote microglial activation and increase the pace of neurodegeneration.

If inflammation is emerging as a unifying mechanism behind many of the "wear and tear" conditions of the modern world, such as CVD, cancer, and dementia, might it also play a similar role in the realm of neuropsychiatric diseases? In this chapter, we provide evidence to support this idea, with a special focus on rapidly accumulating evidence that inflammatory processes contribute to the development of mood and anxiety disorders, which together account for a significant portion of the world's neuropsychiatric morbidity.

10.1.2 Historical Overview of Inflammatory Phenomena in Neuropsychiatric Disorders

So much is lost from the lives of people who suffer with psychiatric illness that it was perhaps natural for initial discoveries regarding these conditions and immunity to focus on yet another loss—in this case, diminished functioning of the humoral and cellular arms of the acquired immune response.[1] Nevertheless, data amassed over the last several decades have led to a dramatic paradigm shift in which the early focus on immunosuppression has been subsumed within, and supplanted by, a growing recognition that many psychiatric conditions—especially mood and anxiety disorders—may be better characterized as conditions of immune activation, especially hyperactivity of innate immune inflammatory responses.

Several strands of research converged in the early 1990s that pointed to a potential role for inflammatory activity in the pathogenesis of psychiatric illness. Basic scientists noticed that laboratory animals subjected to an inflammatory stimulus such as lipopolysaccharide (LPS) evinced behavioral changes indistinguishable from changes seen following a psychological stressor.[2] Combined with data regarding the adaptive value of cytokine-induced behavioral changes in terms of battling infection, these observations gave rise to the concept of "sickness behavior" as a cytokine-based syndrome that shared many symptoms with animal models of depression and with symptoms of major depression (MD) in humans.[3] Around the same time, an influential paper entitled "The Macrophage Theory of Depression" was published, which posited—based at the time on circumstantial evidence—that inflammatory cytokines produced by macrophages might contribute to the development of MD, even in medically healthy individuals.[4] This paper is frequently credited as the launching point for the field by Michael Maes, who, with numerous colleagues, produced almost all the early literature showing links between depression and increased inflammation.[5] Beginning around the turn of the millennium, multiple other groups also began to observe cross-sectional associations between both syndromic and subsyndromic depression and increased inflammation.[6] A final strand in the story of inflammation in psychiatric disease also came to prominence in the 1990s as clinicians began to note that the use of inflammatory cytokines, such as interferon (IFN)-alpha, reliably induced depression, anxiety, and other neuropsychiatric symptoms in previously nonsymptomatic individuals.[7] These findings provided strong evidence that more than just being associated with psychiatric disturbance, cytokines were capable of contributing to the development of symptoms shared by many neuropsychiatric disorders.

10.1.3 Connections between Neuropsychiatric Disorders, Somatic Disorders, and Inflammation

10.1.3.1 Increased Rates of Psychiatric Disease in Medically Ill Patients

Even if one knew nothing of the effects of inflammatory cytokines on mood and behavior, one might suspect a link between inflammation and depression/anxiety, given that inflammation is often considered the *sine qua non* of pathology, and given

the high rate of psychiatric disorders—especially anxiety and depression—in the context of medical illness.[8] Indeed, a recent study of more than 60 countries around the world found that the presence of medical illness is the most powerful predictor of depression in all cultures examined—more powerful than stress, age, and sex.[9] That inflammation might mediate this association is supported by studies finding that rates and severity of depression increase in lockstep with increasing levels of inflammation in patients with a number of medical illnesses.[10] Indeed, an association between depression and immune activation has been observed in a variety of medical illnesses, including cancer,[11] cardiovascular disease,[12–13] congestive heart failure,[14] rheumatoid arthritis,[15] multiple sclerosis,[16] and postviral infection.[17] Whereas 30-day prevalence rates of major depression in the general population are estimated at 5%,[18] rates of current major depression surpass 50% in several medical conditions, and are significantly higher than population norms across a wide range of medical disorders.[19] Examples of illnesses in which rates of current major depression are markedly elevated include cancer (11–50%, average 24%),[20] coronary artery disease (15–25%), human immunodeficiency virus infection (10–36%),[21–23] diabetes (28% in women, 18% in men),[24] Parkinson's disease (40–70%),[25–28] stroke (20% major depression, 19% minor depression),[29] and autoimmune conditions, such as rheumatoid arthritis (13–17%),[30–32] multiple sclerosis (42–54%),[33–34] and systemic lupus erythematosus (16–43%).[35–36] Compounding these already high rates of depression is the fact that a number of current treatment modalities, including chemotherapy, surgery, and dialysis appear to be depressogenic in their own right.[37–39] The risk of depression generally increases as disease severity worsens. Consistent with this finding, rates of current depression rise from 9% in the outpatient setting to approximately 30% in medically hospitalized inpatients.[40] The burden of depression is even more striking when subsyndromal conditions are included, given that many medically ill patients who do not meet criteria for MD nonetheless evince significant depressive symptoms.[41]

10.1.3.2 Neuropsychiatric Disorders as Risk Factors for the Development of Medical Illness

Very recently, an epidemiological study demonstrated that depressive symptoms contribute more to the risk of developing dementia than does the apolipoprotein E4 allele,[42] which is certainly among the best documented genes for any central nervous system disease. This finding is just the latest addition to a vast database showing that mood and anxiety disorders predict the subsequent development of many medical illnesses, including CVD, stroke, cancer, and dementia, as well as the metabolic syndrome in general and type 2 diabetes in particular.[43–52] Mood symptoms also predict the subsequent development of several physical symptoms that represent significant sources of morbidity and financial expenditures on healthcare, including chronic pain and insomnia.[53–54]

In addition to predicting disease development, depression significantly worsens quality of life in medically ill people and, more ominously, greatly increases morbidity and mortality from the underlying disease state. Indeed, even after adjusting for the effects of depression on treatment adherence and other relevant factors, such as disease severity, depressive symptoms (even in the absence of full-blown

MD) increase mortality in patients with human immunodeficiency virus (HIV) disease,[55] cancer,[56] coronary artery disease,[57] congestive heart failure,[58] myocardial infarction,[59] status post-heart transplantation or coronary artery bypass surgery,[60–61] and end-stage renal disease.[39] Depression predicts in-hospital mortality and hastens death in elderly people in nursing homes.[62–63] Depressive symptoms may also interfere with the efficacy of biological treatments. Indeed, patients who develop depressive symptoms following stem cell transplantation have a threefold increased risk of dying within a year of the procedure.[64] Taken together, these clinical data resoundingly support an association among neuropsychiatric disorders (especially depression), inflammation, and disease. In the remainder of this chapter, the specific aspects of these interrelationships will be examined in detail with a continuing focus on depression and anxiety.

10.2 RELATIONSHIP OF INFLAMMATION TO NEUROPSYCHIATRIC DISEASE: MOOD AND ANXIETY DISORDERS

10.2.1 Increased Inflammation in Major Depression

Increasing data suggest that inflammation may play a role in the development of MD in certain depressed patients, including both medically ill and medically healthy individuals.[6] Some of the first data in this regard came from studies of peripheral blood from depressed subjects compared to controls, demonstrating increased inflammatory markers, including proinflammatory cytokines and their soluble receptors, acute phase reactants, and inflammatory mediators such as prostaglandins.[6,65–67] Increased cytokines have also been found in the cerebrospinal fluid of depressed patients.[6] Early findings have been replicated in a multitude of studies, and meta-analyses have indicated that interleukin (IL)-6 and tumor necrosis factor (TNF)-alpha, along with C-reactive protein (CRP), appear to be the inflammatory biomarkers that are most reliably increased in depressed individuals vs. controls.[6,68–70] In addition to mean differences in inflammatory markers between depressed and control subjects, associations between inflammatory markers and depressive symptom severity, as well as individual depressive symptoms, including fatigue, insomnia, and cognitive dysfunction, have been found.[6,71–73] Changes in cytokine production appear to occur across the circadian cycle and may persist in some depressed individuals.[74] Of note, data also suggest that depressed patients who are resistant to standard antidepressant treatment tend to be more likely to exhibit increased inflammatory markers, and increased inflammatory markers prior to treatment have been found to predict nonresponsiveness to antidepressant therapy.[75–76] Depressed patients exposed to early life stress also have been found to exhibit increased peripheral blood concentrations of acute phase reactants and increased inflammatory responses to stressor exposure (see below).[77] Interestingly, patients exposed to early life stress have been shown to exhibit reduced responsiveness to antidepressant medication, although whether this effect is related to inflammatory status has yet to be determined.[78] Finally, data indicate that patients with bipolar disorder also exhibit increases in inflammatory markers, suggesting that the association of inflammation with neuropsychiatric disease spans the spectrum of mood disorders.[79]

Another finding that supports the notion that inflammation and the release of inflammatory cytokines may play a role in depression is that administration of innate immune cytokines such as IFN-alpha and cytokine inducers such as LPS or typhoid vaccination can lead to the development of depressive symptoms, including depressed mood, anhedonia, fatigue, psychomotor retardation, anorexia, impaired sleep (insomnia), and difficulties with memory and concentration.[7,80–81] Indeed, 30–50% of subjects who receive chronic administration of IFN-alpha meet symptom criteria for MD, depending on the dose.[7] Comparison of IFN-alpha-induced behavioral changes with behavioral changes observed in patients with idiopathic MD reveals a striking overlap in symptom expression, with few differences except for greater psychomotor retardation and anorexia in cytokine-treated subjects and greater decreases in self-esteem in patients with idiopathic MD, possibly related to chronicity or concomitant personality disorders.[82]

Alleviation of depressive symptoms following administration of anti-inflammatory therapies also suggests a link between inflammation and depression. For example, in a large double-blind, placebo-controlled trial with over 300 patients per group, patients with psoriasis who received the anti-TNF drug etanercept exhibited a significant improvement in depressive symptoms compared to patients who received placebo.[83] Of note, improvements in depressed mood in the etanercept-treated group were independent of improvement in disease activity, including clearance of skin lesions and reduction in joint pain. In medically healthy patients with major depression, two small double-blind, placebo-controlled trials have revealed that the addition of the cyclooxygenase inhibitors, aspirin and celecoxib (in separate studies), to standard antidepressant medication was superior to antidepressant medication plus placebo.[84–85] Neither of these studies stratified patients on the basis of peripheral inflammatory markers or monitored inflammatory markers in relation to treatment response. Although studies have been somewhat mixed, there are also data to suggest that successful treatment with antidepressant medication is associated with a reduction in inflammatory markers.[6] These findings are consistent with data that antidepressants *in vitro* can reduce immune cell production of proinflammatory cytokines such as TNF-alpha, while increasing the production of anti-inflammatory cytokines such as IL-10.[86] Moreover, the resolution of inflammation in concert with depressive symptoms suggests that the increased inflammatory mediators in depressed patients represent a *state* marker, as opposed to being an enduring *trait* of depression. Taken together, these studies suggest that anti-inflammatory strategies may hold promise for the treatment of certain patients with MD.

10.2.2 Increased Inflammation in Anxiety Disorders

In addition to MD, inflammation has been implicated in the pathophysiology of other neuropsychiatric conditions, including anxiety disorders. Anxiety is an emotional state or reaction that consists of unpleasant feelings of tension, apprehension, and nervousness. The emotional state of anxiety is often accompanied by biological changes, including activation of the autonomic nervous system.[87] Anxiety symptoms as well as anxiety disorders, including panic disorder and posttraumatic stress disorder (PTSD), have been found to exhibit increased inflammatory markers,

including proinflammatory cytokines and acute phase proteins such as CRP.[88] In addition, administration of IFN-alpha or LPS has been shown to increase symptoms of anxiety.[80,89] The most studied anxiety disorder relevant to inflammation is PTSD, likely due the recognition of its widespread and growing impact in the context of natural disasters and modern conflicts in war-torn regions, including the Balkans, Iraq, and Afghanistan.

10.2.2.1 Posttraumatic Stress Disorder

A rich body of research indicates that patients with anxiety disorders, and in particular PTSD, display increased peripheral and central markers of inflammation. Spivak and colleagues were the first to measure cytokines in PTSD patients in 1997, and discovered that compared to healthy, non-trauma-exposed controls, veterans with PTSD displayed increased circulating concentrations of IL-1beta that were correlated with the duration of PTSD.[90] This finding has been bolstered by several additional studies demonstrating increased IL-1beta and other inflammatory markers in PTSD patients, including those with non-combat-related trauma. For example, higher peripheral blood TNF-alpha was found in PTSD patients with mixed trauma experiences vs. non-PTSD controls.[91] Compared to healthy controls, higher peripheral blood IL-6 and soluble IL-6 receptor (sIL-6R) concentrations were found in victims of an automobile accident or hotel fire.[92] Higher IL-6 has also been reported in patients with PTSD subsequent to myocardial infarction (MI) vs. MI patients without PTSD (but only after controlling for depressive features in the same patients).[93] PTSD subsequent to natural disasters has also been associated with inflammatory changes. Compared to non-PTSD refugees, refugees with PTSD triggered by experiences during Hurricane Katrina had higher circulating concentrations of IL-6 that were accompanied by increased sympathetic nervous system activity as manifested by higher heart rate and blood pressure.[94]

Evidence for increased inflammation in PTSD also exists when measuring inflammatory signaling pathways, acute phase reactants, and cytokines in other bodily compartments. In terms of molecular signaling pathway activity in anxiety disorders, patients with PTSD have been shown to exhibit elevated activity of nuclear factor kappa B (NF-κB) in peripheral blood mononuclear cells (PBMCs).[95] PTSD patients had nearly a twofold higher likelihood of having elevated CRP levels, even after controlling for possible confounds such as sex, age, and alcohol use.[96] Alterations in inflammatory markers are evident not just in the peripheral circulation, but in central compartments as well. For example, elevated IL-6 concentrations have been reported in the cerebrospinal fluid (CSF) of combat trauma PTSD patients compared to healthy controls.[97]

In vitro mitogen-stimulated production of proinflammatory cytokines has also been examined in PTSD patients. Rohleder and colleagues reported increased LPS-induced IL-6 and TNF-alpha production in whole blood collected from Bosnian war refugees with PTSD vs. healthy controls.[98] Similar results have been reported, including enhanced LPS-induced production of IFN-gamma in victims of domestic abuse with PTSD.[99]

Besides being associated with PTSD, proinflammatory cytokines and other aspects of immune function may also encourage the expression and severity of key behavioral features of the disorder. Administration of proinflammatory cytokines or

mitogenic agents that induce the cytokine signaling cascade promotes the expression of behaviors that are reminiscent of key behavioral features of PTSD. In animal models, administration of cytokines or mitogens decreases social exploratory behavior, disrupts normal sleep, and changes expression of anxiety-like behaviors.[3,100] As noted above, cytokines or agents that induce cytokines can also induce feelings of anxiety in humans. Reichenberg and colleagues administered a low dose of LPS and later measured anxiety features in healthy adults using the State Anxiety Inventory. Anxiety was increased 1.5 hours after treatment in the LPS group compared to controls, and severity of anxiety was positively correlated with the LPS-induced circulating concentrations of TNF-alpha.[80]

Of note, the frequency and severity of PTSD symptoms (reexperiencing, avoidance, and arousal measured by the clinician administered PTSD scale [CAPS]) have been found to be correlated with several different cytokines, including TNF-alpha, although the strength of these associations was reduced when controlling for time since trauma or comorbid depression.[91]

10.3 IMPACT OF CYTOKINES ON THE BRAIN

Ample data suggest an association between inflammation and inflammatory cytokines and the development of behavioral syndromes, including disorders of mood and anxiety. Accordingly, much attention has been paid to the mechanisms by which peripheral inflammatory processes access the brain and interact with pathophysiologic pathways relevant to behavioral regulation, including neurotransmitter metabolism, synaptic plasticity, and regional brain activity.

10.3.1 Access of Cytokines to the Brain

Cytokines are relatively large molecules that do not freely cross the blood–brain barrier (BBB). Nevertheless, studies have elucidated a number of routes by which cytokine signals can reach the brain, including humoral and neural routes as well as direct penetrance of the BBB by infiltrating immune cells (Table 10.1).

TABLE 10.1
Pathways by Which Peripheral Inflammatory Responses Can Reach the Brain

1. Humoral route
   a. Passage of inflammatory cytokines through leaky regions in the blood–brain barrier located in the circumventricular organs
   b. Passage of inflammatory cytokines via saturable transport molecules on endothelial cells
2. Neural route
   a. Binding of inflammatory cytokines to sensory afferent fibers, which then relay cytokine signals to brainstem nuclei
3. Cellular route
   a. Entry of activated peripheral monocytes/macrophages into brain parenchyma in response to tumor necrosis factor-alpha-induced microglial production of monocyte chemoattractant protein-1

Upon release into the peripheral circulation, cytokines can enter the brain through leaky regions in the BBB located in the circumventricular organs around the third and fourth ventricles.[101–102] The circumventricular organs in mammals include the median eminence and adjacent neurohypophysis, organum vasculosum lamina terminalis, subfornical organ, and the area postrema.[103] These brain regions are characterized by fenestrated capillaries where cytokines can enter brain parenchyma through passive diffusion and activate relevant cell types, including microglia. Circulating cytokines can also access the brain through binding to saturable transport molecules on endothelial cells that make up the BBB.[101,104] Transport molecules for a number of cytokines have been described, including IL-1 and TNF-alpha. The neural route by which cytokines signal the brain involves binding of cytokines to their receptors on sensory afferent nerve fibers that carry signals back to brainstem nuclei, including the nucleus of the solitary tract, which then relays signals to other brain regions, including the paraventricular nucleus via ascending catecholaminergic fibers.[101,105–106] Studies have shown that transection of vagal afferent nerve fibers can block the effects of peripherally administered inflammatory stimuli, including fever, activation of the hypothalamic–pituitary–adrenal (HPA) axis, and alterations in behavior, such as social exploration and other sickness behaviors.[101,105,107] Of note, whether cytokines signal the brain through the humoral or the neural route appears to be a function of the route of cytokine administration as well as the dose.[108] For example, higher doses of intravenously administered inflammatory stimuli appear to be largely transmitted via the humoral route, whereas lower doses of inflammatory stimuli administered locally (e.g., intraperitoneally) are primarily transmitted via the neural route. Finally, peripheral cytokine signals can reach the brain through migration of immune cells across the BBB. For example, in a study using chronic immune activation engendered by ligation of the superior vena cava in mice, TNF-alpha released into the peripheral circulation by the congested and inflamed liver was found to activate microglia in the brain to produce the chemokine monocyte chemoattractant protein (MCP)-1, which in turn was associated with the passage of peripheral blood monocytes into brain parenchyma.[109] TNF-alpha receptor 1 (TNFR1) knockout (KO) mice failed to produce MCP-1, and mice with the gene for MCP-1 knocked out failed to exhibit monocyte/macrophage migration to the brain.

Once cytokine signals reach the brain, there is a rich cytokine network within the brain involving glial elements such as microglia (the richest source of cytokines in the brain) and astrocytes, as well as neurons themselves.[102] Neurons in multiple brain regions have been shown to both produce a variety of cytokines and express cytokine receptors. Brain endothelial cells are also capable of producing a rich array of inflammatory mediators, including nitric oxide (NO), prostaglandins, IL-1, IL-6, and transforming growth factor (TGF)-beta.[101] Of note, increased microglia have been found in the dorsolateral prefrontal cortex and anterior cingulate cortex of suicide victims, suggesting that the depression and anxiety often associated with suicide may be related in part to the expansion of inflammatory glial elements in the brain.[110]

10.3.2 Neurotransmitter Metabolism

One of the primary pathways by which cytokines can influence behavior is through interaction with the metabolism of relevant monoamines and excitatory/inhibitory

small-molecule neurotransmitters. The monoamines, serotonin, norepinephrine, dopamine, and small-molecule neurotransmitters, glutamate, and gamma amino butyric acid (GABA), are well known to play key roles in the regulation of behavior and are all implicated in the pathophysiology and treatment of mood and anxiety disorders.[111] There is a rich literature in laboratory animals demonstrating that acute administration of cytokines or cytokine inducers such as LPS can profoundly alter neurotransmitter turnover.[112] Fewer studies have been conducted in humans, but the data are consistent with the notion that chronic exposure to peripheral inflammatory cytokines can alter neurotransmitter metabolism in the brain in association with changes in behavior.

Chronic subcutaneous administration of IFN-alpha for hepatitis C has been shown to lead to significant increases in brain CSF concentrations of IFN-alpha, which were associated with the activation of a central inflammatory response as reflected by increased CSF IL-6 and MCP-1.[113] Increases in IL-6 were in turn associated with decreases in CSF concentrations of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), which correlated with depression.[113] Evidence of the potential impact of IFN-alpha on serotonin metabolism is also indicated by the marked reduction in the development of depressive symptoms in patients receiving IFN-alpha for malignant melanoma who were treated prior to and during IFN-alpha therapy with the serotonin reuptake inhibitor paroxetine.[114]

Several mechanisms have been proposed by which cytokines such as IFN-alpha and other innate immune cytokines might influence serotonin metabolism. For example, data indicate that cytokine-induced activation of p38 mitogen-activated protein kinase (MAPK) can lead to increases in both the expression and function of the serotonin transporter.[115] Of note, increased activation of p38 MAPK as reflected by increased intracellular phosphorylation of p38 in peripheral blood mononuclear cells was correlated with decreased CSF 5-HIAA in rhesus monkeys abused/neglected as infants.[116] Increased p38 in these animals, who also exhibit increased anxiety-like behavior, was associated with the number of maternal rejections, further supporting a direct relationship between early life stress and immune activation, as noted previously and discussed below.

In addition to effects on the serotonin transporter, there has also been great interest in the impact of cytokines on the enzyme indoleamine-2,3-dioxygenase (IDO), which breaks down tryptophan (TRP), the primary amino acid precursor of serotonin, into kynurenine (KYN).[117] Early studies in patients undergoing treatment with IFN-alpha demonstrated decreased TRP and increased KYN in the peripheral blood of IFN-alpha-treated patients that was associated with the development of depressive symptoms.[118–119] Moreover, inhibition of IDO in mice treated with LPS or an attenuated form of *Mycobacterium bovis*, Bacille Calmette Guerin (BCG), blocked the development of depressive-like behavior while having no effect on cytokine expression.[120–121] Although early hypotheses considered the primary impact of cytokine-induced activation of IDO to be related to its reduction of TRP (and thereby serotonin), more recently, attention has been focused on KYN.[122] Administration of KYN alone has been shown to induce depressive-like behavior in mice.[121] Moreover, KYN is readily taken up into the brain by the large amino acid transporter, and is converted into the neuroactive metabolites,

quinolinic acid (QUIN) in microglia and kynurenic acid (KA) in astrocytes.[117] QUIN is neurotoxic, leading to oxidative stress through lipid peroxidation as well as increased release of the excitatory amino acid glutamate, through its agonist activity at the N-methyl-D-aspartate (NMDA) receptor.[117] QUIN is believed to contribute to neurodegeneration in a number of disorders, including Huntington's disease, Alzheimer's disease, and the dementia associated with infection by HIV.[117] Interestingly, KA also may contribute to alterations in monoamine metabolism. For example, intrastriatal administration of KA has been shown to lead to marked reductions in extracellular dopamine (DA) concentrations.[123]

An additional mechanism by which cytokines may influence DA is through effects on DA synthesis. Intramuscular injection of IFN-alpha to rats has been shown to decrease central nervous system (CNS) concentrations of tetrahydrobiopterin (BH_4),[124] an important enzyme cofactor for tyrosine hydroxylase, the enzyme that converts tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) and is the rate-limiting enzyme in the synthesis of DA. IFN-alpha effects on BH_4 appear to be mediated by stimulation of NO. Indeed, treatment with an inhibitor of NO synthesis was found to reverse IFN-alpha's inhibitory effects on brain concentrations of both BH_4 and DA.[124] IL-6 (which as noted above is increased in patients with depression and anxiety, as well as in the CSF of patients administered IFN-alpha) also has been shown to reduce BH_4 content in sympathetic neurons.[125] Of note, activation of an inflammatory response within the brain has been associated with increased NO production, suggesting that cytokine influences on BH_4 via NO may be a common mechanism for innate immune cytokines and inflammation to reduce DA availability in key brain regions, including the basal ganglia. Finally, similar to the serotonin transporter, MAPK pathways also appear to play a role in the regulation of the expression of the DA transporter (DAT). For example, transient transfection of human (h)DAT–expressing human embryonic kidney (HEK) cells with constitutively active MAPK kinase (MEK) was found to increase the maximum velocity (Vmax) of the human DAT transporter while increasing DAT surface expression.[126] Moreover, inhibition of MAPK signaling was found to decrease DA uptake in a dose- and time-dependent fashion in rat striatal synaptosome preparations and a HEK cell line.[126] Of note, activation of MAPK has also been shown to increase the expression and activity of the norepinephrine (NE) transporter (NET).[115,127] Given the role of the NET in the uptake of both NE and DA, increased DAT and NET expression and activity may contribute to reduced synaptic availability of DA (and NE), secondary to increased sequestration of neurotransmitter.

10.3.3 Synaptic Plasticity

While cytokines in the CNS provide trophic support to neurons and contribute to normal cognitive functions such as memory in laboratory animals,[128–129] significant data indicate that in the context of excessive or prolonged activation, cytokine networks in the CNS can promote an interconnected suite of abnormalities that are increasingly thought to be relevant to the pathophysiology of depression, including diminished neurotrophic support, decreased neurogenesis, increased glutamatergic activation,

oxidative stress, induction of apoptosis in relevant cell types (e.g., astrocytes and oligodendrocytes), and dysregulation of glial/neuronal interactions.[129–143]

Whether as a result of an immune challenge or acute or chronic stress, increases in inflammatory cytokine production have been shown to contribute to decreased neurotrophic support and neurogenesis in brain areas important to behavior and cognition.[3,130–133] For example, LPS administered peripherally produces cognitive impairment and increased hippocampal concentrations of TNF-alpha and IL-1, which are associated with decreased hippocampal expression of brain-derived neurotrophic factor (BDNF) and its receptor, tyrosine kinase-B, as well as reduced hippocampal neurogenesis.[133] In addition, blockade of CNS cytokine activity via administration of IL-1 receptor antagonist (IL-1ra) or transplantation of IL-1ra-secreting neural precursor cells into the hippocampus or the use of IL-1 receptor KO mice has been shown to prevent the effects of acute and chronic stress on behavior, cognition, neurotrophic factors, and neurogenesis.[130–132,144] Of note, *in vitro* studies have suggested that cytokine effects on neurogenesis are mediated in part by activation of NF-κB.[130]

Another pathway relevant to the impact of cytokines on synaptic plasticity is their capacity to increase glutamate release and decrease the expression of glutamate transporters on relevant glial elements, thereby decreasing glutamate reuptake.[134,137,141,145–146] Of note, glutamate released by astrocytes has preferential access to extrasynaptic NMDA receptors, which mediate excitotoxicity and decreased production of trophic factors, including BDNF.[147–148] Cytokines, including TNF-alpha and IL-1, can also induce astrocytes and microglia to release reactive oxygen and nitrogen species that, in combination with QUIN (see above), can amplify oxidative stress and further endanger relevant cell types, including neurons and oligodendrocytes, which are especially vulnerable to oxidative damage.[117,135–136,139,141–143,149–150] Astrocyte and microglial release of cytokines and inflammatory mediators also contributes to mutual amplification of inflammatory pathways within the brain.[137,146] Consistent with the effect of cytokines and central inflammatory processes on glia, loss of glial elements, such as oligodendrocytes and astrocytes in multiple mood-relevant brain regions, including the subgenual prefrontal cortex and amygdala, has emerged as a fundamental morphologic abnormality in MD.[140,151–152]

10.3.4 Regional Brain Activity

Brain imaging studies have begun to elucidate which specific brain regions are targets of the effects of cytokines in humans. One brain region in this regard is the basal ganglia, a brain region rich in DA neurons, which regulate motor activity and motivation. Immunologic stimuli, including both IFN-alpha and typhoid vaccination, have been associated with psychomotor slowing and fatigue in conjunction with changes in neuronal activity in the substantia nigra, putamen, and nucleus accumbens, as measured by functional magnetic resonance imaging (fMRI) and positron emission tomography.[81,153] Given the role of basal ganglia in motivational states and locomotor activity,[154] cytokine-induced effects on the basal ganglia and DA may represent an important mechanism whereby cytokines inhibit behavioral activation and induce depression and fatigue. Indeed, IFN-alpha-induced alterations in basal ganglia activity were significantly correlated with symptoms of fatigue. The impact of cytokines

on the basal ganglia may ultimately support evolutionarily derived pressures to reallocate energy resources from environmental exploration to fighting infection and wound healing.[155]

As noted above, symptoms of anxiety, irritability, and hyperarousal are also apparent following cytokine administration to humans. Such symptoms have been observed after acute administration of LPS as well as chronic treatment with IFN-alpha.[80,89,155–156] Indeed, a significant percentage of patients receiving IFN-alpha therapy have been shown to exhibit marked anxiety, irritability, inability to sleep, and hyperactivity.[156] Similar findings have been reported in rhesus monkeys administered recombinant human IFN-alpha.[157] Of potential relevance to these symptoms is that patients receiving IFN-alpha for hepatitis C exhibit significantly greater activation in the dorsal anterior cingulate cortex (dACC) (Broadman's area (BA) 24) than IFN-alpha-treated controls.[158] The dACC has been shown to play an important role in error detection and conflict monitoring,[159] and increased activity in this brain region has been associated with high-trait anxiety, neuroticism, and obsessive compulsive disorder,[160] all of which are associated with increased anxiety and arousal. Interestingly, activation of the dACC also has been found during a fMRI task of social rejection. Consistent with the role of this brain region in processing social pain, dACC activation was correlated with task-related emotional distress.[160] Combined with its role in error detection and conflict monitoring, the dACC's processing of social pain has been hypothesized to comprise a neural "alarm system," which can both detect and respond to threatening environmental stimuli.[160] Based on the neuroimaging data from IFN-alpha-treated patients, one mechanism by which cytokines may induce anxiety and alarm is through increased activation of neural circuits involving the dACC.[158] Regarding the potential evolutionary significance of these findings, an animal that has been infected or wounded is vulnerable to attack and therefore must maintain increased vigilance to react to intrusions from a predator.[155] Taken together with the effects of cytokines on the basal ganglia, which serve to reduce exploratory behavior, the effects of cytokines on neurocircuits within the brain appear to subserve competing evolutionary survival priorities that promote reduced activity to allow healing, while fostering hypervigilance to protect against future attack.

10.4 MECHANISMS OF INFLAMMATION IN NEUROPSYCHIATRIC DISEASE

Consideration has been given to sources of inflammation that may be relatively unique to patients with neuropsychiatric disorders. Sources that have received special attention include stress, a common precipitant of mood and anxiety disorders, as well as alterations in immunoregulatory neuroendocrine pathways, such as the HPA axis and glucocorticoids, and the autonomic nervous system (ANS). Finally, there has been increasing interest in the role of immunoregulatory T cell subtypes, obesity, and a "leaky" gut.

10.4.1 Psychological Stress

A multitude of studies have demonstrated that psychological stress plays a pivotal role in the development of neuropsychiatric disease, including disorders of mood

and anxiety. Although psychological stress is widely thought to contribute to disease through the "classic" stress response systems, including the HPA axis and ANS, mounting evidence suggests that psychological stress (in part through its impact on stress response systems) is a potent activator of inflammation, and therefore may additionally promote the development of neuropsychiatric and other medical diseases through effects on the inflammatory response.

Psychological stress in adulthood has been shown to be a major risk factor for the development of a variety of neuropsychiatric illnesses.[161] Stressors such as disruption of important social relationships (e.g., via death or divorce), loss of financial status or housing, and caregiving for ill family members are associated with up to a 10-fold increase in the likelihood of developing major depression.[162,163] In addition, exposure to severe adulthood traumas, including rape, automobile accidents, natural disasters, and terrorist attacks, are well-known precipitants of PTSD.[164] Indeed, *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition (DSM-IV) guidelines require identification of a traumatic event in a patient's history in order to make a diagnosis of PTSD, underscoring the connection between PTSD and stress.

As indicated above, one mechanism by which psychological stress may contribute to psychiatric illness is through effects on the inflammatory response.[165] For example, individuals caring for patients with dementia had peripheral blood concentrations of IL-6 that increased over a 6-year period at a rate four times greater than that seen in noncaregiver controls.[166] Increased peripheral blood inflammatory markers have also been found in caregivers of patients with brain cancer.[167] Interestingly, PBMCs from these cancer caregivers were also accompanied by significant increases in the expression of genes containing promoter response elements for NF-κB, suggesting enhanced activity of fundamental inflammatory signaling pathways as a function of chronic stress. Of note, genes containing promoter response elements for the glucocorticoid receptor (GR) were underexpressed in stressed caregivers, indicating that chronic stress may reduce glucocorticoid signaling, thereby disrupting the anti-inflammatory effects of glucocorticoids, which occur in part through inhibition of NF-κB. Chronic stress has also been shown to reduce GR expression in children with asthma.[168]

In addition to the effects of chronic stress, investigators have studied inflammatory markers in response to acute laboratory stressors. Multiple studies have shown that acute stressors increase peripheral blood concentrations of inflammatory mediators, including IL-6, IL-1beta, and CRP.[169] While circulating concentrations of stress hormones like glucocorticoids (e.g., cortisol) and catecholamines (e.g., norepinephrine) are well known to show stress-induced changes within minutes of the initiation of a stressor, circulating cytokines take up to 90 minutes to show stress-induced increases.[169] Interestingly, stress-induced cytokines do not immediately return to prestress levels like cortisol and norepinephrine, but instead may take in excess of 4 hours to return to baseline (Pace and Raison, unpublished observations). Thus, stress-induced activation of the inflammatory system may persist for extended periods of time after stressor cessation, suggesting that increased inflammatory markers may be due, at least in part, to stressor exposure during the hours or days preceding sample collection. Acute stress challenge has also been shown to activate inflammatory signaling

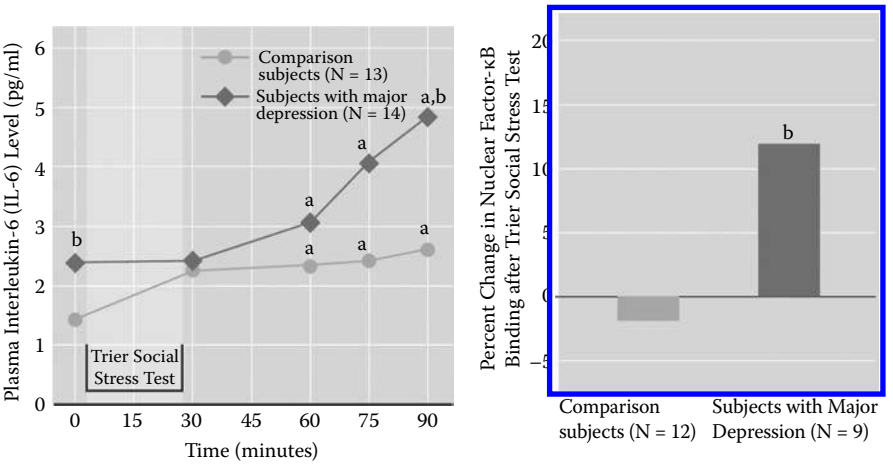

FIGURE 10.1 Patients with major depression and increased early life stress history display elevated baseline and acute psychosocial stress-induced circulating concentrations of interleukin (IL)-6, as well as enhanced psychosocial stress-induced nuclear factor-κB (NF-κB) DNA binding in peripheral blood mononuclear cells. Left panel: Circulating concentrations of IL-6 before and 30, 60, 75, and 90 minutes after the start of the Trier Social Stressor Test (TSST) in healthy controls vs. patients with current major depression and increased history of early life stress. a: Versus 0 minutes within the same group; $p < 0.025$. b: Versus control group at the given time point; $p \leq 0.05$. Right panel: Percent change in nuclear NF-κB DNA-binding from before to 30 minutes after the start of the TSST (ΔNF-κB) in a subset of the same participants. b: Versus control group, $p \leq 0.05$. (Reprinted from T. W. W. Pace, T. C. Mletzko, O. Alagbe, D. L. Musselman, C. B. Nemeroff, and A. H. Miller, *Am. J. Psychiatry* 163:1630–33, 2006. With permission.)

pathways. For example, challenge with a public speaking and mental arithmetic stressor has been found to increase NF-κB-DNA binding in PBMCs.[170–172]

Consistent with increased proinflammatory cytokines under resting conditions, patients with major depression have been found to exhibit exaggerated inflammatory responses to acute stressor challenge. Compared to healthy controls, male patients with major depression (who also had a history of early life stress—see below) showed enhanced responses to a combined public speaking and mental arithmetic challenge task, including significantly increased circulating concentrations of IL-6 and PBMC NF-κB-DNA binding compared to controls (Figure 10.1).[170] Given that stressful events are known precipitants of major depression, and that proinflammatory cytokines encourage the development and severity of major depression (discussed above), these findings suggest that the exaggerated inflammatory response to stress in patients with major depression may also contribute to the maintenance of ongoing illness.

In addition to adulthood stressors, adverse events experienced in childhood or adolescence are also known to be significant risk factors for the development of major depression and PTSD in adulthood. These "early life" stressors include child abuse (emotional, physical, or sexual), neglect (emotional or physical), witnessing maternal violence, and parental loss. Early life stress experiences are rather common

in Western society, with as many as one in four children experiencing such events. A population-based study conducted by the Centers for Disease Control and Prevention with nearly 8,700 participants found that sexual abuse, physical abuse, and witnessing maternal violence were experienced by 21, 20, and 14% of children, respectively.[173] Increasing severity of early life stress experiences is associated with poorer mental health, suggesting a dose–response relationship between early life stress and neuropsychiatric illness.[173] The likelihood of developing major depression in adulthood is fourfold higher for individuals with a history of childhood sexual and physical abuse.[174] Early life stress is also associated with the later development of PTSD. Almost half the women who experience childhood abuse will develop PTSD later in their lives, and exposure to early life stressors has been shown to increase the risk for developing PTSD following subsequent adulthood traumas.[164] Regarding the relationship between early life stress and inflammation, studies have found that adults who experienced childhood physical, psychological, or neglect abuse display increased circulating concentrations of CRP as well as other inflammatory markers.[77] In addition, depressed patients with childhood maltreatment exhibited significantly higher concentrations of CRP than depressed patients without a significant history of child abuse.[175]

10.4.2 Glucocorticoid Resistance and Increased Inflammation

One of the primary mechanisms by which depression may be associated with and contribute to inflammation is glucocorticoid resistance. Glucocorticoids play an essential role in restraining inflammation through inhibitory effects on multiple inflammatory signaling pathways, including NF-κB and activation protein-1 (AP-1), as well as the promotion of ingestion of apoptotic cells by macrophages and their subsequent release of anti-inflammatory cytokines, including TGF-beta and IL-10.[176–177] A hallmark of MD is decreased sensitivity to glucocorticoids as measured both *in vivo* and *in vitro*.[178–179] *In vivo* studies using the synthetic glucocorticoid, dexamethasone (DEX), have reliably demonstrated that patients with MD exhibit reduced glucocorticoid-mediated feedback inhibition of the HPA axis as reflected by nonsuppression of cortisol in the DEX suppression test (DST) and the DEX–corticotropin releasing hormone (CRH) test.[178–179] Interestingly, T lymphocytes from depressed patients with DEX nonsuppression fail to traffic out of the peripheral blood in response to oral DEX administration,[180] suggesting that the potentially neuroprotective effects of T cell migration to the brain during stress, which is mediated by glucocorticoids,[181] may be hampered in depressed individuals. Numerous *in vitro* studies have also found decreased responsiveness of PBMCs, including lymphocytes from patients with MD to the inhibitory effects of glucocorticoids on mitogen-induced proliferation as well as natural killer cell activity.[178–179]

Decreased sensitivity to glucocorticoids in MD is believed to be related in part to alterations in the expression or function of the GR.[178–179] Indeed, a number of studies have reported decreased GR binding in the cytosol of PBMCs from depressed patients.[178–179] In addition, a lack of change in cytosolic GR binding after *in vivo* DEX administration has been found in patients with MD and is consistent with the notion that DEX-induced GR translocation from the cytoplasm to the nucleus may

be impaired in depressed patients.[178–179] In the inactive state, the GR resides in the cytoplasm bound to a complex of heat shock proteins. Upon ligand (glucocorticoid) binding, the GR dissociates from the heat shock protein complex and translocates from the cytoplasm to the nucleus, whereupon it mediates its effects through protein–protein interactions with other transcription factors, such as NF-κB and AP-1, or through DNA binding and the induction of anti-inflammatory factors such as inhibition κ-B, which stabilizes NF-κB in its inactive form in the cytosol.[176] It should be noted that MD has been regularly associated with increased circulating concentrations of glucocorticoids.[178–179] However, given alterations in the expression or function of the GR receptor and their potential contribution to glucocorticoid resistance, it is understandable how increased blood concentrations of glucocorticoids and evidence of increased inflammation can coexist in MD.[178–179] Thus, alterations in the GR and glucocorticoid resistance may provide a primary pathway by which MD contributes to nonresolving inflammation.

Consideration has been given to the mechanisms by which the GR may be impaired in patients with depression. One mechanism that has received much attention is the impact of cytokines and their signaling pathways on GR function (Figure 10.2).

Interestingly, there is a rich literature indicating that cytokines can decrease GR function through inhibition of GR translocation as well as through protein–protein interactions that can inhibit GR-DNA binding in the nucleus.[182–183] For example, *in vitro* studies have shown that IL-1alpha (and beta) can block the translocation of the GR from the cytoplasm to nucleus and inhibit both GR-DNA binding and DEX-induced activation of a reporter gene construct with multiple glucocorticoid response elements (GREs) upstream of its promoter region.[184–185] These effects of IL-1 on GR function were reversed by the IL-1ra, and studies using pharmacologic antagonists and antisense oligonuleotides have demonstrated that IL-1-induced activation of p38 MAPK is involved.[184] Activation p38 MAPK has been found in related studies to phosphorylate the GR, thereby inhibiting translocation.[182] *In vivo* relevance of the role of IL-1 in GR regulation has been shown in mice exposed to chronic social disruption stress, who exhibit glucocorticoid resistance that is associated with increased inflammatory responses and decreased translocation of the GR in isolated splenocytes from DEX-treated animals.[186] These effects of social disruption on GR translocation/function and inflammation were not apparent in IL-1 KO mice. Cytokines have also been shown to induce transcription factors that disrupt GR function through protein–protein interactions.[182] For example, *in vitro* administration of IFN-alpha has been found to decrease DEX-induced GR-mediated reporter gene activity and DEX-induced GR-DNA binding.[187] These effects are mediated by the induction of signal transducer and activator of transcription (STAT) 5, which has been shown to be associated with GR in the nucleus using immunoprecipitation.[187] Moreover, blocking of IFN-alpha-induced STAT5 activation by siRNA reverses the effects of IFN-alpha on GR function.[187] Of note, unlike IL-1, IFN-alpha had no effects on GR translocation. Inhibitory effects on the GR have been demonstrated for a number of cytokines, and activation of MAPK pathways and STAT5 appear to be regularly implicated.[182] Finally, it has been shown that cytokines can increase the expression of the beta isoform of the GR, which is relatively inert compared to the more active isoform of the receptor, GR alpha.[182] Increased GR beta has been found

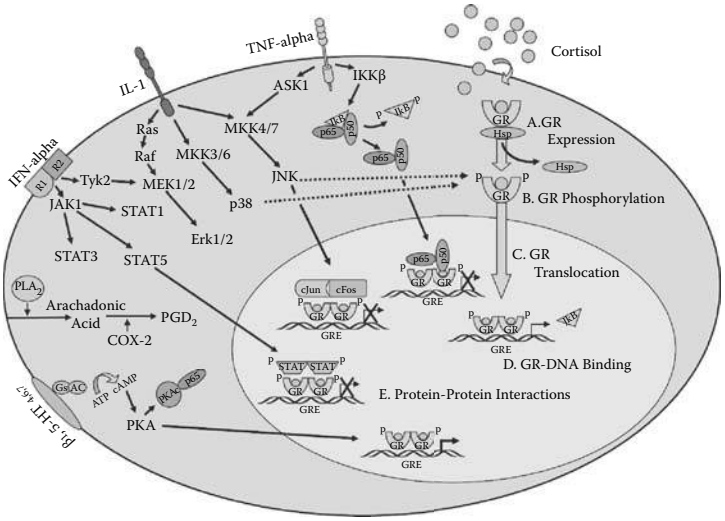

FIGURE 10.2 (See color insert.) Interactions between cytokine and glucocorticoid receptor signaling pathways. Selected cytokines and their signal transduction pathways are depicted in simplified fashion to illustrate representative interactions between cytokine and glucocorticoid receptor (GR) signaling events. Cortisol binds to GR, resulting in dissociation of heat shock protein (HSP) complexes and subsequent phosphorylation. GR then translocates to the nucleus, where it dimerizes and either interacts with other transcription factors or binds to glucocorticoid response elements (GREs) upstream of GR-regulated genes (e.g., inhibitor k-B (IkB)). TNF-alpha binds to its receptor and results in activation of IkB kinase beta (IKKB), which phosphorylates IkB, allowing NF-κB (shown here as p65 and p50 Rel subunits) to translocate to the nucleus. Through protein–protein interactions, activated NF-κB associates with GR, thus interfering with GR-DNA binding. IL-1 binds to its receptor, initiating (a) mitogen-activated protein kinase (MAPK) kinase (MKK)4/7, which culminates in activation of Jun amino-terminal kinase (JNK); (b) MKK3/6, which culminates in activation of p38; and (c) Ras, which results in activation of the extracellular signal-related kinase (Erk)1/2. Of note, MKK4/7 activation of JNK can also occur through TNF-alpha receptor binding. As depicted by the dotted lines, both p38 and JNK can phosphorylate key GR residues, thereby disrupting nuclear translocation of GR. Interferon (IFN)-alpha binds to its receptor, resulting in Janus kinase (Jak) phosphorylation, represented as Jak1 and tyrosine kinase (Tyk)2. Jak1 phosphorylates signal transducers and activators of transcription (STAT) proteins, including STAT1, STAT3, and STAT5. Tyk2 can also activate elements of the Ras signaling pathway, resulting in activation of Erk1/2. Activated STATs translocate to the nucleus, where they can interact with GR through protein–protein interactions, thereby interfering with GR-DNA binding. Phospholipids are hydrolyzed by phospholipase A2 (PLA2) to form arachidonic acid, which is metabolized by cyclooxygenase (COX) 2 to produce prostaglandin D2 (PGD2). Stimulation of serotonergic receptors 4, 6, or 7 (5-HT4, 6, or 7) and beta-adrenergic receptors (beta1) induces a conformational change in G stimulatory (Gs) protein, which then activates adenylyl cyclase (AC). AC, in turn, converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). cAMP then induces a conformational change in protein kinase A (PKA), which translocates to the nucleus, where it is able to enhance GR-DNA binding. In addition, the catalytic subunit of PKA (PKAc) interacts with p65, thereby inhibiting NF-κB nuclear translocation. (Reprinted from T. W. W. Pace, F. Hu, and A. H. Miller, *Brain Behav. Immun* 21: 9–19, 2007. Copyright 2007 Elsevier. With permission.)

in patients with inflammatory disorders, including asthma, ulcerative colitis, and ankylosing spondylitis, especially in patients who are resistant to the therapeutic effects of glucocorticoids.[182,188]

Relevant to increased inflammation in patients with early life stress (see above), data suggest that early adverse events may be associated with epigenetic changes in GR regulation that can reduce the capacity of the GR to upregulate under conditions of challenge. More specifically, postmortem samples from the brain of individuals exposed to childhood abuse were found to exhibit methylation of key regulatory elements in the promoter region of the GR gene, which in turn was shown to limit transcription factor binding responsible for upregulation of the GR.[189] Increased GR gene methylation was also associated with decreased GR mRNA in the postmortem hippocampus of these abused individuals.[189] These data indicate that early life stress may alter GR gene regulation, leading to downregulation of GR expression and ultimately reduced sensitivity to the anti-inflammatory effects of glucocorticoids.

Although there are data to support that GR sensitivity or expression may be paradoxically increased in patients with PTSD,[190–191] it should be noted that increased inflammation in these patients may be more related to decreased glucocorticoid signaling as a function of decreased availability of glucocorticoid hormone. Peripheral blood concentrations of cortisol have been found to be decreased in PTSD in a number of studies. Reduced cortisol levels in PTSD patients have also been consistently reported in urine or saliva.[192]

10.4.3 Autonomic Nervous System Function

Another pathway that may contribute to increased inflammation in mood and anxiety disorders is the ANS. The role of the ANS in stress-related immune functioning is complex. While the sympathetic nervous system (SNS) is known to suppress certain aspects of innate immunity (i.e., natural killer cell activity),[38] data increasingly indicate that the SNS may also activate inflammatory processes even in the absence of any pathogen-related immune stimulus.[10,179] For example, adrenergic agonists have been shown to stimulate the production and release of proinflammatory cytokines, such as IL-1beta, IL-6, and TNF-alpha, from a variety of cell types, including cardiac myocytes, adipocytes, and monocytes/macrophages.[193–197] Conversely, agents that block signaling through adrenergic receptors have been shown to reduce peripheral inflammatory activity in a number of medical conditions.[198–202] The SNS has also been shown to stimulate the production of inflammatory mediators *in vivo*. For example, blockade of alpha- and beta-adrenergic receptors has been shown to abolish proinflammatory cytokine responses to restraint and open-field stress in rodents.[203–204] Ablation of neural projections from the locus ceruleus prevents stress-induced increases of IL-1beta in the CNS, and administration of the beta-adrenergic agonist isoproterenol stimulates production of IL-1beta in the CNS/pituitary gland and the release of IL-1beta and IL-6 into the peripheral circulation.[204] Similarly, stress-induced activation of NF-κB is dependent on NE release and can be abrogated by alpha-adrenergic receptor blockade.[172] In humans, administration of the alpha-adrenergic antagonist prazosin has been shown to block the induction of IL-6 in response to the stress of high altitudes.[205]

Parasympathetic withdrawal in response to stress may also promote inflammation given evidence that vagal activity inhibits NF-κB activation (and the release of TNF-alpha from macrophages) via cholinergic signaling through the alpha-7 subunit of the nicotinic acetylcholine receptor,[206] and direct electrical stimulation of the efferent vagus nerve inhibits the synthesis of TNF-alpha in liver, spleen, and heart.[207] Conversely, vagotomy greatly increases TNF-alpha responses to inflammatory stimuli and sensitizes animals to the lethal effects of LPS.[208] *In vitro* application of acetylcholine (the neurotransmitter of the parasympathetic system) deactivates macrophages, and stimulation of CNS muscarinic receptors *in vivo* inhibits the production and release of the proinflammatory cytokine TNF-alpha during endotoxemia.[209] Moreover, vasoactive intestinal peptide—which is coreleased upon parasympathetic activation—has also been shown to have powerful anti-inflammatory effects.[210–212]

Consistent with a role for the ANS in mediating inflammatory tone are studies reporting an association between increases in resting heart rate and increased resting state markers of peripheral inflammation,[213–214] as well as a number of recently published articles demonstrating a link between resting state indices of inflammation and heart rate variability (HRV) in both healthy and diseased populations. Indeed, in patient and normal populations, consistent correlations have been observed between reductions in overall (i.e., time domain) HRV, high-frequency (HF) and low-frequency (LF) domains, and increased inflammation, with effect sizes being largest for the LF power domain.[215–225] Very recently, IL-6 responses to a laboratory stressor have been shown to be associated with reductions in overall HRV—suggesting that withdrawal of parasympathetic activity may contribute to stress-induced increases in inflammation.[226]

These results suggest that to the degree that mood and anxiety disorders are characterized by ANS alterations known to promote inflammation (i.e., increased sympathetic/reduced parasympathetic activity), these pathways may represent an important mechanism by which inflammation is increased in these disorders. Taken together with glucocorticoid resistance, alterations in ANS activity represent a powerful pathway through which stress and neuropsychiatric disease can promote ongoing inflammation, which in turn can influence the expression of neuropsychiatric disease (Figure 10.3).

10.4.3.1 Major Depression

Although inconsistencies in the data exist, the majority of available evidence suggests that MD is—in general—characterized by an ANS signature comprised of increased SNS signaling and diminished parasympathetic (or cholinergic) tone. Evidence supporting increased SNS/decreased parasympathetic tone in MD includes increased heart rate at rest and in response to stress, increased blood pressure, increased systemic vascular resistance, increased whole body sympathetic activity based on measures of postganglionic NE release and NE clearance, reduced overall HRV, reduced high-frequency HRV (a measure of parasympathetic tone), impaired autonomic information flow (AIF) on 24-hour ambulatory electrocardiograms, impaired baroreflex, higher ventricular repolarization time, and increased incidence of multiple firing within a sympathetic burst based on single-unit muscle sympathetic nerve analysis.[227–239]

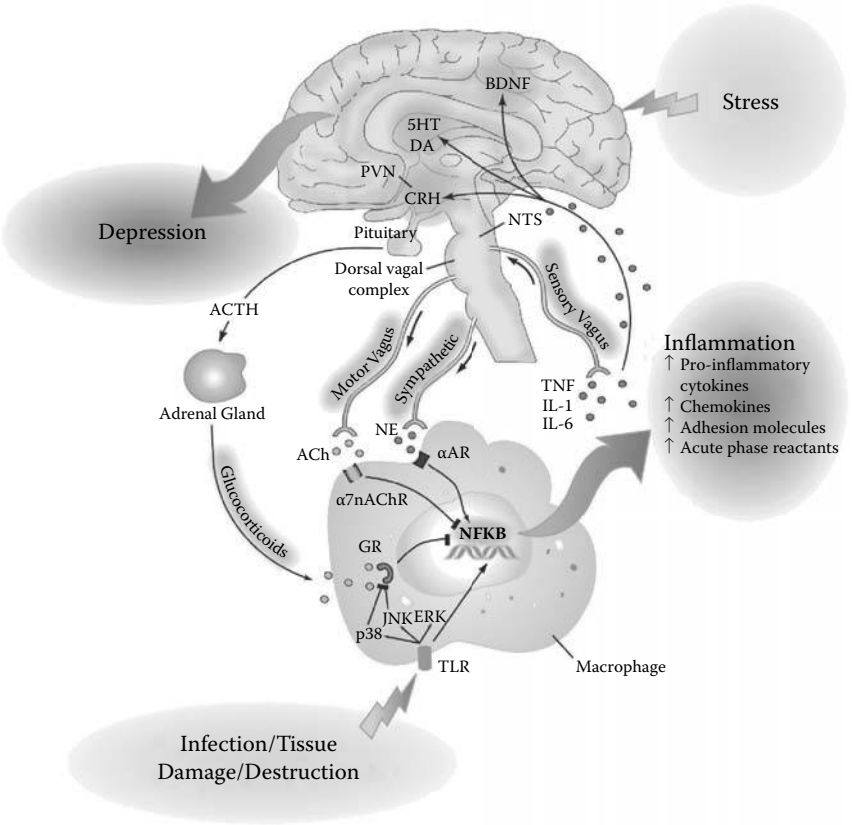

FIGURE 10.3 (See color insert.) Stress-immune interactions and depression. Activation of NF-κB through Toll-like receptors (TLRs) during immune challenge leads to an inflammatory response, including the release of proinflammatory cytokines TNF-alpha, IL-1, and Il-6. These cytokines, in turn, access the brain via leaky regions in the blood–brain barrier, active transport molecules, and afferent nerve fibers (e.g., sensory vagus), which relay information through the nucleus tractus solitarius (NTS). Once in the brain, cytokine signals participate in pathways known to be involved in the development of major depression, including altered metabolism of relevant neurotransmitters such as serotonin (5HT) and dopamine (DA), activation of CRH in the paraventricular nucleus (PVN) and the subsequent production or release of ACTH and glucocorticoids (cortisol), and disruption of synaptic plasticity through alterations in relevant growth factors (e.g., brain-derived neurotrophic factor (BDNF)). Exposure to environmental stressors promotes activation of inflammatory signaling (NF-κB) through increased outflow of proinflammatory sympathetic nervous system responses (release of norepinephrine (NE), which binds to the alpha (alpha AR) and beta (beta AR) adrenoceptors). Stressors also induce withdrawal of inhibitory motor vagal input (release of acetylcholine (Ach), which binds to the alpha-7 subunit of the nicotinic acetylcholine receptor (alpha-7nAChR)). Activation of the mitogen-activated protein kinase pathways, including p38 and Jun amino-terminal kinase (JNK), inhibits the function of glucocorticoid receptors (GRs), thereby releasing NF-κB from negative regulation by glucocorticoids released as a result of the hypothalamic–pituitary–adrenal (HPA) axis in response to stress. (Reprinted from C. L. Raison, L. Capuron, and A. H. Miller, *Trends Immunol.* 27: 24–31, 2006. Copyright 2006 Elsevier. With permission.)

Conversely, although not entirely consistent, data suggest that various forms of treatment, including repeated transcranial magnetic stimulation, electroconvulsive therapy, antidepressants, and psychotherapy, correct sympathetic/parasympathetic imbalances by attenuating SNS activity or increasing vagal tone.[240–245] Epidemiologic studies link intake of omega-3 fatty acid with a reduced depression risk and increased HRV.[246] Interestingly, a recent study suggests that applying biofeedback to directly induce these ANS changes improves depressive symptoms, strongly suggesting that ANS activity may be as much a cause of depressive symptomatology as a result.[247]

10.4.3.2 PTSD

Even more so than MD, PTSD is a condition characterized by abnormalities of the ANS. Many years of studies paint a picture very consistent with increased sympathetic and decreased parasympathetic activity being core biological abnormalities of PTSD,[248] and to a lesser extent, a frequent sequela of trauma exposure in general.[249] Given this, it is no surprise that PTSD has been repeatedly associated with increased inflammatory activity,[90,92,96,250] or that trauma exposure—especially early in life—leads to lifelong increases in inflammatory biomarkers.[175,251]

Findings consistent with increased sympathetic and reduced parasympathetic activity in PTSD include increased heart rate and reduced HRV at rest and in response to traumatic stimuli, increased heart rate and reduced HRV during sleep, increased startle response, and increased urinary and plasma concentrations of NE.[252–260] Sahar and colleagues found that cardiovascular responses to mental challenge in subjects with PTSD are under sympathetic, rather than vagal, control, as is typical in normal individuals.[261] Several findings strongly suggest that this pattern of ANS abnormality may contribute to disease pathogenesis, rather than merely reflecting its presence. First, longitudinal studies suggest that increased sympathetic/reduced parasympathetic activity shortly after a trauma predicts the later development of PTSD.[262–263] Second, interventions designed to reverse this pattern of ANS activity, such as autogenic training and biofeedback, have been shown to improve PTSD and related depressive symptoms.[264–265] Third, while not entirely consistent, some data suggest that administration of a beta blocker immediately after trauma exposure may protect against the development of PTSD.

10.4.4 Immunologic Mechanisms

10.4.4.1 Regulatory T (Treg) Cells

In addition to the anti-inflammatory effects of glucocorticoids and parasympathetic signaling, both the innate and adaptive arms of the immune system have complex regulatory processes built into their function that serve to minimize both autoimmune reactions and overly robust inflammatory responses that might unnecessarily damage host tissues.[266] A central player in regulating adaptive immune processes is T cells that express the CD25 surface marker and are positive for the transcription factor forkhead box P3 (Foxp3).[266] These CD25+Foxp3+ T cells suppress both innate and acquired inflammatory activity through several mechanisms, including direct cell-to-cell contact and via secretion of the anti-inflammatory cytokines

IL-10 and TGF-beta.[267] The central role of CD25+Foxp3+ Treg cells in containing inflammatory processes is highlighted by the fact that in laboratory animals and humans, the absence of these cells results in catastrophic autoimmune reactions.[267–269]

Given the inflammatory nature of mood and anxiety disorders, significant recent interest has been paid to the possibility that Treg activity may be inadequate in these disorders. Several lines of evidence support this possibility. In humans, laboratory stressors have been shown to reduce plasma concentrations of IL-10,[270] as well as circulating CD4+CD25+ Treg cells, which are an important source of IL-10.[270–271] Moreover, acute stress appears to downregulate Foxp3, which is a primary T cell immunoregulatory transcription factor.[271] Chronic traumatic life stress has also been associated with reduced numbers and percentages of circulating CD4+CD25+Foxp3+ Treg cells—a finding in line with many studies showing that PTSD—like MD—is associated with increased circulating levels of proinflammatory cytokines.[92,96,250,272] Finally, MD is associated with a suite of stress system changes known to promote inflammation, including glucocorticoid resistance, sympathetic overdrive, and parasympathetic withdrawal.[179,207,214,227,236,239,273–276] Recent studies suggest that these changes are also capable of suppressing the number and activity of CD4+CD25+Foxp3+ Treg cells.[277–278] Consistent with this, reduced total numbers and percentages of CD4+CD25+ Treg cells in peripheral blood, as well as reduced expression of Foxp3, have been observed in patients with MD.[279]

10.4.5 Obesity and the Leaky Gut

In people who are not medically ill, visceral fat, and its associated immune elements, represents the largest source of inflammatory cytokine production in the body.[177,280] It has long been recognized that adipocytes are capable of producing a host of inflammatory mediators.[280] More recently, it has been appreciated that excessive adipose tissue also serves as a magnet for the migration and activation of both innate (macrophages/monocytes and natural killer cells) and adaptive immune cells.[281–283] This is believed to occur, at least in part, because engorged adipocytes compress blood vessels in the fat, making themselves hypoxic in the process. This promotes mitochondrial dysfunction and ultimately the generation of reactive oxygen intermediates (ROIs), which stimulate an array of inflammatory mediators, including chemokines such as MCP-1, which attract macrophages to the fatty tissue.[280] Moreover, excessive visceral fat also appears to reduce the presence and activity of Tregs that are crucial for dampening both innate and adaptive immune processes.[284]

While many studies examining associations between neuropsychiatric conditions such as depression and inflammation use statistics to factor out the effect of obesity (measured typically as body mass index (BMI)), it has been more recently appreciated that obesity may, in fact, be an important mechanism through which depression promotes increased inflammatory activation.[285–286] This perspective is consistent with the strong bidirectional relationships that exist between excessive fat deposition and MD.[287] Indeed, many studies have shown that obesity strongly predicts the later development of depression,[288–290] and that depression is a risk factor for future obesity.[291–293] Obesity has also been associated with treatment resistance in MD,[294] which is especially interesting given studies demonstrating that increased

inflammation also portends poor response to pharmacologic treatments.[75–76,295–297] Nonetheless, it is not currently known whether increased inflammation mediates the association between excess body weight and treatment nonresponse.

In addition to stimulating the production of cytokines and other inflammatory mediators, obesity may promote inflammation via effects on the composition of the gut microbiota—that vast ecosystem of microbial life that exists in the mammalian gastrointestinal tract and that plays an essential role in the provision of nutrients and protection against pathogenic microbe invasion, and that may also have effects on cognitive function.[298] Recent data indicate that obese individuals support very different gut microbial populations than do normal weight individuals, and that these differences are significantly attenuated following gastric bypass surgery and subsequent weight loss.[299] That such differences might affect physiological processes relevant to MD is supported by data from laboratory animals showing that rodents without the normal complement of gut microbiota demonstrate increased anxiety and evince increased HPA axis responses to stress.[298,300] These "germ-free" animals also show reductions in BDNF in the hippocampus,[300] a classic finding in animal models of depression that is reliably reversed by antidepressant treatment.[301]

Given ample evidence that host factors profoundly influence the composition of the gut microbiota, and that the microbiota can drive host physiology through inflammatory and other chemical mediators,[298] it is increasingly plausible that the gut may be an important source of inflammation in response to a range of environmental adversities known to promote depression. We have already placed obesity in this category. Relevant to the importance of psychosocial stress as a depressogenic risk,[302–303] multiple studies suggest that stress can alter the composition of gut microbiota in ways that may cause increased translocation of bacterial products into the body (i.e., leaky gut) and increase the production of proinflammatory cytokines. For example, in primates, early life stress has been shown to reduce gut colonization by *Lactobacilli* and *Bifidobacteria* (both of which play important roles in maintaining gut endothelial barrier function) and to promote colonization by pathogenic bacteria species.[304] In rodents, maternal separation early in life results in disruptions in the gut microbiota that are accompanied by increased depression/anxiety behavior and increased inflammatory responses. Stress paradigms in adult animals produce similar changes in the microflora and reliably increase various measures of inflammation in the gut, as well as increased translocation of potentially pathogenic bacteria across the gut wall.[305–306] Interestingly, these changes are mediated in part by reduced vagal parasympathetic signaling and by the induction of glucocorticoid insensitivity,[307–308] both of which are classic neuroendocrine hallmarks of neuropsychiatric disorders such as MD.[179] In addition, the stress hormone NE has been shown to increase the uptake of pathogenic bacteria by immune follicles in the gut endothelium and to stimulate the growth and adherence of *Escherichia coli* bacteria,[309] both of which would be expected to produce a local inflammatory response that might serve as a nidus for the systemic inflammation seen in response to psychosocial stress. Consistent with these observations, psychological stress in humans has been associated with reduced fecal *Lactobacilli* levels,[310] and a recent study found increased translocation of Gram-negative gut bacteria with concomitant increases in plasma LPS in patients with MD.[311] The authors of this study suggest that this type of leaky gut

phenomenon (presumably caused, at least in part, by microflora disruption) might be a potential source of the increased inflammatory drive seen in many medically healthy individuals with depression.

Studies addressing potential antidepressant properties of probiotic bacteria in a rigorous manner in humans are few and only suggestive in nature. For example, a small double-blind, placebo-controlled trial found that treatment with trans-galactooligosaccharide (a prebiotic that increases gut *Bifidobacteria*) reduced anxiety in patients with irritable bowel syndrome, although the degree to which reduced anxiety resulted from improved bowel function is unclear.[312] Similarly, 2 months of treatment with a *Lactobacillus* species reduced anxiety, but not depressive symptoms, in patients with chronic fatigue syndrome (CFS) when compared to placebo.[313] A smaller open study in CFS found no effect of probiotic treatment on fatigue, but an improvement in the types of cognitive symptoms that are also core constituents of MD was observed.[314]

10.5 EVOLUTIONARY CONSIDERATIONS: WHY INFLAMMATION IN NEUROPSYCHIATRIC DISEASE?

It is hard for those of us living in the industrialized world at the start of the twenty-first century to grasp how many of our forebears perished from infectious causes, and did so early enough in life to fall afoul of evolution's twin mandates to survive and reproduce. We suggest that this simple truth provides a novel perspective on why MD has the risk factors it does, why it is so prevalent, and why genes that increase the risk for depression are so numerous and so common, despite the fact that depression—like death from infection—is a condition at odds with optimal survival and reproduction. From this perspective, depression may be so common not because it confers any hidden survival advantages in and of itself, but simply because it is so often a by-product of activated inflammatory systems that have been under extremely powerful positive selection pressure since time immemorial.

Genes that confer a heightened risk for depression may have prospered in the human genome not because of their linkage to depression, but because they pleiotropically conferred significant survival advantages in the face of infection as a result of promoting heightened inflammation. Across the long hurly-burly of evolutionary time, reduced infectious mortality trumped increased depression. The trade-off was worth it.[315] The trade-off may have even been worth it in terms of inflammatory responses to psychosocial stress, given the strong association between phylogenetically typical mammalian stressors (i.e., attack by predators, attack by dominant conspecifics) and risk for tissue damage and concomitant wound infection. By proactively switching on inflammatory processes ahead of the conflict, survival may have been enhanced as a result of prepotent immune readiness. In a world in which many stressors meant life or death, optimal survival would likely have accrued in the face of many false alarms, but few failed responses.

But times change, and today we live in a world of false alarms in which our need for inflammation has been substantially reduced. First and foremost, the modern world has developed an "extended phenotype" in regards to our interactions with

the microbial world. That is, many protective functions once the sole province of the immune system have been outsourced to an array of cultural practices, such as sewage and drinking water treatment, mosquito abatement programs, food refrigeration and pasteurization, modern medicine, and widespread public health education and knowledge. In regards to stress, most individuals in the developed world live under rules of law that greatly reduce the risk that psychosocial conflicts will end in physical trauma and infection, and most of our former predators are largely extinct or live their lives behind bars in zoos.

Given these innovations (and losses), and given the high price in tissue damage and emotional suffering that inflammation incurs, we might do well to reduce our inflammatory responses to the adversities of life (microbial and otherwise), and indeed, repeated studies have shown a link between genetic alleles that promote reduced inflammatory/increased anti-inflammatory signaling and human well-being and longevity.[316–317] But we are stuck in a state of evolutionary mismatch, saddled with a shoot-first-ask-questions-later inflammatory response that no longer protects us on a regular basis from an early death, but continues—day in and day out—to inflict needless wear and tear on the body and brain. We would suggest it is for this reason that inflammation plays so central a role in the diseases of aging to which most of us will eventually succumb and that are so highly comorbid with MD. They are comorbid with MD because, like MD, they result—to at least some degree—from inappropriately excessive activation of inflammatory pathways in response to conditions in the modern world toward which such activation is of little value or no avail.

10.6 TRANSLATIONAL IMPLICATIONS

Given the association between inflammation and neuropsychiatric disease and the putative mechanisms involved, it is apparent that there may be a number of strategies and targets relevant for the treatment and prevention of inflammation-induced behavioral changes. Such strategies and targets involve both pharmacologic and behavioral approaches (Table 10.2).

10.6.1 Pharmacological Interventions

10.6.1.1 Promising Pharmacologic Targets and Existing Agents

As indicated above, there are a number of pharmacologic strategies that might be considered for targeting inflammation in the treatment of mood and anxiety disorders. The cytokines themselves are the most obvious targets, and the availability of a host of Food and Drug Administration (FDA)-approved biological agents that inhibit the action of TNF-alpha and IL-1 warrants consideration for clinical trials in treatment-resistant populations of mood and anxiety disorder patients. Data from inflammatory disorders and cancer, as well as patients with sleep disorders, have already suggested the possible efficacy of these agents in terms of symptoms of depression, fatigue, and impaired sleep.[83,318–319] Other relevant targets are the inflammatory signaling pathways themselves, including MAPK and NF-κB. The impact of MAPK pathways (especially p38) on neurotransmitter metabolism and GR function

TABLE 10.2
Treatment Implications: Strategies to Reduce Inflammation and Treat Mood and Anxiety Disorders

1. Pharmacologic
   a. Anticytokine therapies
   b. Antagonists of cytokine signaling pathways (e.g., MAPK and NF-κB)
      i. Natural compounds
      ii. Curcumin
   c. IDO antagonists
   d. Chemokine receptor antagonists
2. Behavioral
   a. Exercise
   b. Diet and nutrition
   c. Sleep hygiene
   d. Stress management
   e. Complementary and alternative strategies
      i. Yoga
      ii. Meditation

has been described,[115,184] and aside from driving inflammatory responses, NF-κB appears to play a central role in the effects of cytokines on synaptic plasticity in general and neurogenesis in particular.[130] Given increasing data implicating KYN and its metabolites, QUIN and KA, in cytokine-induced behavioral changes,[320] the enzyme IDO also represents an intriguing target for strategies that address the unique pathways by which the immune system may influence behavior. IDO antagonists are available (e.g., 1-methyl tryptophan), and are being developed for the treatment of several diseases.[321] As discussed above, the potential role of cells such as monocytes/macrophages trafficking to the brain during peripheral inflammation may also to be relevant for drug development.[109] Molecules that inhibit chemokine receptors may be most important in this regard and are currently being developed for the treatment of inflammatory and autoimmune disorders. Obviously the possibilities are endless; however, the general idea is that drugs in development to target inflammation in a multitude of disease states may also have relevance for neuropsychiatric disorders, emphasizing the need for close collaboration between immunology and the neurosciences as this area of investigation further develops.

10.6.1.2 Natural Compounds

There is a rich tradition for the use of natural compounds to treat a variety of diseases, including cancer. One compound that has received considerable attention, especially as it relates to inflammation and its contribution to disease, is curcumin.

10.6.1.2.1 Curcumin

Curcumin, the active ingredient of the Indian curry spice turmeric, is a compound recognized to have anti-NF-κB effects in a number of experimental contexts. Curcumin and its analogues have therefore garnered considerable interest regarding the discovery

of drugs that inhibit inflammation. Curcumin has been shown to have potent inhibitory effects on constitutive and inducible NF-κB activation, as well as potent inhibitory effects on IKK.[322] These effects are believed to underlie curcumin-induced inhibition of the production of proinflammatory cytokines, including TNF-alpha.[323] In part related to its effects on NF-κB, curcumin also has been shown to exert powerful effects on cyclooxygenase and inducible NO synthase (iNOS).[324] iNOS catalyzes the conversion of L-arginine to NO, which is known as a key factor for the formation of highly reactive oxygen species and, as indicated above, may be relevant to the toxic effects of cytokines in the brain. Curcumin inhibits NO production as well as the expression of iNOS.[325] Curcumin also suppresses the synthesis of prostaglandins,[326] which may contribute to its inhibition of tumor cell growth and metastasis.

Regarding clinical efficacy, curcumin has shown potential as a treatment for arthritis. Rheumatoid arthritis patients treated with curcumin (1,200 mg/day) or phenylbutazone (300 mg/day) displayed identical improvement of symptoms after 2 weeks of drug treatment without any discernible side effects.[327] Curcumin has also shown promise as an intervention for ocular disorders with an inflammatory component. For example, treatment with curcumin reduced uveitis-related symptoms, including redness, lacrimation, poor vision, and vitreous turbidity.[328] Another study investigated the effectiveness of curcumin in the context of idiopathic inflammatory orbital pseudotumor, and found that patients treated with curcumin for up to 22 months had normal eye imaging results.[329] Curcumin has also been found to improve symptoms in patients with inflammatory bowel disease.[330]

Relevant to depression, a series of recent studies by Xu and colleagues suggest that curcumin may have antidepressant properties in rodents as manifested in the tail suspension test and the Porsolt forced swim task.[331–334] Oral administration of curcumin (5 and 10 mg/kg) significantly decreased immobility of mice in a tail suspension test, as well as immobility in the forced swimming task.[333] Results were comparable to those produced by imipramine and, in a later study, fluoxetine.[334] Of note, in these studies, mice were treated with curcumin 45–60 minutes before testing, suggesting that curcumin is able to exert rapid effects on behavior. Subsequent studies have implicated 5-HT1a and 1b receptors, as well as 5-HT2c receptors in curcumin's antidepressant effects in mice. Whether these effects of curcumin are related to effects on inflammatory signaling remains to be determined. Curcumin has exhibited similar antidepressant effects in rats, although in these experiments rats were treated with curcumin for multiple days before testing.[331,333] Despite the effectiveness of curcumin as an anti-inflammatory and possibly antidepressant drug, the compound suffers from a number of issues that limit its potential clinical development, including poor absorption, low serum levels, poor distribution to tissues (low bioavailability), and rapid metabolism.[335] Thus, analogues of curcumin are needed to overcome these shortcomings while offering the benefits of this intriguing natural compound.

10.6.2 Behavioral Interventions

A number of behavioral interventions demonstrate promise to optimize regulation of inflammatory responses while improving mood and anxiety symptoms. These

include physical exercise, dietary interventions, and a variety of complementary and alternative techniques, including yoga, mindfulness, and compassion meditation.

10.6.2.1 Exercise

Physical exercise has been shown to reduce depression and anxiety symptoms,[336] and has been found to be as effective as conventional antidepressant and antianxiety pharmacotherapies in several studies.[337–338] Physical exercise also prevents the development of depressive features subsequent to stressful life events,[339] indicating that exercise is valuable both as a behavioral treatment and as a preventative measure. In addition, exercise exerts significant effects on inflammatory responses. Although exercise activates the inflammatory signaling cascade in the short term, habitual exercise has been associated with reduced inflammation as reflected by reduced concentrations of circulating inflammatory factors, including CRP.[340] Several mechanisms appear to mediate exercise's effects on inflammation. Exercise has been shown to reduce baseline IL-6 and CRP production by decreasing obesity and increasing insulin sensitivity.[341] Exercise also alters skeletal muscle production of proinflammatory cytokines, leading to decreased peripheral blood concentrations of TNF-alpha and IL-6,[342] while increasing the anti-inflammatory cytokine, IL-10.[343] Finally, exercise dampens resting and stress-induced SNS activity, while increasing vagal tone, together leading to enhanced restraint of inflammatory responses.[344] Thus, exercise appears to provide a multipronged approach to the management of the potential impact of inflammation on neuropsychiatric disease.

10.6.2.2 Diet and Nutrition

Diet is another target for influencing inflammation and thereby addressing mood and anxiety symptoms. Indeed, a "prudent" diet rich in vegetables, fruits, and fish has been associated with reduced depressive symptoms.[345] Interestingly, fish may be an especially important component of the prudent diet, as epidemiological studies have found an inverse relationship between the annual consumption of fish and the prevalence of MD.[346] The prudent diet is associated with lower circulating inflammatory markers, including CRP, a relationship that remains significant even after controlling for BMI and other lifestyle variables, such as smoking, sleep patterns, and exercise.[347–348] Reductions in proinflammatory cytokines, including IL-6, have also been associated with adherence to a Mediterranean diet.[349] Diet may influence inflammation through several mechanisms. For example, a diet rich in fatty foods and refined carbohydrates (as well as a lack of exercise) encourages inflammation by impacting body weight and adiposity. As noted above, macrophages that reside in fat tissue are known to produce proinflammatory cytokines and other inflammatory mediators.[350] Second, a high-fat diet promotes endotoxin absorption from the gut, with attendant proinflammatory consequences.[351] Third, a high-fat diet has been found to inhibit the release of anti-inflammatory cytokines, including TGF-beta.[352] Finally, both lower concentrations of omega-3 (*n*-3) polyunsaturated fatty acids (PUFAs) and higher omega-6 (*n*-6):*n*-3 ratios have been associated with increased proinflammatory cytokine production.[353] Fish contains high amounts of *n*-3 PUFAs, while refined vegetable oils are rich in *n*-6 PUFAs. Of note, *n*-3 PUFAs

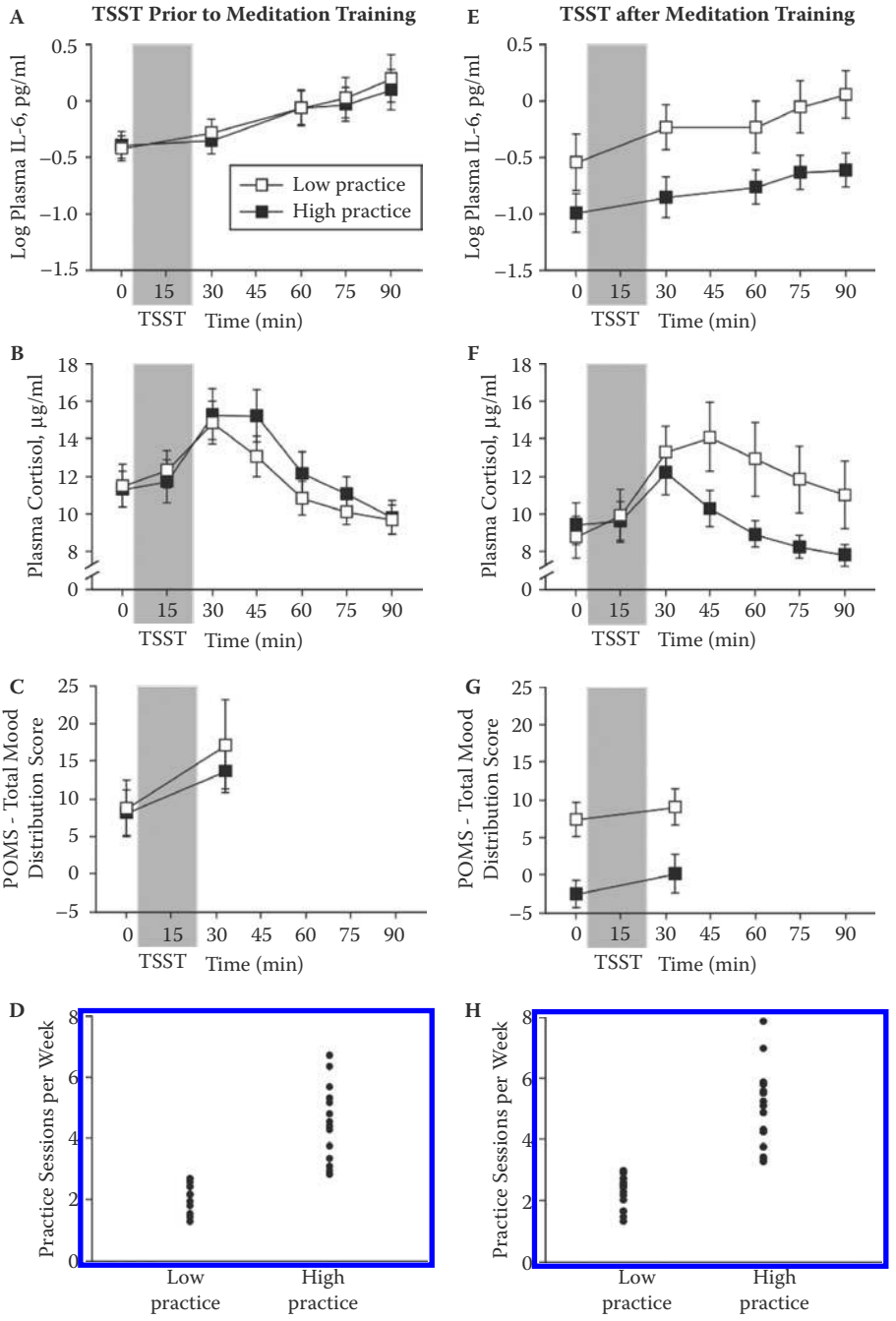

FIGURE 10.4 See Figure 10.4 caption next page.

have been shown to reduce production of arachidonic acid-derived proinflammatory eicosanoids of the prostaglandin 2 series.[354–355]

10.6.2.3 Sleep

In addition to diet and exercise, sleep is another behavioral target that may be relevant to the relationship between inflammation and neuropsychiatric disorders. Sleep disruption has been shown to be associated with increased activation of inflammatory signaling pathways, such as NF-κB, and increased proinflammatory cytokines.[356–357] Moreover, administration of the innate immune cytokine IFN-alpha has been found to disrupt the continuity and depth of sleep, leading to insomnia.[358] These data suggest that a vicious cycle can be established between sleep and inflammation whereby disrupted sleep can induce inflammatory responses, which in turn can further disrupt sleep. Standard behavioral techniques, including cognitive behavioral therapies targeting sleep hygiene, have been shown to improve sleep and may break this cycle in the context of stress, depression, and anxiety.[359]

10.6.2.4 Complementary and Alternative Strategies

Several nontraditional approaches have been shown to positively impact mood and reduce inflammatory responses. Yoga has been found to exhibit antidepressant and antianxiety effects,[360] while also decreasing inflammatory responses in experienced practitioners.[361] Mindfulness meditation, which can be used to disengage from negative and ruminative thinking, benefits patients with MD and anxiety disorders.[362,363] Although the majority of studies investigating meditation and immune effects have focused on acquired immune function (e.g., Davidson et al.[364]) at least one study has assessed inflammatory endpoints. In a study examining the effects of compassion meditation, the amount of meditation practice time was inversely related to plasma IL-6 concentrations induced by a laboratory stress challenge (Figure 10.4).[365]

FIGURE 10.4 Compassion meditation practice optimizes inflammatory, endocrine, and subjective distress responses to a psychosocial laboratory stress challenge. Trier Social Stress Test (TSST) induced plasma interleukin (IL)-6, plasma cortisol, and subjective distress responses in high-practice and low-practice participants challenged with the TSST prior to or after compassion meditation training. High-practice and low-practice meditation groups challenged with the TSST before meditation training (A–D) and after meditation training (E–H) were formed based on a median split of a mean number of practice sessions per week. Regardless of meditation training, challenge with the stressor increased plasma IL-6 (A and E), plasma cortisol (B and F), and distress as measured by the Profile of Mood States (POMS) total score (C and G). In participants who underwent TSST challenge prior to meditation training, IL-6 (A), cortisol (B), and POMS total score (C) responses to the TSST did not differ between high- and low-practice groups. In contrast, in participants who underwent TSST challenge after meditation training, IL-6 (E) and POMS total score (G) responses across the TSST procedure were reduced in high-practice participants compared to low-practice participants. Of note, high- and low-practice participants with training after the TSST showed comparable amounts of overall practice time compared to high- and low-practice participants with training before the TSST (D and H). (Reprinted from T. W. W. Pace, L. T. Negi, T. I. Sivilli, M. J. Issa, S. P. Cole, D. D. Adame, and C. L. Raison, *Psychoneuroendocrinology* 35(2): 310–15, 2010. Copyright 2010 Elsevier. With permission.)

In terms of the mechanisms by which meditation may influence inflammatory function, Transcendental Meditation® and Zen meditation have both been shown to increase HRV,[366] reflective of increased parasympathetic tone. As discussed above, increased parasympathetic tone may attenuate inflammatory responses.

10.7 SUMMARY

The data demonstrating an association between neuropsychiatric disorders and inflammation, as well as the mechanisms involved, are compelling and indicate that neuropsychiatric disorders may not only arise from activation of inflammatory processes, but also significantly contribute to nonresolving inflammation and its impact on the development of a number of medical illnesses. Translational implications of this work are that anti-inflammatory strategies may have special relevance to patients with behavioral disorders, especially patients with neuropsychiatric disorders and medical comorbidities.

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11 Role of Inflammation in Gastrointestinal Diseases

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CONTENTS

11.1 INTRODUCTION

The gastrointestinal (GI) mucosa hosts the largest collection of microbial flora (microbiota) in the human body. Failure to achieve or maintain the balance between the host and its microbiota has detrimental outcomes, leading to a variety of GI diseases related to the activation of dysregulated immune responses and inflammation. The GI mucosa utilizes both innate and adaptive immune mechanisms to protect itself against microorganisms while simultaneously being tolerant toward the normally harmless microflora and antigens consumed in the food. Disturbance of these protective

mechanisms results in diseases, such as inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease, eosinophil-associated GI disorders (EGIDs) (e.g., eosinophilic esophagitis, gastritis, and gastroenteritis), celiac disease, and others.

Chronic intestinal inflammation has been linked to the development of several GI malignancies, including pancreatic, esophageal, gastric, liver, and colorectal cancers. Recent studies suggest that during chronic inflammation, the innate immune system may facilitate GI tumorigenesis in genetically predisposed individuals in response to certain microorganisms. This chapter will review the role of inflammation in GI diseases, focusing on a few examples, and will review the link between inflammation and GI tumorigenesis. Recent studies have elucidated the role of distinct immune cells, cytokines, and other immune mediators in GI tumorigenesis. These mechanisms are discussed herein.

11.2 GASTROINTESTINAL DISEASES RELATED TO INFLAMMATION

11.2.1 Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) comprises a group of inflammatory diseases of the colon and the small intestine. Ulcerative colitis (UC) and Crohn's disease (CD) are two major subtypes of IBD. Both diseases are considered autoimmune diseases, caused by malfunction of the immune system, which develop in genetically predisposed individuals.[1]

Several pathogens have been implicated in the development of IBD and proposed as etiological disease agents, including a number of chronic bacterial and viral infections. Thus, the main hypotheses for disease pathogenesis suggest loss of immune tolerance to normal commensal bacteria coupled with excessive exposure to bacterial antigenic products.[2] This is further supported by the fact that the composition of the gut flora is altered in IBD with an increased presence of aggressive bacteria, such as bacteroides, adherent/invasive *Escherichia coli*, and enterococci.[3]

The exact etiology of CD is unknown. However, it is assumed that a combination of environmental and genetic factors might contribute to disease development.[4] Thus, persistent mycobacterial infection has been suggested as a causative factor for CD,[2] while mutations in a number of genes can increase susceptibility. One such gene is NOD2, encoding for a protein that plays an important role in the immune system and activates nuclear factor kappaB (NF-κB), making it responsive to bacterial lipopolysaccharides. A frameshift mutation in NOD2 generates a truncated NOD2 protein that was found significantly associated with CD.[5] Another susceptibility factor is XBP1, a transcription factor involved in the regulation of the endoplasmic reticulum (ER) stress response. Genetic variants of XBP1 were shown to confer high risk to both CD and UC and link ER stress to intestinal inflammation.[6] These results implicate NOD2 and XBP1, and other genes, as susceptibility factors to CD, and suggest a link between an innate immune response to bacterial components and disease development.

Unlike CD, UC affects only the colon and the rectum, but has a similar unknown etiology and develops on a genetic and environmental background. To date, more than 50 novel IBD susceptible loci have been found, while several promising associations between IBD and gene variants have been described.[7] Of note, sequence variants in

interleukin (IL)-10, an immunosuppressive cytokine, were shown to contribute to UC susceptibility, suggesting that IL-10 function plays a key role in UC pathogenesis.[8]

In recent years, the field of nutrigenomics, assessing gene–diet interactions, has revealed that the genetic susceptibility to UC is impacted by dietary and environmental factors.[9] Nutrients can induce the expression of inflammatory mediators that may affect many cellular processes and tend to affect the immune responses of the host. The role of diet in the management of IBD has been recently reviewed by Rajendran and Kumar,[10] who provided a list of studies examining dietary exclusion and sensitivity to foods in IBD. The most common identified food sensitivities are cereals, milk, eggs, vegetables, and citrus fruits. Notably, Western diets are more strongly associated with CD,[11] which may relate to the increased intake of refined foods and food additives, which may be antigenic.

11.2.2 Eosinophil-Associated GI Disorders

Eosinophil-associated GI disorders (EGIDs) are characterized by having above normal amounts of eosinophils, in one or more specific places anywhere in the digestive system. Thus, EGIDs involve most parts of the GI tract, and include disorders such as eosinophilic esophagitis, eosinophilic gastritis, and gastroenteritis. Eosinophilic inflammation is also a feature of a variety of other GI disorders, including food allergy, allergic colitis, IBD (CD and UC), and parasite infection.[12,13] This series of disorders can affect individuals of all ages worldwide.[14]

Eosinophils are mainly recruited from the bone marrow through the blood circulation, and their infiltration into local tissues is primarily regulated by IL-5 and eotaxin, a specific chemoattractant.[15] Eosinophil presence is thought to contribute directly to GI disease pathogenesis, leading to tissue destruction and clinical symptoms such as diarrhea, vomiting, and mucosal bleeding. For example, infiltration of eosinophils into the mucosal lamina propria during active UC has been shown to contribute to the pathogenesis of the disease.[16] By using IL-5 or eotaxin-deficient mice, Forbes et al.[16] have demonstrated a role for eotaxin in eosinophil recruitment into the colon during experimental UC. This study also showed that eosinophil-derived peroxidase, released upon eosinophil degranulation, is critical in the development of GI dysfunction in this model. Thus, unraveling the mechanisms of eosinophil infiltration into the GI tract is important to the understanding of multiple disease inflammatory processes.

11.2.3 Celiac Disease

Celiac disease is a condition in which the lining of the small intestine is damaged by an allergic reaction to the food protein gluten, which is present in a number of grains. Celiac disease is considered an autoimmune disease mainly because IgA antibodies produced by the immune system against specific gluten components, namely, gliadin, also target and damage the intestinal tissue, resulting in the characteristic flattening of the villi, which impairs absorption of nutrients.

Among adult patients presenting with abdominal symptoms, IgA anti-tissue transglutaminase and IgA anti-endomysial antibodies are known to have high

sensitivity and specificity for diagnosing celiac disease.[17] Clinical presentation of the disease can vary from full-blown malabsorption to subtle and atypical symptoms. Diagnosis currently relies on clinicopathologic studies, including mucosal biopsy, serologic tests, and the effects of a diet free of gluten on the symptoms. Mucosal pathologic features are also variable, ranging from mild abnormalities, including intraepithelial lymphocytosis, to completely flat mucosa.[18]

A common feature of celiac disease and many other organ-specific autoimmune diseases is a central role for T cells in causing tissue destruction.[19] In addition, celiac disease patients have small intestinal intraepithelial lymphocytes that can mediate direct cytotoxicity of enterocytes in an antigen nonspecific manner. Although the immune response to gluten is central to the pathogenesis of the disease, it is becoming clear that intestinal tissue inflammation, induced either by infectious agents or by gluten itself, is crucial for activating T cells and eliciting their tissue-destructive activities.

11.2.4 Gastroesophageal Reflux Disease

Gastroesophageal reflux disease (GERD) is one of the most common problems in clinical practice today. Although many factors are involved in the pathogenesis of GERD, it is generally believed that functional and structural abnormalities of the gastroesophageal junction, as well as an abnormal exposure to gastroduodenal contents, are the main contributors to its pathogenesis. Thus, the antireflux barrier at the gastroesophageal junction is the final determinate of reflux. In the majority of cases, transient lower esophageal sphincter (LES) relaxations appear to be the necessary condition for reflux to occur. In severe cases of GERD, particularly in those with esophagitis and Barrett's epithelium, reduced resting LES pressure plays a contributory role. Novel findings of the inflammatory process in GERD suggest a multifactorial process involving several inflammatory mechanisms. In addition, inflammatory mediators have been shown to contribute to well-known complications of GERD, namely, motility abnormalities, fibrosis, and carcinogenesis.[20] Esophageal dysmotility may be an additive factor leading to increased esophageal acid contact time and predispose patients to developing erosive esophagitis. Both GERD and Barrett's esophagus are recognized as major risk factors in the development of esophageal cancer as they have been associated with inflammation of the esophageal squamous epithelium (e.g., reflux esophagitis). The cellular mechanisms contributing to cancer development in the esophagus are poorly understood. The chronic inflammation that is present in GERD and Barrett's esophagus creates an environment suitable for DNA damage and altered gene expression involved in cellular proliferation and inhibition of apoptosis. Key players in the inflammatory cascade include generation of free radicals, activation of kinases pathways and transcription factors, and production of cytokines and inflammatory enzymes.

11.3 EPIDEMIOLOGY

The decline of infectious diseases in the Western world is paralleled by the rise of allergic disorders, mainly chronic inflammatory and autoimmune diseases.[21] This transition is associated with changes in lifestyle, nutrition, obesity, and increased

exposure to pollutants and antibiotics, which all impact the intestinal microbiota. The 99th Dahlem Conference on Infection, Inflammation and Chronic Inflammatory Disorders (Berlin, June 9–12, 2009) examined the role of microbes (symbionts, commensals, and pathogens) as elicitors, perpetuators, modulators, and terminators of inflammatory responses, and discussed the mechanistic links behind the epidemiological observations, the complexity of a changing microbiome (the collective microbial genomes), and the immunoregulatory consequences of microbial encounter in barrier organs. This panel of experts generated the Dahlem Workshop model, based on background papers, which postulates that gene–environment interactions leading to inflammatory responses in barrier organs (lung, skin, gut) are exposed to environmental stresses. Stress signals are integrated at the site of assault to maintain homeostasis, depending on the genetic predisposition or pathways of immune regulation. If this equilibrium is perturbed, inflammation is not terminated appropriately, and negative consequences (e.g., disease) occur.

Several studies have shown that individual variations in the microbiome influence host health and may be implicated in disease etiology.[22] Altered gut (fecal) microbiota have been associated with several metabolic and inflammatory diseases in humans. One such example is IBD, which is characterized by reduced microbial diversity, implying the necessity of a "complete" microbiome for a healthy microbiota and human host.

The microbiota produces a broad spectrum of metabolites that can interact with the host. These include fermentation products (e.g., short-chain fatty acids), neuroactive molecules (e.g., gamma-amino butyric acid, nitric oxide, hydrogen sulfide, carbon monoxide, ATP, and basic amines), and immunostimulatory bacterial constituents (e.g., lipopolysaccharides, capsular polysaccharides, and DNA).[21]

The molecular interactions between commensal microorganisms and their host are basically different from those triggered by pathogens since they involve tolerance. Frequently, the discrimination between commensals and pathogens is largely context driven: a commensal that finds itself in the wrong anatomical space will elicit a strong innate response. Moreover, a microbe may become a pathogen only by virtue of its ability to damage a host cell and trigger a host response in this way. *Candida albicans* is such an example: this commensal can drive pathology, such as in IBD, only when a particular assembly of pathogen-associated molecular patterns interacts with a defined, and sometimes disturbed, repertoire of pattern recognition receptors (PRRs).[23] Thus, beneficial or deleterious immune responses that either maintain a commensal state or favor damage will determine the pathological consequences.

11.4 ROLE OF BACTERIAL INFECTIONS AND INTESTINAL MICROBIOTA

Bacterial infections of the GI system play an important role in inflammation and the induction of subsequent GI disease. However, most of human interaction with bacteria is beneficial for our survival and comes in the form of GI microflora that prosper in the lumens of the target organs. There exists over 10^9 colony-forming units/ml of bacteria colonizing the oral cavity and lower GI tract, and nearly 10^5

CFU/ml found in the stomach and proximal small bowel.[24] In contrast to causing disease, most individuals derive sustained benefit from the dynamic symbiotic relationship with bacterial flora. Microbiota serve several diverse functions, including immune system stimulation, carbohydrate fermentation and absorption of unutilized energy, increased growth of epithelial and lymphoid tissue, inhibition of pathogenic microbial growth, vitamin synthesis and ion absorption, and allergy prevention. There is also evidence that gut flora may aid in defending against diseases such as IBD, colon cancer, or multisystem organ failure.[25]

But what happens when the balance between host and bacteria is disrupted by disease, inflammation, or drugs? This leads to a change in the microflora barrier protection, allowing pathogenic bacteria to colonize GI mucosa, rapidly multiply, and inflict inflammatory damage. Suddenly, colonization resistance from microflora is rendered inefficient, and the host is vulnerable to enteropathogen invasion. Such is the case when humans face conditions of *Helicobacter pylori*-induced gastritis, antibiotic-induced *Clostridium difficile* overgrowth, and resultant pseudomembranous colitis, or enteritis caused by *Salmonella*, *Shigella*, *Escheria coli*, or *Yersinia*.[26] Each of these bacterial infections deserves attention for the unique mechanisms by which inflammation is produced and clinical manifestations result. This discussion, however, will focus on *H. pylori*, which over the last decades has gained unparalleled attention for its exceptional inflammatory process and ability to provoke GI disease.

11.4.1 *Helicobacter pylori* and Gastritis

Helicobacter pylori is a Gram-negative microaerophilic rod that plays a leading role in gastric inflammation, peptic ulcer disease, and the ultimate development of gastric cancer.[27] Ever since it was discovered in 1982 by Warren and Marshall, *H. pylori* and its association with GI disease has been the subject of incredibly extensive research. *H. pylori* is the most well-known risk factor for developing peptic ulcer disease, with infection documented in 95% of duodenal ulcers and 60–80% of gastric ulcers. In addition, because of its relationship with gastric carcinoma and gastric-associated lymphoid tissue lymphomas, *H. pylori* has been classified as a definite carcinogen by the World Health Organization's International Agency for Research on Cancer.[28]

The transmission of *H. pylori* is not well understood but is thought to be transmitted from person to person through oral–oral or fecal–oral mechanisms, and is more prevalent in developing countries where unsanitary and overcrowded living conditions and unclean food and water may exist. In developing areas of the world, such as parts of Asia, Africa, and Latin America, *H. pylori* infection is frequently acquired during childhood, where the majority of infected individuals will remain asymptomatic, silently transmitting the infection to their surrounding peers. Without successful eradication therapy, *H. pylori* manifests as a chronic lifelong infection, putting the individual at risk for future inflammatory sequelae. It is thought that over 80% of the population in developing parts of the world is infected with *H. pylori* by age 20, as compared to 20–50% in industrialized countries, with an overall world prevalence of around 50%.[29]

Even in industrialized nations, the prevalence of *H. pylori* varies between subpopulations. In the United States, for example, individuals in lower socioeconomic

brackets display higher rates of *H. pylori* in comparison to those in higher socioeconomic classes. Also, with regard to race, African Americans, Hispanics, and Asians are more commonly infected with *H. pylori* than Caucasians of the same age. Age differences can also be seen, where in the United States, it is estimated that 20% of people younger than 30 and 50% of those older than 60 are infected with *H. pylori*. It is thus evident that *H. pylori* spreads itself not only over various geographical areas, but among people of varying age, race, and socioeconomic background.[30–32]

Infection with *H. pylori* leads to a state of chronic gastritis. *H. pylori* infects the antrum or corpus of the stomach, and depending on the site of colonization, various pathologies arise. Antral-predominant gastritis accounts for 10–15% of infected subjects and typically results in duodenal ulcers and no increased cancer risk, whereas corpus-predominant gastritis is found in 1% and leads to gastric ulcers, atrophic changes, intestinal metaplasia, dysplasia, and predisposition to gastric cancer. The majority (>80%) of infected subjects, however, display a mixed or benign type of chronic gastritis that does not result in gastric disease and carries little clinical significance.[28]

When comparing chronic antral and corpus gastritis, antral gastritis is usually associated with hypergastrinemia and hyperchlorydia, while corpus gastritis manifests with hypergastrinemia and hypochlorydia. It is believed that antral gastritis is the result of the initial insult by *H. pylori*, where the high acid output significantly contributes to both duodenal and prepyloric ulcers. With continued inflammation, the bacteria migrate proximally into the corpus, aided by a shift to inhibition of gastric acid secretion and hypochlorydia. This state of decreased acid secretion further potentiates parietal cell destruction, corpus atrophy, and metaplasia. Although a high percentage of individuals infected with the bacterium are asymptomatic, a small percentage will have gastric mucosa that is susceptible to entering the cascade of inflammatory changes.[33,34]

These inflammatory changes involve the interaction between *H. pylori* virulence factors, host immune response, and environmental agents. When *H. pylori* migrates into the lumen of the stomach, the bacterium is able to effectively burrow into the mucosa through the release of urease and the use of multiple polar flagella exhibiting rapid corkscrew motility.[35] Large amounts of urease effectively convert urea into ammonia and carbon dioxide, alkalinizing the surrounding bactericidal environment and providing a protective shell for *H. pylori*.[27] This production of cytosolic and cell surface-associated urease and the generation of carbon dioxide are the bases of the urea breath test used to diagnose *H. pylori* infection. It is not only *H. pylori*'s utilization of urease, but also its mobile and chemotactic ability that aid in mucosal colonization. Using chemotaxis, *H. pylori* is directed to swim away from the acidic environment of the stomach lumen, nestling itself into the mucosal lining and paving the epithelial cell surface.[36] Furthermore, it is thought that the bacterium is able to increase its swimming speed to lessen contact time with the low pH. Therefore, the combination of urease, polar flagella, speed, and environmental sensing all work in unison to enhance contact with the mucosal surface, allowing maximum amounts of bacteria to induce inflammation.[28]

After colonizing the mucosal stomach layer, binding to epithelial cells is accomplished by adhesins on *H. pylori*. Specifically, the blood group antigen-binding

adhesion (BabA) is an outer membrane protein that binds to Lewis B blood group and fucosylated ABO blood group antigens expressed on the stomach epithelial cell surface.[37,38] There is also an interaction between sialic-binding adhesin A (SabA), which binds the sialyl-Lewis-X epithelial glycoprotein receptor.[39] Once colonization and adhesion are complete, *H. pylori* injects several other virulence factors that contribute to the observed gastritis. Of these, the most important include vacuolating cytotoxin A (VacA), the outer inflammatory protein (OipA), and the well-established cytotoxin-associated gene in the pathogenicity island (CagA PAI).[28]

VacA has several inflammatory mechanisms contributing to gastritis. Many polymorphisms exist among the VacA alleles, which subsequently result in various levels of cell toxicity.[29] VacA is known for its action in destroying cytoskeletal structure through vacuole formation, increased cell permeability, damage of cell cycle genes, and inhibiting the host immune response. VacA interferes with phagocytosis and antigen presentation, decreasing T cell response. These actions predispose to gastritis and peptic ulceration.[40,41]

VacA further induces apoptosis of gastric cells through a mitochondrial-dependent pathway. Through the activation of the pro-apoptotic proteins Bax and Bak of the Bcl-2 family, cytochrome C is released from the inner mitochondrial membrane, leading to cell death.[42] It is also thought that VacA enhances inflammation through upregulation of prostaglandin E2 production through inducing cyclooxygenase 2 (COX-2).[43] COX-2 overexpression is thought to play an important role in perpetuating the cycle of chronic inflammation from *H. pylori* infection and has implications in the development of gastric cancer. Specifically, COX-2 may initiate cellular changes early on in gastric carcinogenesis, and this has brought attention to the use of COX-2 inhibitors having a preventative role in gastric carcinoma development.[44]

Regarding *H. pylori*'s arsenal of virulence factors causing gastric disease, the most emphasis is placed on the role of CagA PAI. The CagA PAI is a 40 kb segment of DNA on the *H. pylori* genome that codes for the CagA protein. This CagA cytotoxin, along with several other proteins encoded along the same segment, helps create the type IV secretion system. This system is a membrane-spanning structure that plays a vital role in transferring the CagA cytotoxin across the bacterial membrane into gastric epithelial cells. CagA-positive strains of *H. pylori* are known to propel a more intense inflammatory response that has been linked to more severe peptic ulceration, atrophic gastritis, and the eventual development of gastric carcinoma. Interestingly, according to epidemiological studies, CagA-positive strains of *H. pylori* are found in 30–40% of the Western countries, whereas nearly all strains of *H. pylori* infecting individuals in East Asian countries are CagA-positive.[27]

CagA gains access to the epithelial cells through the interaction with another product of the pathogenicity island, CagL, and a host integrin, which when united, accelerates the delivery of the cytotoxin into the cell.[30] While inside the plasma membrane, CagA then undergoes tyrosine phosphorylation by Src kinases and further interacts with SHP-2, the cytoplasmic protein tyrosine phosphatase, forming an elongated cell shape referred to as the hummingbird phenotype. This interaction between CagA and SHP-2, along with the induction of the MAP kinase mitogenic signaling pathway, further promotes gastric cell inflammation, transformation, and carcinogenesis.[29]

Phosphorylated CagA and its interaction with host proteins cause tissue damage by several mechanisms. CagA works by disrupting cytoskeletal structure and epithelial cell tight junctions, stimulating abnormal cell signaling, and increasing pro-inflammatory cytokines such as IL-1, IL-6, IL-8, and TNF-α. CagA is able to effectively intertwine with several junction proteins, such as ZO-1, JAM, and E-cadherin, the result of which is disruption of tight and adherens epithelial junctions. This leads to a loss of cell polarity and modulation of cell differentiation, leading to a path of potential carcinogenesis.[28] It is thought that the loss of cell polarity and morphogenic cell changes are specifically influenced by CagA's inhibitory interaction with PAR1/MAPK kinase, an important protein in epithelial cell polarity. Furthermore, through inhibition of PAR1, the CagA-SHP-2 interaction is stabilized, strengthening the hummingbird phenotype, with ensuing cell damage and inflammation.[45]

With regard to the process by which CagA increases pro-inflammatory cytokines, NOD1 and NF-κB are implicated in this process. Nucleotide-binding oligomerization domain protein 1 (NOD1) is an innate intracellular recognition receptor that effectively detects specific peptidoglycan mucopeptides in Gram-negative bacteria such as *H. pylori*.[46] When NOD1 interacts with *H. pylori*, NF-κB is activated, leading to increased gene transcription of pro-inflammatory cytokines such as IL-8. IL-8 functions as a chemoattractant, leading to neutrophil and lymphocyte recruitment as well as effecting cell multiplication, migration, and angiogenesis.[47]

OipA is an outer membrane protein that is thought to enhance the pro-inflammatory response of the gastric mucosa to *H. pylori*. When expressed in conjunction with CagA, an enhanced inflammatory response develops.[29] It has also been demonstrated that OipA expression induces cytokines such as IL-8, whose pro-inflammatory function causes neutrophil infiltration and subsequent mucosal injury.[48]

IL-1 is another pro-inflammatory cytokine that is upregulated in *H. pylori* infection. IL-1 polymorphisms IL-1B and IL-1RN are associated with both an increased inflammatory response and a two- to threefold increased risk of gastric cancer. This is mediated through a state of chronic atrophic gastritis characterized by hypergastrinemia and hypochlorydia.[49,50] This scenario of decreased gastric acid production and increased gastrin secretion describes cases of corpus gastritis, which in contrast to antral gastritis, is known to progress from inflammation to intestinal metaplasia to gastric cancer.

In conclusion, *H. pylori* represents one of the most common bacterial infections in the world. Although the majority of infected individuals display no clinical manifestations, the rest are at risk for chronic gastritis leading to peptic ulcer disease and gastric carcinoma. However, it is only through the unique interplay of bacterial virulence factors, host response, and environmental changes that *H. pylori* succeeds in establishing chronic inflammation, making this bacteria–host relationship and the underlying molecular mechanisms a continued topic of fascination to the scientific community. As knowledge further builds upon itself and global awareness becomes more effective, we will continue to work toward the eradication of *H. pylori*, subsequently aiding in the prevention of gastritis, peptic ulcer disease, and gastric cancer.

11.5 ROLE OF INFLAMMATORY MEDIATORS

Inflammation is a primary defense process against exogenous stimuli, such as viruses, bacterial pathogens, foods, and irritants. While acute inflammation is the initial response of the body to harmful stimuli, chronic or pathological inflammation results from prolonged stimulation of the immune system. Several studies have shown that GI tumorigenesis is closely associated with chronic inflammation, and many of the cellular alterations that accompany chronic inflammation, such as oxidative stress, gene mutations, epigenetic changes, and inflammatory cytokines, are shared with carcinogenic processes, thereby forming a critical interplay between chronic inflammation and carcinogenesis. Chronic inflammation is characterized by a continued active inflammatory response and tissue destruction. The immune cells contributing to its pathology (e.g., macrophages, neutrophils, and eosinophils) are involved directly or by producing inflammatory cytokines that may influence the carcinogenesis process.[51] Chronic inflammation promotes carcinogenesis by inducing gene mutations, inhibiting apoptosis, or stimulating angiogenesis, and cell proliferation.[52] Inflammation also induces epigenetic alterations that are associated with disease development. Several key genes in the inflammatory process are known, including COX-2, NF-κB, TNF-α, TGF-β, and others, providing a mechanistic link between inflammation, GI diseases, and cancer. Given the extensive literature in the field, only two important key molecules are mentioned herein.

11.5.1 Nuclear Factor KappaB

NF-κB transcription factors have a key role in many physiological processes (e.g., innate and adaptive immunity) and disease conditions. Due to its important role in mediating inflammatory signals, a lot of attention has been focused on NF-κB and its upstream activator, IkB kinase (IKK), and their involvement in inflammation and carcinogenesis.[53] NF-κB is not a single gene but represents a family of closely related transcription factors that includes five genes: NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA (p65), c-Rel, and RelB. NF-κB regulates the expression of genes, many of which play important roles in the regulation of inflammation and apoptosis. Although RelA, RelB, and NF-κB1 genetic alterations are rare in human cancer, these proteins are constitutively activated in a wide variety of human tumors and have been associated with tumor progression associated with inflammatory processes.[54]

One such example is IBD, and based on several studies, it is known that constitutive NF-κB activation in IBD increases the risk of colorectal cancer (CRC) in patients with long-standing active disease. Moreover, IBD is characterized by increased expression of pro-inflammatory NF-κB target genes.[55] The chronic mucosal inflammation in IBD is caused by increased activation of effector immune cells, which produce high levels of pro-inflammatory cytokines such as TNF-α, IL-6, and interferon-gamma (INF-γ), resulting in colonic tissue damage. Activation of NF-κB is markedly induced in IBD patients, and through its ability to promote the expression of various pro-inflammatory genes, NF-κB significantly affects mucosal inflammation. Several studies have shown that NF-κB inhibition protects against chronic intestinal inflammation in experimental animal models. In addition, recent findings suggest that NF-κB not only has

pro-inflammatory functions but also is tissue protective. Interestingly, deficiency in Toll-like receptor 5, a strong activator of NF-κB, results in spontaneous colitis and exacerbates mucosal inflammatory responses to *Salmonella* infection.[56]

11.5.2 Tumor Necrosis Factor-Alpha

There is growing evidence supporting a role for various cytokines, released by epithelial and immune cells, in the pathogenesis of chronic inflammation and associated GI cancer. Cytokines are essential mediators of the interactions between activated immune cells and nonimmune cells, including epithelial and mesenchymal cells. Tumor necrosis factor-alpha (TNF-α) is a potent pro-inflammatory cytokine thought to be involved in the pathogenesis of IBD and other GI inflammatory diseases. Recent data indicate that TNF-α promotes tumor development in models of experimental colitis. Popivanova et al.[57] have recently shown that blockade of TNF-α reduces the formation of colorectal tumors in mice lacking the TNF receptor, p55 (TNF-Rp55 knockout). Combined treatment with azoxymethane and dextran sodium sulfate (AOM/DSS), which causes severe colonic inflammation and the subsequent development of multiple tumors, induced the intracolonic expression of TNF-α, which in turn regulated the trafficking of inflammatory cells, a major source of COX-2. These findings are of particular interest because they suggest that blocking of TNF signaling can reverse tumorigenesis, probably by reducing the infiltration of inflammatory cells and, consequently, the circulating levels of COX-2.

A role for TNF-α in the pathogenesis of bacterial-associated gastroduodenal disease has also been suggested. Using TNF receptor 1 (TNF-R1) knockout mice Thalmaier et al.[58] have shown that TNF-R1-mediated TNF-α signals might support a systemic humoral immune response against *H. pylori* infection, which is the main causative agent of chronic gastritis and is closely associated with peptic and duodenal ulcer disease, as well as gastric carcinoma, as further described in Section 11.7.1.

11.6 INVOLVEMENT OF GENETIC ALTERATIONS IN THE PATHOGENESIS OF INFLAMMATORY GI DISEASES

There are several major mechanisms that participate in the development and progression of inflammation and carcinogenesis of the GI tract. These include genetic alterations such as single nucleotide polymorphisms (SNPs), epigenetic alterations (e.g., DNA methylation, histone modifications), and microsatellite instability (MSI).

An SNP is a DNA sequence variation occurring when a single nucleotide in the genome differs between members of a species. Notably, a pathogenic link has been identified between inflammatory mediators, inflammation-related gene polymorphisms, and carcinogenesis. Few examples are mentioned herein. COXs are important enzymes involved in the physiological maintenance of the mucosal integrity that play a role in inflammation and tumor development in the GI tract. Previous studies support the involvement of COX-2 polymorphisms in either gastric or colorectal carcinogenesis. Thus, carriers of certain COX-2 SNPs have an increased risk for these types of cancer.[59] Other inflammation-related polymorphisms, namely, IL-1β

(rs1143627), COX-2/PTGS2 (rs5275), and IL-8 (rs4073), have been shown to contribute to the risk of cancer development in the upper aerodigestive tract. However, their role is seemingly minor.[60]

There have been few studies showing an association of genetic polymorphisms with *H. pylori* infection that can result in chronic gastritis, precancerous lesions, and gastric cancer. Genetic polymorphisms in the CYP2E1 gene, a variant of cytochrome p450, seem to be more prevalent in patients with gastritis and gastric cancer, but have not been proven as risk factors. On the other hand, individuals who carry a G → T substitution in the IL-1β gene (rs1143627) have lower gastric acid secretion, thereby compromising the survival of *H. pylori*. Another polymorphism in the myeloperoxidase (MPO) gene, 463 G → A substitution, results in reduced transcriptional activity and less available enzyme.[61] MPO is secreted during activation of neutrophils and plays an important role in the defense of the organism by catalyzing the production of hypochloric acid, which is utilized by neutropils to kill bacteria and other pathogens. MPO has been speculated to be a major oxidative stress pathway, and therefore an SNP affecting the enzyme level clearly plays a role in the immune response of the host. Izzoti et al.[61] have recently reviewed the role of genetic factors in the clinical outcome of *H. pylori* infection and concluded that the major factors playing a pathogenic role in *H. pylori*-related noncancer diseases are: (1) host polymorphisms in genes involved in inflammation and protection against oxidative damage, (2) host exposure to dietary genotoxic agents, and (3) bacterial genetic polymorphisms. Thus, there is an increasing body of evidence that suggests that mutagenesis-related mechanisms play a pathogenic role in the appearance of noncancer diseases following infection with *H. pylori*.

A polymorphism in the insulin growth factor 1 (IGF-1) gene, rs6214 A → G substitution, is located in the untranslated region of the gene and does not result in any changes in the protein sequence. However, the ancestral allele (A) is associated with a lower risk to esophageal adenocarcinoma (EAC) and its precursor, Barrett's esophagus. Thus, ancestral homozygote individuals have the lowest risk, while having one A allele (heterozygote) confers a lower risk than having no A alleles at all (G homozygote). Another SNP that was found to be associated with Barrett's is rs6898743, located in the growth hormone factor (GHR) gene, which causes a substitution of C to G. This SNP, unlike the previous one, seems to be protective.[62]

The human leukocyte antigen (HLA) is a gene that encodes to a protein that is an essential component of the immune system function. The HLA gene is a heterodimer, composed of two α and β chains named DQA and DQB, respectively. The HLA gene has several polymorphisms that eventually result in four distinct HLA complexes.[63] A study by Howell and colleagues[64] has shown that some HLA SNPs in DQA or DQB confer an approximately 200-fold increased risk to celiac disease and may influence the development of a number of cancers. Another gene that was found to be associated with celiac disease is the cytotoxic T lymphocyte-associated gene (CTLA-4), which plays an important regulatory role in the immune response. The polymorphism, CTLA-4 A → G substitution at position 49, is associated with celiac disease development in several populations.[65]

The transition from chronic inflammation to gastric cancer may also be accelerated by polymorphisms in the innate immune response genes. Specifically, the Toll-like

receptor 4 (TLR4) is a pattern recognition receptor on gastric epithelial cells that when bound by *H. pylori*, activates several inflammatory signaling pathways. Some of these include activation of NF-κB and the MAP kinase pathway.[28,66] A functional polymorphism at position +896 in exon 4 of the TLR4, known as TLR4+896A>G, results in an aspartic acid residue being replaced by glycine. This mutation renders individuals susceptible to a more severe inflammatory response and the more pronounced development of gastric atrophy, an initial precursor lesion in the process of noncardia gastric carcinogenesis.[67]

11.6.1 Epigenetic Alterations

Epigenetics comprise heritable changes in gene expression that are not caused by changes in the primary DNA sequence. These genetic changes occur normally, but within the wrong context, they may affect carcinogenesis. Several epigenetic alterations, reported in inflammatory GI diseases and precancerous lesions of the GI tract, have been shown to affect DNA repair system genes, tumor suppressor genes, oncogenes, cell cycle regulators, growth factors, and adhesion molecules.[68] Moreover, evidence suggests that epigenetic abnormalities, particularly altered DNA methylation, play a crucial role in the development and progression of human GI malignancies.

In mammalians, DNA methylation is an aberrant loss or gain of a methyl group to the 5-position of the cytosine of a CpG dinucleotide. Between 70 and 80% of all CpGs are methylated. Unmethylated CpGs are clustered in "CpG islands" that are present in the 5′ regulatory regions of many genes. In many disease processes such as inflammation and cancer, gene promoter CpG islands acquire abnormal DNA methylation, which results in heritable transcriptional silencing. DNA methylation has been described in several human malignancies. Cancer cells exhibit overall hypomethylation and regional DNA hypermethylation of the CpG islands at the tumor suppressor gene promoters. Alterations in DNA methylation are frequently seen even at the early and precancerous stages, and associated with chronic inflammation and several other factors. There are several well-known genes, such as p16, APC, BRCA1, E cadherin, retinoblastoma (Rb), p53, and others, that undergo this type of control, resulting in their loss of function and impaired protein production, which consequently may affect disease development.[52]

Another mechanism of epigenetic regulation is by histone posttranslational modifications, which include modifications in the lysine residues of histones, such as lysine acetylation, lysine and arginine methylation, and lysine ubiquitination, and sumoylation. There are two states of the lysine residue: the acetylated state activates gene expression, while the deacetylated form inhibits it. Dysregulation of histone deacetylases (HDACs) plays a crucial role in the early stages of human carcinogenesis. Aberrant modifications of histone H4 are associated with hypomethylation of the CpG sequences. These modifications accumulate during cancer progression, indicating their importance in it.[69] Interestingly, overexpression of HDACs appears to be more common in gastric and colon carcinomas.[70] Overexpression of HDACs leads to abnormal gene expression, such as repression of tumor suppressor genes and upregulation of oncogenes, resulting in unregulated cell proliferation and angiogenesis.

The acetylation of several inflammatory mediators has been reported. Acetylation of histone H4 of the pro-inflammatory COX-2 gene promotes its transcriptional activation, while acetylation of NF-κB induces the release of the pro-inflammatory cytokines IL-6 and IL-8.[52] 15-Lipoxygenase-1 (15-LOX-1) participates in cell differentiation and regulation of the inflammatory process. 15-LOX-1 is silenced in many cancers. The silencing is performed by histone modifications that cause its transcriptional inactivation; thus, histone modification of the 15-LOX-1 seems to be important for its transcriptional silencing in colon cancer.[71]

Inflammatory responses are also affected by reduced histone acetyltransferases (HATs) and their altered enzymatic activity. Thus, dysregulation of both HATs and HDACs has been implicated in IBD.[72] Several studies have shown an improvement in chronic inflammations due to the use of HDAC inhibitors (HDIs), which appear to possess strong anti-inflammatory effects in several inflammatory diseases, thereby suppressing pro-inflammatory cytokines such as IL-1 and IL-6. Bäckdahl et al.[73] have recently shown that UC patients treated with an HDI exhibited a marked inhibition of NF-κB activation.

Since inflammation is a critical component of tumor progression, many genetic alterations can activate mutual genes that are necessary for inflammation and may act as oncogenes in the malignant process. Genes that are needed in the inflammatory process, such as various growth factors, may cause tumor progression by unregulated cell proliferation and angiogenesis. Nevertheless, despite the recent advances in the field of epigenomics, the link between cellular signaling and epigenetic modulation is still not clear.

11.7 FROM CHRONIC INFLAMMATION TO MALIGNANT TRANSFORMATION

11.7.1 Chronic Atrophic Gastritis and Gastric Cancer

It is well established that the greatest risk factor for developing nonautoimmune chronic atrophic gastritis is infection with *H. pylori*.[74] Furthermore, as discussed above, *H. pylori*-induced corpus-predominant atrophic gastritis is closely linked to the development of gastric cancer.[75] Specifically, chronic inflammation caused by *H. pylori* is a strong risk factor for noncardia intestinal type gastric cancer, whereby in the face of chronic atrophic gastritis, gastric cells become vulnerable to intestinal metaplasia, dysplasia, and subsequent adenocarcinoma.[76]

Aforementioned is the important interplay between virulence factors, host response, and the environment in perpetuating gastric inflammation and gastric cancer. Gastric carcinogenesis is the result of several simultaneous insults to the gastric mucosa, with virulence factors CagA, VacA, OipA, and BabA, along with pro-inflammatory cytokines IL-1, IL-8, and TNF-α, playing a leading role in *H. pylori* pathogenesis. In addition to these factors, when discussing the transformation from chronic inflammation to cancer, it is important not to dismiss the functions of gastrin, apoptotic proteins, growth factors, nitric oxide synthase, and COX in *H. pylori*-induced carcinoma.[29,77]

Several animal studies have linked hypergastrinemia to the development of gastric cancer. In Mongolian gerbils, hypergastrinemia was associated with overexpression of anti-apoptotic proteins such as survivin and Bcl-2, as well as stimulation of growth factors and the COX-2-PG system. Upregulated in CagA-positive *H. pylori* infection, COX-2 itself may also inhibit apoptosis, while at the same time increasing PGE2 receptors, inflammation, and malignant invasion.[78] Therefore, it can be said that an interconnected relationship exists between gastrin, COX-2, anti-apoptotic proteins, and CagA, whereby the resulting effects are altered cellular growth and gastric cell structure, rendering atrophic gastric mucosa susceptible to parietal cell loss, gastric ulcers, and malignant transformation.[78] These discoveries have fueled the idea of vaccination using anti-gastrin immunogen (G17DT), a complex of gastrin with diphtheria toxin. G17DT elevates endogenous production of anti-gastrin antibodies, decreasing gastrin levels and negating carcinogenic transformation. When combined with chemotherapy, it has been demonstrated in a phase II clinical trial that G17DT administration was correlated with prolonged median survival and time to progression in individuals suffering from advanced gastric and gastroesophageal cancer.[79] Therefore, targeting gastrin production through antibodies that decrease gastric acid secretion and inhibit gastrin from interacting with the cholecystokinin 2 receptor may represent a new modality of attacking gastric carcinogenesis.[80]

Along with gastrin, increased expression of epidermal growth factor (EGF), hepatocyte growth factor (HGF), TGF-α, and the c-Met gene has been linked to gastric carcinogenesis in individuals with chronic atrophic gastritis. The c-Met protein, also known as HGF receptor, is a member of the tyrosine kinase growth factor receptor family, and HGF is the only known ligand that binds it. Upon HGF binding, c-Met induces several biological responses, activating oncogenic pathways that lead to tumor growth, angiogenesis, and invasion. Higher expression of c-Met is linked to a poorer prognosis in those with gastric cancer and increased risk of tumor metastasis.[29]

Individuals with long-standing chronic atrophic gastritis may also display elevated inducible nitric oxide synthase (iNOS) along with activated myeloperoxidases and NADPH oxidases. This leads to the generation of reactive oxygen and nitrogen free radicals, which over time damage DNA, inhibit DNA repair enzymes, modulate transcription factors, deregulate apoptosis, promote angiogenesis, and increase oncogene expression.[81] In addition, pro-angiogenic cytokines such as vascular endothelial growth factor (VEGF) are known to be elevated in individuals with gastric cancer, and may be correlated with higher expressions of iNOS. This indicates that there is an interplay between iNOS and VEGF, where high levels of iNOS enhance tumor progression by stimulating angiogenesis.[82]

Recent research points to activation-induced cytidine deaminase (AID) as a potential modulator in enhancing conversion from atrophic gastritis to gastric cancer. AID is a B cell–specific DNA editing enzyme that contributes to the process of somatic hypermutation and class switch in immunoglobulin genes. Overactivation of AID through the NF-κB signaling pathway may lead to mutations in tumor suppressor genes such as p53, thereby promoting gastric malignancy.[27]

In conclusion, the progression from chronic atrophic gastritis to gastric cancer is a multistep process that involves the interaction of numerous factors. Gastric cancer is the fourth most common cancer worldwide and the second leading cause

of cancer-related death.[27] It is therefore vital to continue our understanding of the relationship between *H. pylori*-induced chronic atrophic gastritis and gastric cancer as a means to work for enhanced prevention and treatment strategies. Eradication therapy for *H. pylori* is a wonderful start, but alternate treatment modalities must be established for those individuals displaying atrophic changes and an advanced infection unresponsive to antibiotics alone.

11.7.2 Pancreatitis and Pancreatic Cancer

Chronic pancreatitis (CP) is an inflammatory disease of multifactorial origin that over time leads to pancreatic insufficiency and malabsorption, diabetes mellitus due to endocrine failure, and persistent pain. Unfortunately, as in many other inflammatory conditions, chronically inflamed tissue in the pancreas can serve as a foundation for the later development of adenocarcinoma. The cumulative risk of pancreatic cancer in subjects with CP was found to be 4% (95% CI = 2–5.9%) after 20 years.[83] This risk is at least 10 times lower in individuals without CP,[84] but infinitely higher in persons with the hereditary form of chronic pancreatitis due to an autosomal dominant mutation of the cationic trypsinogen gene, where the risk of pancreatic cancer by age 70 approaches 40%.[85–87] Pancreatic ductal adenocarcinoma (PDAC) is the most common subtype of pancreatic cancer associated with CP, serving as the fourth leading cause of cancer-related deaths in the United States in both men and women, with a 5-year survival of <5%.[88] Its high mortality rate is largely due to tumor aggressiveness and propensity for early local invasion and metastases. Understanding the molecular link between chronic pancreatitis and pancreatic cancer may help guide patient management in order to prevent this devastating outcome.

Chronic inflammation in the pancreas occurs as a result of continuous injury, which may be due to recurrent infections (repeated attacks of severe acute pancreatitis), continued exposure to toxins (alcoholic pancreatitis), genetic mutations (hereditary pancreatitis), or autoimmune reactions (autoimmune pancreatitis). Approximately 70% of chronic pancreatitis can be attributed to alcohol abuse, while other factors and unknown mechanisms account for the remaining 30%.[89] Additionally, cigarette smoking has been shown to promote the development of chronic pancreatitis, especially in susceptible individuals, as well as increase the risk of diabetes and pancreatic calcifications.[90] Smoking also independently increases the risk of pancreatic cancer in patients with and without underlying chronic pancreatic inflammation.[91] Studies of patients with hereditary pancreatitis showed a twofold increase in incidence of PDAC in smokers compared to nonsmokers, as well as a 20-year earlier onset of cancer.[92]

Regardless of the etiology, CP is characterized by extensive fibrosis that occurs as a result of recurrent episodes of parenchymal cell injury and necrosis, followed by regeneration and fibrosis, a process called the necrosis-fibrosis sequence.[89] Recently, precancerous lesions have been described in the pancreas. These precursor lesions, termed pancreatic intraepithelial neoplasms (PanINs), are connected to pancreatic cancer development analogous to the adenoma–carcinoma sequence of CRC, and are present in nearly all cases of chronic pancreatitis. They are associated with accumulation of genetic mutations and progressive levels of cellular and architechtural atypia

resulting in PanIN 1, 2, or 3.[84,87] The genetic alterations that seem to be involved in this process will be discussed shortly.

The role of CP as a significant risk factor for pancreatic cancer was initially established in 1993.[83] Although the exact relationship between CP and PDAC remains uncertain, fibrosis, which can be described as a series of pathologic changes in the extracellular matrix composition, characterizes the underlying pathophysiology of both entities. The exact mechanism of fibrosis is still largely unknown, but recent evidence suggests that chronic inflammation results in DNA damage (via formation of oxygen radicals) and subsequent mutations, which in turn change the local microenvironment in the pancreas in favor of fibrosis and tumor growth.[87,91] Several molecular mechanisms are associated with this change. Inflamed tissues induce the production of nitric oxide via NO synthase, which contributes to oncogenesis by inhibiting cell apoptosis, damaging DNA and proteins, inhibiting cellular repair functions, and promoting angiogenesis.[93] Multiple inflammatory mediators and growth factors are released from inflamed tissue in the pancreas as part of the regenerative process, including PDGF, TFG-β, FGF-β, IL-1β, IL-6, TNF-α, endothelin-1, activin, and connective tissue growth factor (CCN2), among others.[89,94] These factors, when continuously secreted, aid in survival and growth of premalignant cells.

A crucial role in pancreatic fibrogenesis is played by the pancreatic stellate cells (PSCs), star-shaped cells that constitute approximately 4% of all pancreatic cells and are similar in structure and function to the hepatic stellate cells (HSCs) of the liver.[95] In the normal pancreas, the PSCs remain in the quiescent state and can be identified by the presence of vitamin A-containing lipid droplets in their cytoplasm, much like HSCs. Based on their periacinar, periductal, and perivascular localizations, quiescent PSCs likely function to maintain acinar and ductal cells, as well as serve some unknown vascular function.[94] In response to pancreatic injury or inflammation, these PSCs undergo activation to become myofibroblast-like cells that express α-smooth muscle actin (α-SMA) in their cytoplasm. Activated PSCs are major participants in a fibroblastic reaction described as desmoplasia, which is composed of extracellular matrix (ECM) proteins, immune cells secreting various cytokines and growth factors, myofibroblasts, and ECM-metabolizing enzymes.[96] This desmoplastic reaction is unique to pancreatic cancer and always densely surrounds tumor cells. Activated PSCs expressing α-SMA are observed surrounding both PanINs of chronic pancreatitis and cancer cells, suggesting their crucial role in desmoplasia.[97]

Several important functions of PSCs have been identified. First, upon activation they synthesize and secrete important ECM components, such as collagen types I, III, and V, laminin, and fibronectin, which play important roles in fibrinogenesis.[94,96] They also produce ECM-degrading enzymes of the matrix metalloproteinase (MMP) family and their inhibitors, termed tissue inhibitors of metalloproteinases (TIMPs).[94] In this way, PSCs tightly regulate ECM turnover and maintenance of pancreatic tissue architecture. While transient activation of PSCs may therefore serve a healing function by releasing all these molecules, their prolonged activation results in oversecretion of MMPs and TIMPs, contributing to the progression of inflammation and carcinogenesis. The fine balance between production and degradation of the ECM is the main determinant of fibrosis progression and regression.[89,96,98]

In recent *in vitro* experiments PSCs have also been shown to release soluble factors that directly promote malignant transformation in pancreatic duct epithelial cells.[97] Incubation of human pancreatic duct epithelial (HPDE) cells with human PSC medium resulted in a significant increase in the number of colonies compared with HPDE cells incubated with control medium. Examination of the resultant HPDE cells showed a sevenfold increase in the expression of fibroblast growth factor 7 (FGF-7), and a fourfold increase in pleiotrophin, suggesting that these factors, among others, contribute to the growth of pancreatic epithelial cells. PSCs secrete numerous other chemokines and cytokines as well as growth factors and other inflammatory mediators. Interestingly, many of these mediators further promote PSC activation via autocrine loops. For example, TGF-1β produced by activated PSCs increases TGF-1β mRNA expression through signaling pathways involving Smads, a group of molecules that function as intracellular signaling modulators of TGF-β family members.[87] Other cytokines released by PSCs are IL-1β, IL-6, TNF-α, IL-8, and PDGF,[89] all of which further perpetuate PSC activation.

Recent research also shows a direct relationship between PSCs and pancreatic cancer cells, where PSCs enhance the proliferation of cancer cells and cancer cells recruit and activate additional PSCs.[99] *In vitro* studies demonstrate that pancreatic cancer cells induce activation of PSCs, evidenced by increased proliferation, ECM synthesis, and migration. This suggests that pancreatic cancer cells continuously recruit PSCs to facilitate in-tumor cell growth as well as local and peripheral invasion. *In vivo* studies, which involve injecting nude mice with a mixture of pancreatic cancer cells and PSCs directly into the pancreas, show accelerated tumor growth and increased propensity for local and distant metastases. Additional evidence suggests that PSCs facilitate angiogenesis and are capable of transendothelial migration by intravasating and extravasating to and from blood vessels. This promotes the idea that PSCs can actually accompany pancreatic cancer cells to distant sites in the body and aid in survival and growth of metastatic cells.

Genetic alterations play an important role in inflammation-induced pancreatic oncogenesis. Mutant expression of the K-Ras proto-oncogene is found in nearly all cases of PDAC as well as in many instances of CP.[84] Additionally, patients with CP often contain PanIN lesions that possess K-Ras mutations.[100] High levels of mutated K-Ras augment the activity of the Ras signaling pathway, leading to acinar cell death and metaplasia associated with pancreatic fibrosis and inflammation. Ji et al.[101] propose that the levels of Ras activity ultimately control the development and progression of both chronic pancreatitis and pancreatic cancer. They may be elevated via extrinsic (inflammatory stimuli due to tissue damage) or intrinsic (somatic K-Ras mutations) factors, and often both. Once the Ras pathway activity level reaches a certain threshold, cells in the pancreas undergo metaplasia, PSCs are recruited, and PanINs begin to form. Elevated levels of Ras also generate an inflammatory process that resembles CP, which further promotes progression to PDAC by creating a genetically unstable environment with loss of tumor suppressors and accumulating K-Ras mutations.[84,100] Longer duration of chronic pancreatitis is associated with higher levels of K-Ras mutations, increased development of PanINs, and higher likelihood of progression to PDAC.[102]

Another significant link between inflammation and pancreatic carcinogenesis lies in the constitutive expression of the transcription factor NF-κB found in pancreatic cancer.[87] This transcription factor, which is also overexpressed in many other cancers, regulates a number of genes associated with oncogenesis, including genes responsible for cell cycle regulation (Cyclin D1, *c-myc*), angiogenesis (VEGF), and other genes responsible for tissue invasion, regulation of apoptosis, and cytokine expression.

Expression of COX-2 also significantly contributes to pancreatic carcinogenesis and is significantly increased in both pancreatic cancer and precursor PanINs.[87,99,103] Overexpression of COX-2 increases synthesis of prostaglandins, which promote cytokine synthesis, cell proliferation, and suppression of immune system surveillance. This finding may have a therapeutic value, suggesting that COX-2 inhibitors may play a role in cancer reduction.

In conclusion, much remains to be discovered in the link between chronic pancreatitis and pancreatic cancer. Further understanding of the molecular mechanisms connecting these two diseases will help in the development of therapeutic strategies to prevent or reverse the inflammatory process and decrease the incidence of pancreatic cancer. As such, counseling patients regarding alcohol and cigarette abuse is important in trying to reduce the incidence of acute attacks of pancreatitis, as well as the long-term consequences of chronic pancreatitis and pancreatic cancer.

11.7.3 HBV/HCV-Related Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer death worldwide, with 80% of cases occurring in developing countries (Far East and South Asia), where the prevalence of viral hepatitis is higher[104] and rapidly fatal in almost all cases, with survival generally less than 1 year from diagnosis. There is now an increasing body of evidence that, in a very high percentage of cases, chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) is required for the development of HCC. Other risk factors for this cancer include dietary exposure to aflatoxins, smoking, and alcohol abuse.[104] Immune-related risk factors include primary biliary cirrhosis and autoimmune hepatitis (AIH).[105] The incidence of HCC in patients with AIH is relatively low despite the fact that AIH patients are commonly treated with immunosuppressive drugs, such as steroids and others, which may potentially increase the risk of malignant transformation. However, immunosuppressive steroids act through inhibitory effects on cytokines, which are important for inflammation. An increased production of some cytokines, IL-1β and TNF-α, has been demonstrated to coincide with the presence of liver cancer.[106] Thus, downregulation of these cytokines by immunosuppressive therapy may be speculated to contribute to protection from the development of HCC.

Aflatoxins, a group of mycotoxins produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, are some of the most important of the environmental toxins that contribute to the pathogenesis of HCC, particularly in regions and developed countries where the consumption of contaminated foods is common. An association between aflatoxin B1 and a specific AGG-to-AGT transversion mutation at codon 249 of the p53 gene in human HCC has been shown, providing mechanistic support to a causal link between exposure and disease.[107] However, the mechanisms

underlying the interaction between hepatitis viruses and aflatoxins are not clear as yet. A number of potential mechanisms have been described, including the fixation of AFB1-induced mutations in the presence of liver regeneration and hyperplasia induced by chronic HBV infection, the predisposition of HBV-infected hepatocytes to aflatoxin-induced DNA damage, an increase in susceptibility to chronic HBV infection in aflatoxin-exposed individuals, and oxidative stress exacerbated by coexposure to aflatoxins and chronic hepatitis infection.[108]

Viral hepatitis due to chronic HBV/HCV infection affects more than 500 million individuals worldwide.[109] HBV can be identified in over 90% of cases of HCC. In most or all of them, the HBV DNA is integrated into the DNA of the host cell. Individuals who are HBV carriers for a long period, in certain regions, particularly if infected in their youth, have a greater risk of developing cancer than those who have not been chronically infected. This makes the HBV carrier group the highest known risk category for a common cancer. HCV is the most important risk factor for HCC in Western European and North American countries, based on epidemiological studies showing that up to 70% of patients with HCC have anti-HCV antibodies in the serum.[109] HCC has a higher prevalence in patients with HCV-associated cirrhosis than in nonviral etiologies of chronic liver disease, while only a few cases of HCV-associated HCC have been reported in the noncirrhotic liver. As a result, the role of HCV in HCC development has been debated. HCV is a single-stranded RNA virus without a DNA intermediate in its replicative cycle. Therefore, the integration of HCV nucleic acid sequences into the host genome seems unlikely. A possible explanation for HCV-associated HCC is that the virus causes necro-inflammatory hepatic disease with vigorous regeneration, fibrosis, and eventually cirrhosis. Of note, treatment of hepatitis C with INF-α can lead to sustained clearance of HCV, suggesting that interferon therapy leads to a decrease in the incidence of HCC.[110]

Other than chronic virus infection, development of HCC is also triggered by factors that lead to chronic hepatic injury and deregulation of the normal process of wound healing, which promote persistent stimulation of pro-angiogenic processes that lead to significant structural changes in the liver and functional changes in hepatic physiology.[111]

It should be mentioned that the second most important type of liver cancer is cholangiocarcinoma, whose main known cause is infestation with the liver flukes, *Opistorchis viverrini* and *Clonorchis sinensis*, which are frequent in some areas in Southeast Asia. Cholangiocarcinoma will not be discussed in this chapter.

11.7.4 JCV-Related Colorectal Cancer

Another virus that has been recently linked to the development of lower GI tract cancers is the JC virus (JCV) that was found associated with CRC and might contribute to its pathogenesis by several mechanisms.

JCV is a nonenveloped circular double-stranded DNA virus related to the polyomavirus family that is well adapted to humans and is widespread in the general population. JCV infection occurs early in childhood and lasts throughout life. The extent of the exposure to JCV infection is indirectly known by sero-prevalence rates detected in different populations worldwide, ranging from 44 to 90%.[112] The infection

may occur by fecal contamination and is usually persistent and subclinical, but is reactivated under conditions of immunosuppression. Infection with JCV appears to occur through the GI tract, but an oral–fecal transmission might be possible as well. The detection of antibodies from childhood to adults suggests that these antibodies are unlikely to be protective, but are indicative of exposure, probably multiple or sustained, to JCV.[112]

JCV cannot replicate by itself, but needs to "push" the host cells into the S phase, thereby controlling cell cycle. A main JCV protein involved in this process is the large T-antigen (T-Ag). Large T-Ag also has the ability to transform cells, upon incorporation into the genome, by modulating crucial cell signaling pathways involved in cell cycle and apoptosis.[113] Some studies have shown that 80–90% of CRC cases express viral genomic DNA and the large T-Ag protein, which can also be found in benign adenomas, with a lesser frequency, but not in normal colonic tissue.[114] The contribution of JCV infection to the development of CRC is still controversial. Nevertheless, a positive correlation between CRC and the presence of antibodies against JCV is known, and high titers of anti-JCV antibodies were found in patients with advanced CR neoplasia.[114] It has been suggested that in CRC patients, the virus is activated and may induce chromosomal instability (CIN) or other chromosomal alterations. Moreover, it was shown that the large T-Ag protein, present exclusively in the nuclei of CRC cells, may induce hypermethylation and silencing of several tumor suppressor genes, including p16, PTEN, APC, and others.[115] Thus, the association between T-Ag expression and promoter methylation in CRC suggests that this viral oncogene may induce methylator phenotype, and that JCV may be involved in CRC through multiple mechanisms of genetic and epigenetic instability.[115]

JCV was shown to alter the transcription of a variety of genes in the infected cells, including proteins whose gain or loss causes unbalanced mitoses leading to aneuploidity (abnormal chromosomal content), which is frequently seen in CRCs.[114] A study by Link et al.[116] has recently shown that infection of CRC cells with JCV and the presence of large T-Ag encourage the transcriptional activation of crucial genes and associated signaling pathways, such as AKT and MAPK, which results in increased cell migration and metastatic potential. Thus, JCV T-Ag expression in CRC associates with a metastatic phenotype, which may partly be mediated through the AKT/MAPK signaling pathway.

11.8 SUMMARY

As reviewed in this chapter, chronic inflammation in the GI tract plays a critical role in the development of associated GI diseases and is strongly associated with cancer development, involving several multifactorial processes related to environmental exposures, diet, inherited gene polymorphisms, infections, or dysfunctions of the immune response.

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12 Pathogenesis of Insulin Resistance, the Metabolic Syndrome, and Inflammation

An Issue of Modern Lifestyle

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CONTENTS

12.1 INTRODUCTION

Excessive consumption of macronutrients is an essential part of the causation and maintenance of obesity. Since being overweight and obese are associated with excessive caloric consumption, and the latter is becoming universal, dependent largely on affordability of food, we need to examine the effects of excessive food consumption. Excessive caloric consumption is a practice not only in Western countries, but is now also a rapidly growing problem in developing countries with increasing prosperity.

Work over the past decade has brought out several novel features that elucidate the link between macronutrient intake and oxidative and inflammatory stress,[1–2] abnormal vascular reactivity, and the induction of transient hypertension. That a fat-rich meal leads to an acute hypertriglyceridemia, and that a long-term increase in carbohydrate consumption leads to chronic hypertriglyceridemia have been known for a long time.[3]

12.2 EFFECT OF A HIGH-FAT, HIGH-CARBOHYDRATE MEAL ON LIPID CONCENTRATION

It is well known that the intake of a high-fat meal leads to an increase in plasma triglyceride concentration. The clearance of triglycerides is dependent upon the action of insulin and insulin sensitivity.[3] It has recently been shown in at least two studies that the magnitude of postprandial hypertriglyceridemia may be a factor determining atherogenesis and cardiovascular risk.[4] In this context, it is relevant that rosiglitazone, an insulin sensitizer, also increases the clearance of triglycerides and free fatty acids (FFAs).[5]

12.3 EFFECT OF A HIGH-FAT, HIGH-CARBOHYDRATE MEAL ON OXIDATIVE AND INFLAMMATORY STRESS

A high-fat, high-carbohydrate meal induces an increase in reactive oxygen species (ROS) generation by both polymorphonuclear neutrophils (PMNs) and mononuclear cells (MNCs).[6] This effect is particularly pronounced in males. In association with this, there is an increase in the expression of $p47^{phox}$, the essential subunit of NADPH oxidase, the enzyme that generates the superoxide radical from molecular O_2[6]. Such a meal also induces an increase in activity of inhibitor of kB (IkB) kinases (IKKs), IKKα and IKKβ, which in turn phosphorylate IkBα and cause its ubiquitination and proteasomal destruction.[6] This allows the nuclear factor kB (NF-κB) sequestered in the cytosol to translocate to the nucleus and to set up the transcription of

pro-inflammatory genes.[7] Tumor necrosis factor alpha (TNFα) and interleukin (IL)-1β have been shown to be induced by such meals.[8–9] Which macronutrient is responsible for the induction of oxidative and inflammatory stress has been elucidated by the observations that both glucose and saturated fat (cream) induce an increase in ROS generation, an increase in the expression of $p47^{phox}$, an increase in IKKα and IKKβ, a decrease in IkBα, and an increase in intranuclear NF-κB.[10] Both induce TNFα and IL-1β.[10] The only study using pure protein (casein) showed an increase in ROS generation, but the magnitude of increase was far smaller than that observed with glucose or saturated fat.[11] Thus, it would appear that the major dietary components inducing an increase in oxidative and inflammatory stress are carbohydrates (glucose) and fat.

12.4 EFFECT OF A HIGH-FAT, HIGH-CARBOHYDRATE (HFHC) MEAL ON PUTATIVE MEDIATORS OF INSULIN RESISTANCE

12.4.1 Suppressor of Cytokine Signaling-3 (SOCS-3)

Recent work has shown that the intake of a HFHC meal leads to an increase in the expression of a key protein that interferes with both insulin and leptin signal transduction.[9] The suppressor of cytokine signaling-3 binds to and causes the ubiquitination and proteasomal degradation of insulin receptor substrate 1 (IRS-1) in experimental animals.[12,13] Since IRS-1 is the main link between the tyrosine phosphorylated β-subunit of the insulin receptor and phosphoinositide 3 (PI3) kinase, the loss of IRS-1 would lead to an impairment of insulin signal transduction and the induction of insulin resistance.[14] SOCS-3 has also been shown to interfere with leptin signal transduction at the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) level through the prevention of the translocation of STAT into the nucleus.[15] Thus, the induction of SOCS-3 would also potentially lead to a resistance to leptin.

SOCS-3 is known to be induced by the pro-inflammatory cytokines, TNFα, IL-6, and IL-1β.[16] Our recent work has shown that a HFHC meal induces TNFα and IL-1β while at the same time inducing an increase in SOCS-3 mRNA and protein.[9] This increase in the expression of TNFα, IL-1β, and SOCS-3 occurs within 1 h of the intake of the meal and continues for at least 5 h. Our more recent data show that both glucose and saturated fat (cream) induce an increase in SOCS-3.

12.4.2 Pro-Inflammatory Kinases

Several pro-inflammatory kinases have been shown to interfere with insulin signal transduction through the serine phosphorylation of IRS-1.[14,17] This prevents the binding/association of IRS-1 to PI3 kinase, and thus blocks the insulin signal. Our recent work has shown that the intake of the HFHC meal induces an increase in the expression of IKKβ, protein kinase C (PKC)-β2, and Jun amino-terminal kinase (JNK)-1.[14,18,19]

12.4.3 Toll-Like Receptors (TLRs)

TLRs are a class of pathogen recognition receptors that recognize and bind to pathogens and trigger inflammatory responses.[20] In addition to binding pathogens through

the recognition of pathogen-associated molecular patterns (PAMPs), they have also been shown to recognize and bind damage-associated molecular patterns (DAMPs) associated with endogenous products derived from tissue damage. TLR-4 and TLR-2 classically bind endotoxin (lipopolysaccharide (LPS)) and lipopeptides and peptidoglycans of Gram-positive bacteria, respectively.[21,22] However, by binding to DAMPs, they trigger inflammatory responses that lead to insulin resistance in experimental animals. The deletion of TLR-4 and TLR-2 is known to protect animals from the induction of insulin resistance following a high-fat diet.[23]

It is therefore of great interest that a HFHC meal results in the induction of both TLR-4 and TLR-2.[9] Both could potentially contribute to insulin resistance. In addition, our recent work has shown that the intake of a HFHC meal or cream results in an increase in the plasma concentration of endotoxin (LPS).[24] The combination of an increase in LPS and its receptor, TLR-4, would contribute to further NF-κB activation and the transcription of pro-inflammatory genes like TNF, IL-6, and IL-1, all of which can potentially interfere with insulin signal transduction and thus cause insulin resistance.[25,26] This intriguing combination would enhance inflammation and would contribute not only to insulin resistance but also to atherogenesis.

In this context, it is relevant that the injection of 3 ng/kg LPS into insulin-sensitive normal subjects result not only in inflammation, but also insulin resistance at 24 h after the injection.[27] In addition, in adipose tissue, there was an induction of SOCS-3, which interferes with insulin signal transduction. This action of endotoxin has also been observed in a recent study.[28] Clearly, endotoxemia probably plays an important role in the pathogenesis of insulin resistance.

12.4.4 Protein Tyrosine Phosphatase-1B (PTP-1B)

PTP-1B is a tyrosine phosphatase closely linked to the insulin receptor. It dephosphorylates the tyrosine residues of the activated insulin receptor, and thus inactivates it and limits insulin action.[29] Its expression has been shown to be increased in the obese.[30] Our recent work shows that it is induced by a HFHC meal in parallel with the induction of SOCS-3, which also interferes with insulin action. Indeed, the inhibition of PTP-1B is currently being exploited as a strategy for the treatment of type 2 diabetes.

If a single HFHC meal induces a significant increase in the expression of SOCS-3 and the kinases that reduce IRS-1 quantity and activity, while also increasing PTP-1B,[19] it is important to determine whether their expression is chronically increased in obese insulin-resistant patients who are in a state of chronic oxidative and inflammatory stress. Indeed, the expression of SOCS-3 is increased in the obese by three times that observed in matched normal subjects.[31,32] It is relevant that the increase in SOCS-3 expression is significantly related to body mass index (BMI) and homeostatic model assessment of insulin resistance (HOMA-IR), while it is inversely related to insulin receptor phosphorylation.[32] Similarly, the expression of IKKβ and PKC-β2 in the obese is also significantly higher than that in normal subjects.[32] These kinases cause serine phosphorylation of IRS-1, and thus impair insulin signal transduction, as stated above.[33]

12.5 EFFECT OF A HFHC MEAL ON VASCULAR REACTIVITY

It was shown over a decade ago by Vogel's group that a HFHC meal impairs flow-induced endothelium-mediated vasodilation, and that this may be due to oxidative stress.[34,35] As discussed above, this meal induces an increase in ROS generation with a parallel increase in NADPH oxidase subunits and indices of inflammation. Inflammation has also been shown to impair endothelial responses. An increase in ROS generation, including the superoxide radical, leads to a diminished bioavailability of nitric oxide (NO) since superoxide forms peroxynitrite in combination with NO.[36] Peroxynitrate is not vasodilatory and is also toxic to tissues.

As mentioned above, obesity is associated with chronic oxidative stress and is also characterized by impaired flow-induced endothelium-mediated vasodilation. Whether repeated HFHC meals also lead to a chronic pro-constrictive prehypertensive state is a possibility that has to be considered.

12.6 EFFECT OF A FAT INFUSION ON VASCULAR REACTIVITY AND BLOOD PRESSURE

The infusion of lipid (triglyceride) with heparin in normal subjects to increase FFA concentrations to a similar level to those observed in the obese has been shown to induce a rapid impairment of vascular reactivity within 1 h in parallel with the induction of oxidative stress and inflammation at the cellular and molecular levels.[37] Recent work has reported a sharp increase in blood pressure with the increase in plasma FFA concentrations.[38] Clearly, macronutrients, given either orally or intravenously, result in oxidative stress, inflammation, and altered vascular responses. It is possible that such altered responses may contribute to hypertension in the long term.

It is clear that a single HFHC meal is able to induce significant oxidative stress and inflammation. This is associated with the induction of SOCS-3 and several pro-inflammatory kinases and PTP-1B, which can interfere with insulin signal transduction. These molecules are also known to be increased in the obese and contribute to insulin resistance. This meal also impairs vascular reactivity and may predispose to hypertension. These observations are relevant to the pathogenesis of the metabolic syndrome.

12.7 THE EFFECT OF REPEATED MEALS

Since a single moderate-sized HFHC meal in normal subjects leads to the extensive changes in oxidative and inflammatory stress and vascular behavior observed chronically in the insulin-resistant obese, it is important to determine whether the repeated intake of such meals will induce an additive effect. If, indeed, these meals have prolonged effects with a lasting increase in oxidative and inflammatory stress and the induction of the mediators of insulin resistance accompanied by changes in vascular behavior, we should be able to establish a macronutrient/obesity-based pathway for the development of the metabolic syndrome.

12.8 REVERSAL OF OXIDATIVE AND INFLAMMATORY STRESS WITH CALORIC RESTRICTION AND WEIGHT LOSS

Caloric restriction and weight loss result in a marked reduction in oxidative and inflammatory stress in the obese.[39,40] Within 1 week of caloric restriction, there is a significant reduction in ROS generation, which is reduced by 50% at 4 weeks. Similarly, the concentrations of carbonylated proteins fall significantly within 1 week. Indices of lipid peroxidation, thiobarbituric acid-reactive species (TBARS), and hydroxyoctadecadienoic acid (HODES) also diminish. In normal subjects, acute caloric restriction, like a fast for 24 or 48 h, also results in a marked reduction in oxidative stress.[40] In the obese, there is also a reduction in SOCS-3 expression at the mRNA level at 4 weeks (unpublished data). A reduction in plasma TNFα concentrations was observed following weight loss over a decade ago.[41] In addition to a reduction in TNFα concentrations, several other cytokines and pro-inflammatory mediators, like C-reactive protein (CRP), also fall.[42] There may be an associated fall in the serine kinases that phosphorylate IRS-1 and inactivate it. These changes would restore sensitivity to insulin. Since TNFα induces the expression of SOCS-3, its reduction would also contribute to a reduction in SOCS-3 expression.[13] Similarly, plasma CRP concentrations also fall after caloric restriction and weight loss.

12.9 HIGH-FAT DIET IN EXPERIMENTAL ANIMALS

The intake of a high-fat diet (HFD) has been known to be associated with not only an increase in body weight, but also the induction of insulin resistance. There is a concomitant increase in the expression of SOCS-3, which would potentially interfere with insulin signal transduction at the IRS-1 level.[12] It is of interest that TLR-4 and endotoxemia play an important role in the pathogenesis of insulin resistance in relation to high-fat diets in experimental models.[43]

Glucose-stimulated insulin secretion from the islet cells is decreased in HFD-fed mice. Pancreatic insulin content and pro-insulin mRNA are also reduced.[44,45]

Adipocytes isolated from HFD-fed rats bound less insulin and showed a decreased number of insulin receptors, and a decrease in the maximally insulin-stimulated activity of the glucose transport system and intracellular glucose metabolism.[46–48] Similar effects of HFD have been observed in the muscles.[49] A decrease in the binding of insulin to its receptors results in impairment of the action of several enzymes, such as pyruvate dehydrogenase and tyrosine kinase, as well as a decrease in the active form of glycogen synthase.[45] Decreased insulin-stimulated glucose uptake in high-fat feeding was attributed to a decrease in phosphorylation of the β-subunit of the insulin receptor.[50]

The concentrations of intramuscular triglycerides and fatty acid intermediates (fatty acyl-CoAs, diacylglycerol (DAG), and ceramide) are elevated in skeletal muscle of HFD-fed mice. These could attenuate insulin signaling through inhibiting akt/protein kinase B (PKB), or could activate pro-inflammatory pathways, including mammalian target of rapamycin (mTOR), JNK, IKK, and PKC pathways resulting in insulin resistance.[17] Skeletal muscle from insulin-resistant HFD-fed mice and Zucker diabetic fatty rats shows an increase of partially oxidized acylcarnitines,

reflecting excessive rates of β-oxidation of fatty acids and mitochondrial overload, which contributes to skeletal muscle insulin resistance.[51]

It has been suggested that chronic nutrient excess can trigger endoplasmic reticulum (ER) stress in adipose tissue and liver.[17] Knockout (KO) of X-box protein-1 (xbp1), a gene encoding a transcription factor that mediates the expression of ER chaperons, leads to increased ER stress, JNK activation, and IRS-1 Ser 307 phosphorylation, resulting in impaired insulin action, hyperinsulinemia, and glucose intolerance of HFD-fed mice.[52] Treatment with orally active chemical chaperons reverses these effects on ER stress and JNK and improves tissue insulin sensitivity.[53]

The pioneering work of Ferrante's group has shown that the macrophages resident in stromal elements of adipose tissue are the major contributors and regulators of inflammation in this tissue.[54] Stromal macrophages actively secrete the chemokine, monocyte chemotactic protein-1 (MCP-1), which attracts monocytes from the circulation by binding to its receptor, chemokine (C-C motif) receptor 2 (CCR-2), expressed by these cells.[55] Macrophages recruited to adipose tissue during induction of obesity with HFD feeding are pro-inflammatory, as indicated by increased expression of cytokines and inflammatory pathway genes.[56] Myeloid cell KO of IKKβ or JNK1 protected mice from HFD-induced glucose intolerance, hyperinsulinemia, and insulin resistance in skeletal muscle, adipose tissue, and the liver. The mice developed the same degree of obesity as wild-type, demonstrating that without a myeloid cell-driven inflammatory component, obesity itself did not cause insulin resistance.[17,57] Clearly, therefore, a high-fat diet chemo-attracts myeloid-derived monocytes from the circulation into the adipose tissue, where in the pro-inflammatory state they generate more MCP-1, and through IKKβ and JNK-1, two major inflammatory mediators, induce systemic insulin resistance. It is relevant that while the deletion of IKKβ from myeloid-derived cells leads to systemic insulin resistance,[58] the deletion of this gene from adipose tissue and skeletal muscle leads to insulin resistance only in that tissue.

It is of interest that TLR-4 and endotoxemia play an important role in the pathogenesis of insulin resistance in relation to high-fat diets in experimental models. Dietary fats enhance absorption of LPS.[59] There is an increase in circulating LPS derived from gastrointestinal bacteria in HFD-fed mice.[17] The deletion of TLR-4 leads to protection from HFD-induced insulin resistance. In adipocytes, LPS activates TLR-4 in preadipocytes and alters the expression of multiple cytokines (TNFα and IL-6) that inhibit insulin signaling. Also, LPS promotes expression of NF-κB and activation of the mitogen-activated protein kinase (MAPK) pathway.[59] Of relevance in this context is the induction of endotoxemia following a single HFHC meal, along with an increase in plasma lipopolysaccharide binding protein (LBP) concentrations and the expression of TLR-4 in the human.[9]

Mice that were chronically infused with LPS and those that were fed a HFD gained the same amount of body weight. As compared with control mice, both mouse models showed similarly increased levels of insulin and glucose.[59] Infusion of LPS and HFD elicited similar inflammatory responses in muscle adipose and hepatic tissues.[59] Mice with KO of CD14, which is a coreceptor for LPS facilitating the binding of LPS to TLR-4, were protected from HFD-induced insulin resistance.[60]

Thus, the increase in LPS and the induction of CD14 and TLR-4 play an important role in the pathogenesis of insulin resistance.

12.10 ANTI-INFLAMMATORY FOODS

Since the popular HFHC meals from fast food chains induce oxidative and inflammatory stress even in modest amounts (900 calories) and a high-fiber and high-fruit meal does not, it is important to ask whether some foods do not exert such effects and whether there are some that are actually antioxidant and anti-inflammatory.[9] There are foods like berries, which are known to have high antioxidant content. However, these foods have not been shown to exert antioxidant and anti-inflammatory effects in humans or animals, *in vivo*.

Our recent work has shown at least two food/nutritional products to be anti-inflammatory. The intake of orange juice, not prepared from concentrates, suppresses ROS generation, $p47^{phox}$ expression, and intranuclear NF-κB binding, and increases plasma concentrations of LPS and LPB, as well as the expression of CD14 and TLR-4 induced by a HFHC meal.[18,61] In addition, it prevents the increase in SOCS-3 expression induced by a HFHC meal.

The other nutritional product that appears to have profound anti-inflammatory effects is resveratrol.[62] When taken as an extract of *Polygonum cuspidatum* by normal subjects for a period of 6 weeks, it suppresses ROS generation, $p47^{phox}$ expression, intranuclear NF-κB binding, the expression of JNK-1, SOCS-3, and PTP-1B, and the plasma concentrations of TNFα.[62] Our most recent work shows that the intake of a resveratrol containing polyphenol preparation of muscadine grapes prior to the intake of a HFHC meal prevents the meal-induced increases in oxidative and inflammatory stress, endotoxemia, and the increase in the expression of TLR-4, CD14, SOCS-3, and PTP-1B.[19]

Pomegranate juice has also been shown to reduce experimental inflammation in cartilage.[63] It needs to be tested in humans. With the emergence of anti-inflammatory food products, it is clear that an important revolution in eating and lifestyle habits can be stimulated. Some of these products could also be tested potentially as therapeutic agents in the areas of infective and metabolic inflammation. The latter would be relevant to insulin resistance and type 2 diabetes.

12.11 ACUTE ENDOTOXEMIA AND INSULIN RESISTANCE

There are recent data demonstrating that the injection of LPS (3 ng/kg) into normal subjects induces insulin resistance 24 h later, as measured by a frequently sampled intravenous glucose tolerance test (FSIGT) and HOMA-IR.[27] This phenomenon is associated with an increase in the expression of SOCS-3 in adipose tissue and peripheral blood leukocytes in association with the induction of pro-inflammatory cytokines like TNFα and IL-6. Thus, LPS-induced acute inflammation leads to acute insulin resistance. The induction of an increase in HOMA-IR following LPS (2 ng/kg) has recently been confirmed by our group.[28] While the injection of LPS leads to the induction of insulin resistance, it is relevant that LPS concentrations increase significantly after a high-fat, high-carbohydrate meal.[9] Whether there is

a cumulative increase in endotoxemia following the repeated intake of such meals, matching the effects induced by the injection of endotoxin needs to be investigated.

12.12 THE ROLE OF PHYSICAL INACTIVITY AND EXERCISE

Physical inactivity is an independent risk factor for insulin resistance and type 2 diabetes.[64] Physical inactivity has adverse effects on skeletal muscle metabolism, manifesting in poor oxidative capacity, lower glycogen storage, and decreased capillary density. The role of physical inactivity in inducing insulin resistance and undesirable metabolic effects can be demonstrated even in acute experiments. Thus, strict bed rest for a period of 5 days in normal subjects has now been shown to consistently result in a significant increase in insulin resistance and systolic blood pressure. In addition, there is a decrease in brachial artery diameter and a reduction in flow-mediated dilation of the brachial artery.[65] A similar response to inactivity is seen even in endurance runners.[66] The deleterious characteristics of inactivity can be restored by regular exercise.

Physical exercise increases energy expenditure, thus causing weight loss. However, many studies have shown that physical exercise has metabolic benefits independent of loss of adiposity.[67,68] These include an improvement in insulin sensitivity, glucose uptake, and lipid metabolism. Regular exercise has consistently been shown to improve insulin sensitivity.[68,69] The mechanisms underlying these improvements have been under investigation for decades. Dube et al. studied the effects of moderate exercise on insulin sensitivity in previously sedentary obese individuals.[70] Aerobic training for 4 months led to an improvement in insulin sensitivity (21%), increase in intramyocellular triglyceride (21%) and glycogen (16%) content, increase in mitochondrial oxidative capacity (20%), and a 25% decrease in total diacylglycerol and ceramide content in the muscle. Capillary density in muscle increased by 7%, and the percentage of type I slow oxidative fibers increased (11%). Apart from the change in ceramide content, none of the changes correlated significantly with the increase in insulin sensitivity. The increase in intramyocellular triglyceride is paradoxical because insulin-resistant states such as obesity and type 2 diabetes are associated with higher intramyocellular triglyceride content. However, the triglycerides per se may not confer insulin resistance; but rather, the increases in triglyceride content provide substrate for energy metabolism in the exercise-trained state. On the other hand, accumulation of diacylglycerol and ceramide in the muscle may be more directly relevant to insulin signaling. The decrease in these metabolites with exercise may be due to the increase in oxidative capacity of muscle.

A single bout of exercise is also associated with a significant improvement of insulin sensitivity for 3–4 h.[71] The underlying mechanisms are not well understood. Acute exercise does not appear to enhance insulin receptor tyrosine kinase activity, IRS-1 tyrosine phosphorylation, IRS-1-associated PI3 kinase activity, or glycogen synthase kinase-3 activity. Thus, although exercise leads to an increase in membrane glucose transporter (GLUT4) translocation in response to insulin stimulation, the signaling step is yet unknown. Acute exercise leads to inhibitory phosphorylation of Akt substrate of 160 kDa (AS160) and activation of atypical protein kinase C (aPKC).[71,72] These proteins are known to be involved in GLUT4 translocation. Since

these proteins are also stimulated by insulin, they may act as one site of convergence between insulin- and contraction-stimulated glucose uptake.

The main effect of exercise on glucose uptake in working muscle is, however, insulin independent. Many factors play a role in exercise-induced translocation of the GLUT4.[72–74] AMP-activated protein kinase (AMPK) is one such mediator. AMPK is an intracellular fuel sensor that is activated upon depletion of cellular ATP. AMPK phosphorylates AS160, which enhances GLUT4 translocation, as mentioned above. Calcium-calmodulin-dependent protein kinase and nitric oxide also regulate exercise-induced glucose uptake.

Lipid metabolism is improved by exercise, leading to increased capacity to oxidize and utilize fatty acids.[75] This is another mechanism by which exercise improves insulin sensitivity. AMPK mediates the increased fatty acid utilization following exercise by phosphorylating and inhibiting acetyl CoA carboxylase.[76–79] Exercise increases mitochondrial biogenesis, possibly mediated by PGC1 alpha.[80–83] Thus, multiple intracellular pathways are responsible for the improvements in insulin sensitivity and substrate utilization following acute and chronic exercise. Associated with these phenomena is the reduction in cardiovascular risk, such as improvements in blood pressure and lipids.[84,85] Some studies have shown that physical activity also leads to a reduction in inflammatory mediators such as CRP and IL-6. This reduction was independent of weight loss in some.[86–88]

Since certain ethnic groups like Asian Indians have increased insulin resistance even in the absence of overt adiposity, it is likely that insulin resistance in these groups is related to genetic factors.[89] One important factor that probably contributes to the pathogenesis of insulin resistance in this racial group is upper abdominal adiposity, which is often not associated with overt obesity. It is possible that the effect of macronutrient intake in these groups is even more powerful. This requires careful investigation.

12.13 NOVEL ACTIONS OF INSULIN AND INSULIN RESISTANCE

Since insulin induces vasodilation through the increased expression and activation of endothelial nitric oxide synthase (eNOS) and the release of NO, any interference with its signal transduction and action would potentially exert a vasoconstrictor effect, as is manifest in the reduction of flow-mediated dilation.[90,91] This would potentially contribute to the pathogenesis of hypertension. Similarly, insulin resistance would interfere with the action of insulin in inhibiting lipolysis and promoting lipogenesis in adipose tissue. Increased lipolysis would result in increased FFA concentrations and further interfere with insulin action and, as mentioned earlier, promote hypertension. The increased FFA concentrations at the hepatocyte level would promote triglyceride synthesis and secretion, leading to hypertriglyceridemia. Since insulin increases triglyceride clearance through the activation of lipoprotein lipase, resistance to its action would also increase the plasma concentration of triglycerides.[92,93]

Insulin is known to promote apolipoprotein A and high-density lipoprotein (HDL) biosynthesis by hepatocytes, *in vitro*. This process would be relatively inhibited in

insulin-resistant states, and thus hepatic HDL secretion would be diminished.[94,95] HDL concentrations also fall in insulin-resistant states because triglyceride overloaded particles are vulnerable to lipolytic action of lipoprotein lipase. This allows the residual HDL particles to be filtered by the kidney, and thus HDL levels fall further.

Insulin also exerts an anti-inflammatory action, which would be reduced in insulin-resistant states, which would thus be pro-inflammatory and potentially pro-atherogenic.[96] The pro-inflammatory state would also account for the increased CRP and plasminogen activator inhibitor-1 (PAI-1) concentrations observed in insulin-resistant states. It is relevant that the infusion of insulin leads to consistent and rapid reduction in both CRP and PAI-1 concentrations.[97]

12.14 FUTURE DIRECTIONS FOR RESEARCH

The fact that we now have three simple strategies to induce insulin resistance, through repeated intake of high-fat and high-carbohydrate meals, total bed rest for a short time, or the injection of a small amount of endotoxin, allows for future research to dissect the underlying mechanisms. In this context, it is also relevant that HFHC meals induce a low-grade endotoxemia, an increase in the plasma concentration of LBP, and the expression of TLR-4, the receptor for endotoxin (LPS). The expression of SOCS-3 is also increased by both HFHC meals and the injection of endotoxin. Thus, inflammatory mechanisms are integral to the induction of insulin resistance and are now known to be features of the metabolic syndrome.

12.15 THE RATIONAL MANAGEMENT OF INSULIN RESISTANCE, THE METABOLIC SYNDROME

Since being overweight and obese, the essential components of the metabolic syndrome, are dependent upon an excessive caloric intake, and excessive caloric intake has been shown under experimental conditions to induce insulin resistance, abnormal vascular reactivity, hypertriglyceridemia, oxidative stress, and inflammation, the fundamental approach to the rational treatment of this syndrome is clear: a reduction in caloric intake. Since total bed rest induces insulin resistance within a few days and exercise leads to insulin sensitization, physical activity and exercise are also cardinal to the treatment of this syndrome. Weight loss is known to reduce blood pressure and hypertriglyceridemia. Thus, lifestyle changes can reverse the major features of the metabolic syndrome. These issues are also relevant to type 2 diabetes and atherosclerotic cardiovascular disease.

However, since changing lifestyle consistently over a prolonged period of time is often not easy, drugs may have to be used to control each element of the syndrome: hypertension, hypertriglyceridemia, a low HDL-cholesterol concentration, hyperglycemia, insulin resistance, and excessive weight/obesity. This therapeutic approach does not mean that the origin of the syndrome is through diverse mechanisms. It simply implies that the therapeutic approach incorporating the basic etiological mechanisms is extremely difficult to follow.

12.15.1 Increased Renin–Angiotensin–Aldosterone System (RAAS) Activity in Obesity

Alterations in the renin–angiotensin system have been described in obesity. The expression of the angiotensinogen gene has been shown to be diminished in the adipose tissue of the obese, while that of renin, angiotensin converting enzyme, and type 1 angiotensin II receptor is enhanced significantly.[98] These changes could contribute to an increased activity of the RAAS and thus to hypertension. On the basis of these observations, it has been suggested that the rational treatment strategy for hypertension associated with obesity should be based on drugs that interfere with RAAS.[99] There are hitherto no studies demonstrating a diminution in RAAS following caloric restriction or increased exercise.

12.15.2 Reversal of Insulin Resistance and the Metabolic Syndrome Following Surgery for Obesity

Surgical procedures have recently been shown to lead to marked weight loss and a concomitant resolution of the features of the metabolic syndrome.[100] Thus, there is a reduction of abdominal adiposity, hyperglycemia, hyperinsulinemia, hypertension, and hypertriglyceridemia. With the progressive resolution of these features in association with weight loss, there is a concomitant reduction in medications that these patients need to take in relation to these features. In the postsurgical period, while there is a marked and often a dramatic weight loss, there are also subtle changes in incretin and other mechanisms that may contribute to the reversal of the features of the syndrome.

12.16 CONCLUSIONS

On the basis of the data and the discussion above, we can conclude that insulin resistance is an entity arising out of the abuses of modern lifestyle, with a marked increase in caloric intake far beyond our requirements and a marked decrease in physical activity. We have demonstrated how a single HFHC meal is pro-inflammatory, and how it lays the foundations of insulin resistance, abnormal vascular reactivity, and hypertension and hypertriglyceridemia. Bed rest and lack of exercise also lead to the induction of insulin resistance and abnormal vascular reactivity within a very short time. The combination of the intake of "toxic" food and the lack of exercise would lead to the induction of insulin resistance and the other features of the metabolic syndrome. In this context, it is important to mention that the toxic meal induces low-grade endotoxemia associated with an increase in lipopolysaccharide binding protein (LBP), CD14, and TLR-4, the receptor for endotoxin, while the injection of a small amount of endotoxin into normal subjects leads to insulin resistance within 24 h. The best rational and specific treatment of insulin resistance is therefore a significant reduction in caloric intake and an increase in exercise. The failure to

achieve this leads to the markedly enhanced risk of the metabolic syndrome, type 2 diabetes, and atherosclerotic cardiovascular disease.

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13 Role of Inflammation in Cancer Development

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CONTENTS

13.1 INTRODUCTION

Inflammation essentially is described as the complex, vascular, and immune system responses to injury or infections that occur as a consequence of pathogens, irritants, and other noxious stimuli (Mantovani 2009; Mantovani et al. 2008; Aggarwal et al. 2009c). The role of inflammation in cancer was first proposed by Rudolf Virchow in 1863, when he observed the presence of leukocytes in neoplastic tissues (Aggarwal and Gehlot 2009; Aggarwal 2003). Since Virchow's initial observation that linked inflammation and cancer, both the cause and consequence of inflammation in cancer continue to be elucidated (Grivennikov et al. 2010; Kundu and Surh 2008). The inflammatory milieu promotes a cellular environment that favors the development of genomic aberrations and the initiation of carcinogenesis (Mantovani 2009). While

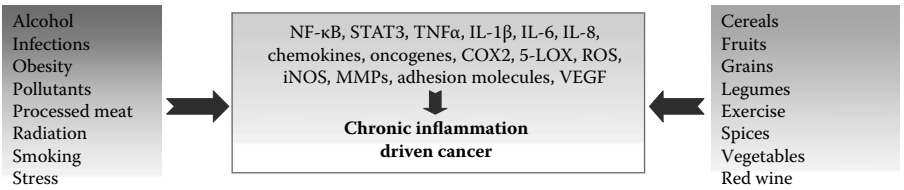

FIGURE 13.1 Various lifestyle and environmental related factors mediate chronic inflammation driven cancers through activation of various signaling intermediates and chemopreventive dietary agents/exercise have shown great potential to suppress inflammation and tumorigenesis.

acute inflammation is primarily a self-limiting process and has therapeutic consequences, inadequate resolution of inflammatory responses often leads to various chronic ailments, including cancer (Aggarwal et al. 2009c).

It is widely believed that environment and lifestyle-related factors play an important role in chronic inflammation eventually leading to cancer (Aggarwal et al. 2009c; Anand et al. 2008; Karin et al. 2006). For example, almost 30% of all cancers have been attributed to tobacco smoke, 35% to diet, 14–20% to obesity, 18% to infections, and 7% to radiation and environmental pollutants (Anand et al. 2008). Though sufficient knowledge has been generated in understanding the critical role of cigarette smoke, alcohol consumption, obesity, radiation, bacterial, and virus infection in the development of chronic diseases that affect human health, knowledge gaps still exists in gene–nutrient interaction and in the development of cancer. Dietary factors such as red meat intake have been implicated with cancer risk. Charbroiling red meat leads to the generation of heterocyclic amines such as 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine, as well as polycyclic aromatic hydrocarbons (Lauber and Gooderham 2007). These compounds have been shown to induce mutations and epithelial cell damage, inflammation, and proliferative inflammatory atrophy leading to cancer development (Nakai et al. 2007). On the other hand, diets rich in fruits and vegetables, legumes, and exercise have been shown to reduce the risk of cancer (Aggarwal et al. 2008; Bardia et al. 2009). Thus, chronic inflammation-driven cancer can be considered a preventable disease that requires major lifestyle-related changes (Figure 13.1).

Chronic inflammation has been linked to various steps involved in tumorigenesis, including cellular transformation, survival, proliferation, invasion, angiogenesis, and metastasis (Porta et al. 2009; Demaria et al. 2010). Within the tumor microenvironment, various proinflammatory mediators participate in a complex signaling process that facilitates extravasations of tumor cells through the stroma, thereby promoting tumor progression (Colotta et al. 2009; Balkwill and Mantovani 2001). Inflammation acts as a key regulator of tumor promotion and progression by several mechanisms, including accelerated cell proliferation, evasion from apoptotic cell death, and stimulation of tumor neovascularization (Aggarwal et al. 2006b). The mechanisms for cancer development in the presence of chronic inflammation involve the continuous presence of cytokines, chemokines, reactive oxygen and nitrogen species, oncogenes, cyclooxygenase-2, 5-lipoxygenase, matrix metalloproteinases, and activation of important transcription factors such as nuclear factor-κB (NF-κB) and signal transducer and

activator of transcription 3 (STAT3) (Aggarwal and Gehlot 2009; Aggarwal et al. 2006b; Balkwill and Mantovani 2010). In this chapter, we will focus on the role of various proinflammatory mediators in carcinogenesis and provide further insight into the existing intricate link between chronic inflammation and cancer.

13.2 ROLE OF TUMOR NECROSIS FACTOR IN INFLAMMATION-DRIVEN CARCINOGENESIS

Tumor necrosis factor (TNF-α) was first isolated as an anticancer cytokine more than two decades ago, but when its antitumor activity was tested on cancer patients, a paradoxical tumor-promoting role of TNF-α became apparent (Aggarwal 2003; Sethi et al. 2008b; Balkwill 2009). Evidence since then has indicated that when expressed locally by the cells of the immune system, TNF-α has a therapeutic role. However, when dysregulated and secreted in the circulation, TNF-α can mediate a wide variety of diseases, including cancer (Aggarwal 2003; Balkwill 2006; Szlosarek et al. 2006) TNF-α has now been shown to be one of the major mediators of inflammation. The proinflammatory role of TNF-α has been linked to all steps involved in tumorigenesis, including cellular transformation, survival, proliferation, invasion, angiogenesis, and metastasis (Sethi et al. 2008b).

Numerous reports indicate that TNF-α induces cellular transformation, proliferation, and tumor promotion, with human TNF-α being 1,000 times more effective than the chemical tumor promoters okadaic acid and 12-O-tetradecanoylphorbol-13-acetate (Komori et al. 1993). Although initially thought to be a product only of macrophages, TNF-α has now been shown to be produced by a wide variety of tumor cells, including those of B cell lymphoma (Digel et al. 1989, 1990), megakaryoblastic leukemia (Liu et al. 1998), adult T cell leukemia (Tsukasaki et al. 2001), breast carcinoma (Montesano et al. 2005), colorectal cancer, lung cancer, squamous cell carcinoma, pancreatic cancer (Kalthoff et al. 1993; Schmiegel et al. 1993), ovarian carcinoma (Wu et al. 1993; Takeyama et al. 1991; Naylor et al. 1993), cervical epithelial cancer (Duarte et al. 2005), glioblastoma (Aggarwal et al. 1996), and neuroblastoma (Goillot et al. 1992, Nabors et al. 2003). In most of these cells, TNF-α acts as an autocrine growth factor; however, in few cell types TNF-α acts as a paracrine growth factor and induces the expression of other growth factors, which mediate proliferation of tumors. For instance, in cervical cells TNF-α induces amphiregulin, which induces the proliferation of cells (Woodworth et al. 1995), whereas in pancreatic cells TNF-α induces the expression of epidermal growth factor receptor (EGFR) and transforming growth factor (TGF-α), which mediate proliferation (Schmiegel et al. 1993). In a genetic model of liver cancer, TNF-α produced by myeloid cells promoted inflammation-associated tumors (Pikarsky et al. 2004), and also in a chemically induced model of colorectal cancer, macrophage TNF-α has been implicated in inflammation and subsequent tumor development (Balkwill 2009). Endogenous and exogenous TNF-α showed enhancement of metastasis in an experimental fibrosarcoma metastasis model (Orosz et al. 1993).

TNF-α is expressed by human ovarian carcinoma *in vivo*. Approximately 80% of the patient biopsies were found to be positive for TNF-α (Takeyama et al. 1991).

The gene for TNF-α was also found to be expressed in 45 of 63 biopsies of human epithelial ovarian cancer (Naylor et al. 1993). Macrophages are important in the induction of new blood vessel growth during wound repair, inflammation, and tumor growth. It has been demonstrated that macrophage-induced angiogenesis is mediated by TNF-α (Leibovich et al. 1987). In inflammation and wound repair, TNF-α could augment repair by stimulating new blood vessel growth; TNF-α might stimulate tumor development by promoting vessel growth in the tumor. The role of both TNF-α and its receptors has been examined in cancer development. Studies have shown that TNF receptor 1 (TNFR-1)-mediated signaling is required for skin cancer development induced by NF-κB inhibition (Lind et al. 2004). This suggests a critical role of local TNFR-1-mediated signaling and associated inflammatory response cooperating with repressed keratinocyte NF-κB signaling in driving skin cancer development. An essential role of TNFR p55 has been determined in the liver metastasis of intrasplenic administration of colon 26 cells (Kitakata et al. 2002). TNF-α is an essential cytokine in carcinogenesis and tumor promotion in mouse skin and, very possibly, for carcinogenesis in humans as well (Suganuma et al. 1996). It has been further shown that a proinflammatory cytokine is required for *de novo* carcinogenesis and that TNF-α is important to the early stages of tumor promotion (Moore et al. 1999). Thus, strategies that neutralize systemic TNF-α may be useful in cancer treatment and prevention.

13.3 ROLE OF INTERLEUKINS IN CHRONIC INFLAMMATION AND CANCER

Several interleukins have been linked with inflammation and subsequent cancer development. These interleukins include IL-1, IL-6, IL-8, and IL-17. IL-1α, expressed in both normal tissue and several tumor cells, is a regulatory cytokine that can induce the activation of transcription factors, including NF-κB and AP-1, and promotes the expression of genes involved in cell survival, proliferation, and angiogenesis (Wolf et al. 2001). IL-1β is involved in the generation of IL-17-producing CD+T cells, and the IL-23/IL-17 axis has been reported to promote skin carcinogenesis (Langowski et al. 2006). Direct evidence of the role of IL-1β in human cancer has also been obtained in multiple myeloma. IL-1β released by myeloma cells induces the production of IL-6 by bone marrow stromal cells, and functions as a growth factor for myeloma cells (Lust et al. 2009). IL-1β also upregulates HIF-1α protein through a classical inflammatory signaling pathway involving NF-κB and COX-2, culminating in upregulation of VEGF, a potent angiogenic factor required for tumor growth and metastasis (Jung et al. 2003).

IL-6 is another major proinflammatory cytokine that has been implicated in inflammation-associated carcinogenesis (Hong et al. 2007, Naugler and Karin 2008b). IL-6 modulates the expression of genes involved in proliferation, survival, and angiogenesis via the JAK-STAT signaling pathway (Lin and Karin 2007). An elevated level of IL-6 has been found in the pathogenesis of various cancers (Cozen et al. 2004; Kai et al. 2005; Schneider et al. 2000). Autocrine IL-6 production in renal cell carcinoma (RCC) was linked with the involvement of p53. RCC cell lines containing mutant p53 produced higher levels of IL-6 than those containing wild-type p53

(Angelo et al. 2002). Conversely, mice lacking IL-6 are less susceptible to development of plasmacytoma, which is a malignant disorder of plasma cells (Gado et al. 2001). Furthermore, analysis of biopsy specimens from inflammation-associated gastric cancers has revealed that the levels of IL1β and IL-6 are highly elevated in tumors, compared to adjacent normal mucosa (Kai et al. 2005). The serum levels of IL-6 have been found to be significantly increased and positively correlated to tumor burden in colon cancer patients (Chung and Chang 2003).

Expression of IL-8 by human melanoma cells upregulates MMP-2 activity and increases tumor growth and metastasis (Singh and Varney 2000). Huang et al. (2002) have reported that neutralizing antibodies to IL-8 inhibit angiogenesis, tumor growth, and metastasis of human melanoma, suggesting the potential utility of anti-IL-8 as a modality to treat melanoma and other solid tumors either alone or in combination with conventional chemotherapy or other antitumor agents. The expression of IL-8 by human ovarian cancer cells correlates directly with disease progression (Xu and Fidler 2000). The acidic pH induced elevation in IL-8 expression in human ovarian carcinoma cells, and transcription factors, AP-1, and NF-κB was found to be responsible for acidic pH-induced transcriptional activation of the IL-8 gene (Xu and Fidler 2000). Using a nude mice tumor xenograft model, Sparmann and Bar-Sagi (2004) showed that IL-8 is a transcriptional target of Ras signaling. Ras-dependent IL-8 secretion was required for the initiation of tumor-associated inflammation and neovascularization (Sparmann and Bar-Sagi 2004). Another cytokine IL-17 has been reported to act as a growth factor in cutaneous T cell lymphoma (Asarch et al. 2008). This proinflammatory cytokine produced by the Th17 subtype of T cells has recently been recognized as a key player in inflammation and cancer (Lin and Karin 2007). The role of IL-17 in inflammation-associated cancer depends mainly on its proangiogenic property. For example, IL-17- overexpressing human cervical cancer (Tartour et al. 1999), fibrosarcoma (Numasaki et al. 2003), and human non-small-cell lung cancer preferentially exhibit higher oncogenic growth *in vivo* (Numasaki et al. 2005).

13.4 ROLE OF CHEMOKINES IN CHRONIC INFLAMMATION AND CANCER

Chemokines are soluble chemotactic cytokines that are grouped into four classes based on the positions of key cysteine residues: C, CC, CXC, and CX3C (D. Wang et al. 2009, Lu et al. 2006, Aggarwal and Gehlot 2009). Chemokines play pleiotropic roles in cancer progression, including angiogenesis, inflammation, cell recruitment, and migration, and have a well-known role in regulating the recruitment and trafficking of leukocytes to sites of inflammation (Raman et al. 2007). Like cytokines, chemokines also act by interacting with specific receptors expressed by both infiltrated leukocytes and tumor cells in an autocrine or a paracrine fashion (Allen et al. 2007). Several studies have reported the involvement of chemokines and chemokine receptors in cell proliferation, migration, and invasion and metastasis of different types of tumors (Kollmar et al. 2007; Owen et al. 1997; Yuecheng and Xiaoyan 2007).

The chemokine receptors CXCR4 and CCR7 are highly expressed in human breast cancer cells, malignant breast tumors, and metastasis (Muller et al. 2001). Their

respective ligands CXCL12/SDF-1α and CCL21/6 exhibit peak levels of expression in organs representing the first destinations of breast cancer metastasis (Muller et al. 2001). In breast cancer cells, signaling through CXCR4 or CCR7 mediates actin polymerization and pseudopodia formation and subsequently induces chemotactic and invasive responses (Muller et al. 2001). *In vivo*, neutralizing the interactions of CXCL12/CXCR4 significantly impairs metastasis of breast cancer cells to regional lymph nodes and lung (Kim et al. 2008). Overexpression of CXCL-1/GROa, CXCL-2/GROb, or CXCL-3/GROg can promote soft agar colony formation and transformation of melanocytes in culture, as well as tumorigenicity of transplanted melanoma cells in nude mice (Owen et al. 1997). CXCR4 ligand, CXCL12 (stromal cell-derived factor 1), was found to be expressed in ovarian cancer cell line IGROV (Scotton et al. 2002). CXCR4 activation also induced EGFR transactivation in an ovarian cancer cell line (Porcile et al. 2004). It has been demonstrated that CXCR4 and SDF-1 induce proliferation in ovarian cancer cells, and this correlated with epidermal growth factor (EGF) receptor transactivation. The functional chemokine receptor CCR3 has been shown to be upregulated in human RCC (Johrer et al. 2005). Mip-3α and its receptor, CCR6, promote pancreatic cancer cell invasion (Kimsey et al. 2004). Colocalization of Mip-3α and its CCR6 receptor promotes pancreatic cancer cell invasion of type IV collagen (Kimsey et al. 2004). Extensive studies suggest that inflammatory processes may be involved in the development or progression of prostate cancer. CXCL14 (BRAK) RNA expression has been observed in normal and tumor prostate epithelium and focally in stromal cells adjacent to cancer (Schwarze et al. 2005). Malignant melanoma, which has a metastatic pattern similar to that of breast cancer, and was correlated with a high incidence of skin metastases, shows high expression levels of CCR10 in addition to CXCR4 and CCR7 (Scala et al. 2006). Thus, chemokines and their receptors have a critical role in determining the metastatic destination of tumor cells. A list of various interleukins and chemokines associated with cancer development is summarized in Table 13.1.

13.5 ROLE OF ONCOGENES IN CHRONIC INFLAMMATION-DRIVEN CANCER

Oncogenes are altered versions of normal cellular genes, the so-called proto-oncogenes, involved in the regulation of cell growth (Weinberg 1994; Croce 2008). The discovery of oncogenes, almost 30 years ago, provided a critical breakthrough in our understanding of the molecular and genetic basis of cancer. Oncogenes have also provided important knowledge concerning the regulation of normal cell proliferation, differentiation, and apoptosis (Croce 2008). It has increasingly become evident that pleiotropic effects of oncogenes also include the induction of a pro-tumor microenvironment, through the persistent promotion of an inflammatory milieu (Borrello et al. 2008; Croce 2008; Grivennikov and Karin 2010b). For example, Liu et al. (2004) have shown that HRAS- and KRAS-G12V induce the expression of various cytokines, including IL-1α, IL-1β, IL-6, CXCL8, and IL-11, in human ovarian cells. Interestingly, Ras-transformed ovarian cells show dependency on cytokines for survival since antibodies blocking IL-1β and CXCL8 induce

TABLE 13.1
Role of Cytokines, Interleukins, and Chemokines in Tumorigenesis

Cancer	Inflammatory Mediator	Mechanism(s)	Reference
Bladder cancer	IL-6	Transformation	Cai et al. 2007
Multiple myeloma	IL-6 poly	Proliferation	Ishikawa et al. 2006
Colorectal cancer	IL-6	Increased risk	Knupfer and Preiss 2010
Melanoma	IL-18	Metastasis	Vidal-Vanaclocha et al. 2006
Cervical carcinoma	TNF-α	Growth	Duarte et al. 2005
Pancreatic carcinoma	IL-1α	Metastasis	Melisi et al. 2009
Prostate cancer	IL-8 poly	Angiogenesis	McCarron et al. 2002
Lung carcinoma	IL-1a	Angiogenesis	Jung et al. 2003
NHL	TNF-α	Autocrine growth	S. S. Wang et al. 2009
Melanoma	IL-8	Tumor growth	Singh and Varney 2000
Glioblastoma	IL-8	Angiogenesis	Brat et al. 2005
RCC	IL-6	Autocrine growth	Angelo et al. 2002
Pancreatic carcinoma	IL-1β	Chemoresistance	Arlt et al. 2002
Ovarian tumors	IL-8	Disease progression	Xu and Lam 2001
Tumor	IL-8	Growth, angiogenesis	Sparmann and Bar-Sagi 2004
Lung carcinoma	IL-1β	Growth	Saijo et al. 2002
Breast cancer	CXCR4, CCR7	Metastasis	Muller et al. 2001
Melanoma	CXCR4, CCR7,	Metastasis	Muller et al. 2001
Ovarian carcinoma	CXCR4/CXCL12	Invasion and growth	Xu and Fidler 2000
RCC	CCR3	Higher risk	Johrer et al. 2005
Pancreatic carcinoma	MIP-3a, CCR6	Cell invasion	Kimsey et al. 2004
Ovarian carcinoma	CXCR4, SDF1	Proliferation	Porcile et al. 2004
Prostate carcinoma	CXCL14	Inhibits tumor growth	Schwarze et al. 2005

apoptosis in Ras-transformed cells (Liu et al. 2004). Furthermore, transcription factor NF-κB is activated in Ras-transformed ovarian epithelial cells, and this activation is responsible for the increased expression of CXCL8 (Yoneda et al. 1998). A cause–effect relationship between Ras oncogenes and tumor-associated inflammation and neovascularization was reported by Sparmann and Bar-Sagi (2004), who demonstrated that CXCL8 is a transcriptional target of Ras signaling in HeLa cells. Using a tumor xenograft model, they further showed that HRAS-G12V-dependent CXCL8 induction is necessary for tumor growth. Moreover, Ras-mediated production of CXCL8 by human cancer cells leads to recruitment of mouse inflammatory cells, which produce growth and angiogenic factors. Indeed, inhibition of CXCL8 reduced the recruitment of host inflammatory cells to tumor and led to a substantial decrease in tumor vasculature and extensive tissue necrosis (Sparmann and Bar-Sagi 2004). These findings indicate a role for CXCL8 in Ras oncogene-dependent tumor angiogenesis. Furthermore, Ancrile et al. (2007) have demonstrated that IL-6 acts downstream of Ras in a paracrine fashion to promote angiogenesis. They have demonstrated that HRAS-G12V induces IL-6 expression in different cell types, and

that genetic ablation of the IL-6 gene or treatment with a neutralizing IL-6 antibody retards Ras-driven tumorigenesis.

Recent reports suggest that Myc oncogene can also orchestrate a complex inflammatory program (Meyer and Penn 2008). Pelengaris and colleagues (2002) have shown that activation of Myc by 4-hydroxytamoxifen can induce rapid tumor onset. The sustained expansion of β cells induced by Myc activation is accompanied by concurrent expansion of islet vasculature (Pelengaris et al. 2002). Myc activation in β cells rapidly induces expression and release of the proinflammatory cytokine IL-1β, which in turn mediates release of VEGF-A from mast cells and onset of tumor angiogenesis (Shchors et al. 2006). Mast cell activation is required not only for angiogenesis during outgrowth of Myc-dependent islet tumors, but also for tumor maintenance, and the inhibitors of mast cell function trigger hypoxia and cell death of tumor and endothelial cells (Soucek et al. 2007). Moreover, four mutually exclusive genetic lesions have been identified in papillary thyroid carcinoma, covering about 80% of the cases: rearrangements of Ret or Trk genes and activating mutation of Ras or B-raf genes (Arighi et al. 2005). Thus, several extrinsic oncogene-independent inflammatory pathways are activated in human carcinomas and are likely to play a key role in various stages of carcinogenesis.

13.6 ROLE OF OXIDATIVE STRESS IN CHRONIC INFLAMMATION AND CANCER

Reactive oxygen intermediates, also generically referred to as oxidants, are derivatives of molecular oxygen, such as superoxide, hydrogen peroxide, hypochlorous acid, singlet oxygen, and the hydroxyl radical (Hwang and Bowen 2007; Schetter et al. 2010; Klaunig et al. 2010). Under normal circumstances, phagocyte-derived oxidants serve a protective function by killing invading bacteria and parasites (Aggarwal et al. 2006b). However, they can also have detrimental effects, causing tissue damage and contributing to the development or progression of numerous diseases, including cancer (Babior 2000). Chronic inflammation is accompanied by increased production of tissue-reactive oxygen and nitrogen intermediates. ROS can alter signal transduction cascades as well as induce changes in transcription factors, such as NF-gen nuclear factor-erythroid-derived 2/related factor 2 (NF-E2/rf2 or Nrf2), and activator protein 1 (AP-1), that mediate immediate cellular stress responses (Closa and Folch-Puy 2004; Klaunig et al. 2010; Kwak and Kensler 2010). The pro-neoplastic activity of reactive oxygen species is mainly due to their ability to cause DNA damage (Marnett 2000). Proteins and lipids are also significant targets for oxidative attack, and modification of these molecules can increase the risk of mutagenesis (Schraufstatter et al. 1988). Oxidative damage to DNA has also been linked to aflatoxin B-induced p53 and Ras gene mutations in hepatocarcinogenesis (Shen and Ong 1996) in UV-induced mouse and human skin cancers (Nishigori et al. 2004). In addition, p15INK4B and p16INK4A tumor suppressor genes appear to be targets of ROS-induced renal cell carcinoma in rats (Tanaka et al. 1999). Agents that either scavenge reactive oxygen intermediates or prevent their formation inhibit induction of DNA damage, mutagenesis, and transformation by inflammatory

phagocytes. This forms the basis for the theory that dietary antioxidants can inhibit the development or progression of cancer (Aggarwal and Shishodia 2006; Aggarwal et al. 2008, 2009b).

13.7 ROLE OF REACTIVE NITROGEN INTERMEDIATES IN CHRONIC INFLAMMATION AND CANCER

Inducible nitric oxide synthase (iNOS) is one of three key enzymes generating nitric oxide (NO) from the amino acid l-arginine (Bogdan 2001a). iNOS gene expression and subsequent mRNA translation are controlled by various agonists, especially proinflammatory mediators. The most prominent cytokines involved in iNOS stimulation are TNF-α, IL-1β, and IFN-γ (Bogdan 2001b). The expression of iNOS is regulated by transcription factors, including NF-κB, AP-1, STAT3, IL-6, interferon-regulatory protein 1, and high-motility group I (Y) protein (Nathan 1992). iNOS has been implicated in different stages of cellular changes that lead to malignancy: transformation of normal cells, growth of transformed cells, angiogenesis triggered by angiogenic factors released from tumor cells or from the surrounding tissue, and metastasis of malignant cells (Geller and Billiar 1998).

During chronic inflammation, induced expression of iNOS in macrophages and epithelial cells leads to production of NO (Lirk et al. 2002). In a variety of human malignant tumors, e.g. breast, lung, prostate, bladder, colorectal cancer, and malignant melanoma, expression of iNOS can be observed (Lirk et al. 2002; Jaiswal et al. 2001). For example, Chun et al. (2004) have reported topical application of phorbol ester–induced iNOS expression and subsequent NO production, which in turn induced COX-2 expression via NF-κB activation in mouse skin. Pretreatment of mouse skin with aminoguanidine suppressed chemically induced mouse skin papilloma formation, suggesting that iNOS and NO play an important role in tumorigenesis (Chun et al. 2004).

The overexpression of iNOS has been detected in Barrett's mucosa, a premalignant condition arising from chronic reflux esophagitis and colorectal adenomas or carcinomas (Wilson et al. 1998). Analysis of clinically isolated prostate cancers has shown that strong iNOS expression is positively correlated with rapid cancer cell proliferation, dedifferentiation, and progression to advanced stage cancer (Aaltoma et al. 2001). In another study, Rieder et al. (2003), using the biopsy specimens from patients with stomach carcinoma and *H. pylori*-induced gastritis, demonstrated that elevated expression and activity of iNOS are associated with the development of intestinal metaplasia. The overexpression of iNOS in colon tissues from patients with ulcerative colitis suggests that iNOS may contribute to the pathogenesis of colitis-related neoplasia (Wink et al. 1998). Similarly, overexpression of iNOS was associated with enhanced DSS-induced colon carcinogenesis in APC min+ mice, compared to APC+/+ mice (Tanaka et al. 2006). Further studies are required to elucidate the role of the NO/iNOS pathway in inflammation-driven tumorigenesis and to establish the utility of iNOS inhibitors as chemoprevention agents.

13.8 OVEREXPRESSION OF CYCLOOXYGENASES CAN MEDIATE INFLAMMATION-DRIVEN CANCER

Cyclooxygenase (COX-2), an inducible enzyme regulated by NF-κB, is known to mediate tumorigenesis (Surh and Kundu 2007; Aggarwal et al. 2006b). COX-2, the inducible isoform of prostaglandin H synthase, has been implicated in the growth and progression of a variety of human cancers. COX-2 has been shown to regulate angiogenesis induced by colon cancer cells (Tsujii et al. 1998). Cyclooxygenase regulates colon carcinoma-induced angiogenesis by two mechanisms: COX-2 can modulate production of angiogenic factors by colon cancer cells, while COX-1 regulates angiogenesis in endothelial cells. Takeda et al. (2003) demonstrated that most COX-2-expressing cells in the polyps are stromal fibroblasts. It has been also reported that COX-2 and mPGES were induced in the COX-1-expressing fibroblasts in human familial adenomatous polyposis polyps (Einspahr et al. 2003). Administration of the COX-2-selective inhibitor rofecoxib or the functional inactivation of the COX-2 in adenomatous polyposis coli knockout mice, a murine model of human adenomatous polyposis, reduced the number and size of intestinal polyps (Oshima et al. 1996, 2001), thereby indicating the correlation between the abnormal upregulation of COX-2 and tumorigenesis.

COX-2 expression in human tumors can be induced by growth factors, cytokines, oncogenes, and other factors. IL-1 has been reported to upregulate COX-2 in human colorectal cancer cells via multiple signaling pathways (Liu et al. 2003). Paradoxically, Tang et al. (2002) showed that COX-2 overexpression inhibits death receptor 5 expression and confers resistance to TRAIL-induced apoptosis in human colon cancer cells. Forced HER2 expression in MCF-7 cells was shown to upregulate COX-2, although no association was found in human tumors. Expression of COX-2 in breast cancer correlates with poor prognosis, and COX-2 enzyme inhibitors reduce breast cancer incidence in humans. COX-2 overexpression in the mammary gland of transgenic mice induced mammary cancer (Kundu and Fulton 2002).

COX-2 has also been implicated in the progression of human lung adenocarcinoma. COX-2 mRNA steady-state levels were high in well-differentiated adenocarcinoma samples but low in poorly differentiated adenocarcinoma, squamous cell carcinoma, and small-cell lung cancer. COX-2 overexpression enhanced the *in vitro* expression of both CXC ligand CXCL8 and CXCL5, NSCLC angiogenic peptides in the NSCLC cell lines (Pold et al. 2004). COX-2 appears to play an important role in gastrointestinal carcinogenesis, and COX-2 overexpression has been demonstrated both in esophageal adenocarcinomas and in the metaplastic epithelium of Barrett's esophagus (Abdalla et al. 2005). Inhibition of COX-2 suppresses growth and induces apoptosis in human esophageal adenocarcinoma cells (Souza et al. 2000). COX-2 expression was strong in the squamous cell carcinomas (SCCs) and weak in esophageal adenocarcinomas (ADCs) (Hashimoto et al. 2007). COX-2 expression levels in tumor specimens from patients with low- and high-grade astrocytomas indicated a correlation between the percentage of COX-2 expression and patient survival (Shono et al. 2001). These findings indicate that high COX-2 expression in tumor cells is associated with clinically more aggressive gliomas, and is a strong predictor of poor survival.

13.9 OVEREXPRESSION OF 5-LIPOXYGENASE MEDIATES CHRONIC INFLAMMATION AND CANCER

5-Lipoxygenase (5-LOX) is a key enzyme in the metabolism of arachidonic acid to leukotrienes (Pidgeon et al. 2007). Several studies suggest that there is a link between 5-LOX and carcinogenesis in humans and animals (Pidgeon et al. 2007; Cuendet and Pezzuto 2000; Shureiqi and Lippman 2001). In addition to the important role of leukotrienes as mediators in allergy and inflammation, these compounds are also linked to pathophysiological events in the brain, including cerebral ischemia, brain edema, and increased permeability of the blood–brain barrier in brain tumors. Abundance of the mRNA for arachidonate 5-LOX, which is the rate-limiting enzyme in leukotriene synthesis, has been investigated in a series of human brain tumors (Boado et al. 1992). The 5-LOX transcript is expressed in human brain tumors, and 5-LOX gene product may play a role in human tumor-induced brain edemas (Boado et al. 1992).

The arachidonic acid-metabolizing enzymes COX-2 and 5-LOX are also overexpressed during the process of colonic adenoma formation promoted by cigarette smoke. Ye et al. (2005) investigated whether there existed a relationship between COX-2 and 5-LOX and whether dual inhibition of COX-2 and 5-LOX had an anticarcinogenic effect in the colonic tumorigenesis promoted by cigarette smoke. They found that pretreatment of colon cancer cells with cigarette smoke extract promoted colon cancer growth in the nude mouse xenograft model, and inhibition of COX-2 or 5-LOX reduced the tumor size (Ye et al. 2005). Studies also indicate that exposure to the mainstream smoke of unfiltered cigarettes enhanced 5-LOX protein expression in the inflammation-associated colonic adenomas (Ye et al. 2004). Such expression was accompanied by an upregulation of MMP-2 and VEGF, the key angiogenic factors for tumorigenesis. 5-LOX inhibitors decreased the incidence of colonic adenoma formation and reduced angiogenesis, MMP-2 activity, and VEGF protein expression (Ye et al. 2004). Overexpression of 15-lipoxygenase-1 (15-LOX-1) in human prostate cancer cells has been found to increase tumorigenesis (Kelavkar et al. 2001). Inhibitors of 5-LOX (MK-886) can prevent NNK-induced formation of tumors (Rioux and Castonguay 1998). Possible mechanisms of action of these inhibitors include inhibition of tumor growth and 5-LOX-mediated activation of NNK. 1-([5-(3-Methoxy-4-ethoxy carbonyloxyphenyl)-2′,4′-pentadienoyl] aminoethyl)-4-diphenylmethoxypiperidine (TMK688) is a potent and orally active 5-LOX inhibitor having antihistamine activity in its moiety (Jiang et al. 1994). Oral administration of TMK688 has been shown to inhibit two-stage skin carcinogenesis as well as complete skin carcinogenesis (Jiang et al. 1994).

13.10 ROLE OF MATRIX METALLOPROTEINASES (MMPs) IN CHRONIC INFLAMMATION AND CANCER

Matrix metalloproteinases (MMPs) are a multigene family of zinc-dependent endopeptidases that share a similar structure and which collectively have the capacity to degrade extracellular matrix (Cruz-Munoz and Khokha 2008). MMPs are now

TABLE 13.2
Role of Inflammatory Enzymes (COX-2, 5-LOX, and MMP-9) in Carcinogenesis

Tumor	Enzyme	Reference
Breast cancer	COX-2	Kundu and Fulton 2002, Chang et al. 2005
Cervical cancer	COX-1	Sales et al. 2002
Ovarian tumors	COX-2, iNOS	Klimp et al. 2001, Gupta et al. 2003
Glioma	COX-2	Shono et al. 2001
Prostate cancer	COX-2	Subbarayan et al. 2001
Melanoma	COX-2	Denkert et al. 2001, Goulet et al. 2003
Esophageal adenocarcinoma	COX-2	Souza et al. 2000
Esophageal SCC and AC	COX-2	Hashimoto et al. 2007
Urinary bladder	COX-2	Wild et al. 2005
Pancreatic cancer	COX-2	Farrow and Evers 2002
Head and neck SCC	COX-2	Mendes et al. 2009
Lung carcinoma	COX-2	Su et al. 2004, Heuze-Vourc'h et al. 2003
Gastric carcinoma	COX-2	Forones et al. 2008
Colorectal cancer	COX-2	Einspahr et al. 2003, Tang et al. 2002
Brain tumors	5-LOX	Zhang et al. 2006, Boado et al. 1992
Colorectal cancer	COX-2, 5-LOX	Ye et al. 2005
Pancreatic cancer	MMP-9	Bergers et al. 2000
Skin cancer	MMP-9	Coussens et al. 2000

implicated as key modulators of many biological processes during pathophysiological events, such as skeletal formation, angiogenesis, cellular migration, inflammation, wound healing, and cancer (Egeblad and Werb 2002; Roy et al. 2009). MMPs have also been implicated in the epithelial-to-mesenchymal transition (EMT), a hallmark of cancer progression to metastasis (Thiery 2002). Studies have also demonstrated that MMPs are involved in the angiogenic switch, one of the earliest stages of tumor growth and progression. It has also been shown that MMP-9 can be a regulator of the angiogenic switch in a pancreatic tumor model (Bergers et al. 2000). MMP-9/gelatinase B is upregulated in angiogenic dysplasias and invasive cancers of the epidermis in a mouse model of multistage tumorigenesis elicited by HPV16 oncogenes (Coussens et al. 2000). MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis (Coussens et al. 2000). In gene expression profiles associated with poor outcome of patients with breast tumors, 2 of the 70 genes identified were found to be MMP-1 and MMP-9 (van 't Veer et al. 2002). In another study, patient survival, gene overexpression, and RNAi validation data showed that MMP-1 is the second most important gene in a 95-gene expression profile in determining the metastatic potential of breast cancer to produce lung metastases (Minn et al. 2005). MMP-7 also promotes cancer invasion by proteolytic cleavage of the extracellular matrix substrates and activates other MMPs, such as proMMP-2 and proMMP-9, to facilitate tumor invasion. The roles of COX-2, 5-LOX, and MMPs in tumorigenesis are summarized in Table 13.2.

13.11 ROLE OF TRANSCRIPTION FACTOR NF-κB IN CHRONIC INFLAMMATION AND CANCER

The transcription factor nuclear factor-kappa B (NF-κB), discovered by David Baltimore in 1986, is present in the nucleus and binds the promoter of the immunoglobulin kappa chain in B cells. In mammalian cells, the NF-κB family of transcription factors is composed of homodimers and heterodimers derived from five distinct subunits, RelA (p65), c-Rel, RelB, p50 (NF-κB1), and p52 (NF-κB2). All family members share a highly conserved Rel homology domain (RHD; ~300 aa) responsible for DNA binding, dimerization domain, and interaction with IκBs, the intracellular inhibitor of NF-κB (Karin 2006b; Sethi and Tergaonkar 2009; Sethi et al. 2008a). In unstimulated cells, the majority of NF-κB complexes are kept predominantly cytoplasmic, and in an inactive form, by binding to a family of inhibitory proteins, the IκBs. Generally, the inactive NF-κB–IκBα complex is activated by phosphorylation on two conserved serine residues within the N-terminal domain of the IκB proteins. Phosphorylation of these conserved serine residues in response to stimulation leads to the immediate polyubiquitination of IκB proteins by the SCF-β–TrCP complex. This modification subsequently targets IκB proteins for rapid degradation by the 26S proteasome (Karin 2006a). Activation of the NF-κB signaling cascade results in complete degradation of IκB, allowing translocation of NF-κB to the nucleus, where it induces transcription (Vallabhapurapu and Karin 2009; Shen and Tergaonkar 2009; Ahn et al. 2007; Ahn and Aggarwal 2005). NF-κB is activated by many divergent stimuli, including proinflammatory cytokines (e.g., TNF-α, IL-1), T and B cell mitogens, bacteria, lipopolysaccharide (LPS), viruses, viral proteins, double-stranded RNA, and physical and chemical stresses. Activated NF-κB binds to specific DNA sequences in target genes, designated as κB elements, and regulates transcription of over 400 genes involved in inflammation, immunoregulation, tumor cell proliferation, invasion, metastasis, angiogenesis, chemoresistance, and radioresistance (Mantovani 2010; Wong and Tergaonkar 2009; Li and Sethi 2010). Mutations in the genes coding for the IKKα and IKKβ catalytic subunits, Karin and his colleagues (Luo et al. 2004; Maeda et al. 2005; Sil et al. 2004) examined the role of the NF-κB pathway in tumorigenesis. They found that mice lacking IKKβ only in hepatocytes or hematopoietic-derived Kupffer cells showed a marked increase in hepatocarcinogenesis induced by diethylnitrosamine (Maeda et al. 2005). Interestingly, although tumorigenic function of IKKβ was found to be mediated via NF-κB, the metastatic function of IKKα was found to be independent of NF-κB (Sil et al. 2004). Moreover, they showed that processes that occur within inflammatory cells are essential for cancer development and progression (Maeda et al. 2005). To determine the role of NF-κB in inflammation-induced tumor growth, Luo et al. (2004) used an experimental murine cancer metastasis model in which a colon adenocarcinoma cell line metastasizes to the lungs when stimulated by LPS. They found that endotoxin-induced metastatic growth response depends on TNF-α production by host hematopoietic cells, leading to NF-κB activation in tumor cells. Inhibition of NF-κB in the colon led to tumor regression. The latter depends on TRAIL receptor induction in NF-κB-deficient cancer cells. These findings support the role of the proinflammatory NF-κB pathway in tumor development.

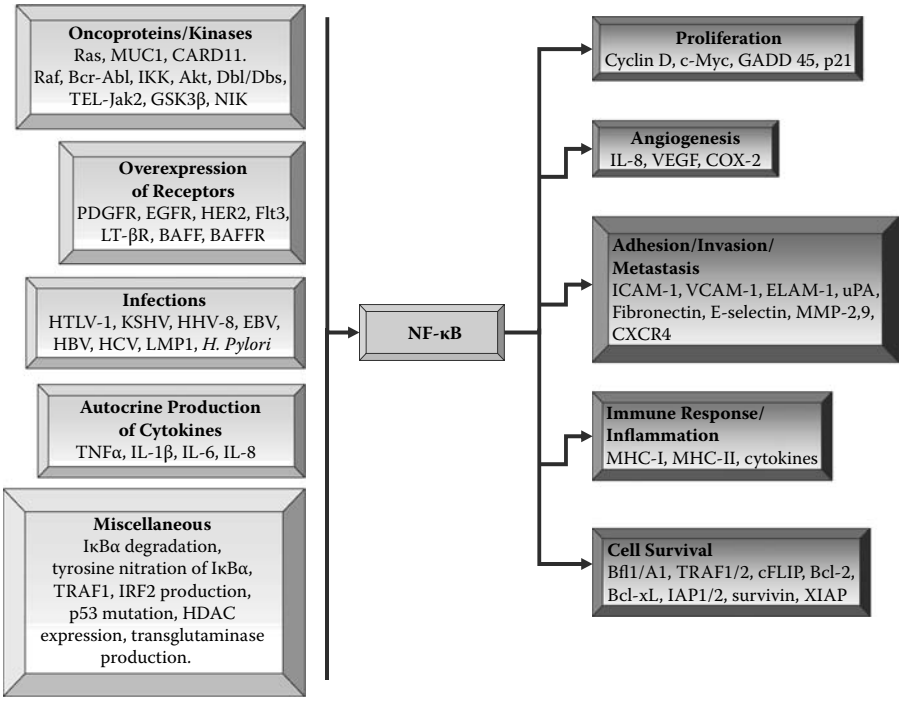

FIGURE 13.2 Mechanisms of constitutive activation of NF-κB in tumorigenesis.

Numerous studies have indicated that tumor cells exhibit an elevation in constitutive production of the proinflammatory cytokines TNF-α, IL-1α, IL-6, GM-CSF, and KC (Grivennikov et al. 2010; Baud and Karin 2009). Production of tumor-promoting cytokines by immune/inflammatory cells that activate NF-κB, along with other transcription factors such as AP-1 and STAT3 in premalignant cells to induce genes that stimulate cell proliferation and survival, is a major tumor-promoting mechanism (Grivennikov et al. 2010; Baud and Karin 2009). For instance, inhibition of TNF-α production by nonparenchymal cells (Kupffer and endothelial cells) prevented NF-κB activation in hepatocytes and early tumors and reduced tumor multiplicity (Pikarsky et al. 2004). Greten et al. (2004) reported that deleting IKKβ in myeloid cells caused suppression of NF-κB, activation, and diminished expression of inflammatory cytokines, thus leading to a significant decrease in tumor size. The host environment promotes the constitutive activation of NF-κB and proinflammatory cytokine expression during metastatic tumor progression of various cancers (Sethi et al. 2008a; Naugler and Karin 2008a; Karin 2008). What causes the constitutive activation of NF-κB in various tumor cells is not fully understood. Many different mechanisms have been described, including overexpression of growth factor receptors, mutation of IκBα such that it cannot bind to NF-κB, constitutive activation of Ras protein, high proteolytic activity directed to IκBα, and autocrine secretion of inflammatory cytokines (Sethi et al. 2008a) (Figure 13.2). In most of these tumor cells, constitutive activation of NF-κB is responsible for proliferation, because inhibition of NF-κB leads to

abrogation of proliferation (Bargou et al. 1997). Constitutive activation also has been linked to chemoresistance and radioresistance (Li and Sethi 2010).

Metastasis of cancer cells is a complex process involving multiple steps, including invasion, angiogenesis, trafficking of cancer cells through blood vessels, extravasations, organ-specific homing, and growth (Nguyen et al. 2009). Chemokines (such as SDF-1α and their receptors, such as CXCR4) and adhesion molecules (such as endothelial leukocyte adhesion molecule-1, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1) are thought to play a critical role in motility, homing, and proliferation of cancer cells at specific metastatic sites (Nguyen et al. 2009). NF-κB promotes breast cancer cell migration and metastasis by inducing the expression of the chemokine receptor CXCR4 (Helbig et al. 2003). NF-κB regulates the motility of breast cancer cells by directly upregulating the expression of CXCR4. The cell surface expression of CXCR4 and the SDF-1α-mediated migration are enhanced in breast cancer cells isolated from mammary fat pad xenografts compared with parental cells grown in culture (Helbig et al. 2003). Inhibition of NF-κB signaling also inhibits angiogenesis and tumorigenicity of human ovarian cancer cells by suppressing expression of vascular endothelial growth factor (VEGF) and IL-8 (Huang et al. 2000). Thus, the activation of NF-κB represents the central event in linking the process of chronic inflammation to different stages of carcinogenesis (Konturek et al. 2004).

13.12 ROLE OF TRANSCRIPTION FACTOR STAT3 IN CHRONIC INFLAMMATION AND CANCER

The transcription factor signal transducer and activator of transcription 3 (STAT3) was originally identified as a DNA binding protein that responds to stimulation by epidermal growth factor and IL-6, and has an important role in their signaling (Ihle 1996; Yu and Jove 2004). Upon activation, STAT3 undergoes phosphorylation-induced homodimerization, leading to nuclear translocation, DNA binding, and subsequent gene transcription (Bowman et al. 2000). The phosphorylation is mediated through the activation of nonreceptor protein tyrosine kinases called Janus-like kinase (JAK). JAK1, JAK2, JAK3, and TYK2 have been implicated in the activation of STAT3 (Yu and Jove 2004; Yu et al. 2009). In addition, the role of c-Src kinase has been shown in STAT3 phosphorylation (Ihle 1996). In normal cells, the duration of STAT3 activation is temporary, usually lasting from a few minutes to several hours (Yu and Jove 2004). In these cells, STAT3 plays crucial roles in the development of various organs and in cell proliferation (Yu and Jove 2004). In contrast, constitutive activation of STAT3 has been observed in many kinds of solid tumors and hematological malignancies (Aggarwal et al. 2009a, 2009c), and this persistently active STAT3 is thought to contribute to oncogenesis by modulating the expression of a variety of genes involved in cell proliferation, invasion, metastasis, and angiogenesis (Turkson and Jove 2000; Aggarwal et al. 2006a). Moreover, conditional STAT3 ablation in enterocytes, keratinocytes, and other epithelial cell types was found to inhibit tumor development and progression (Chan et al. 2004; Kataoka et al. 2008; Grivennikov et al. 2009; Bollrath et al. 2009).

Chronic inflammatory conditions that drive carcinogenesis can also be attributed to genetic alterations that directly affect the STAT3 pathway (Yu et al. 2009). The importance of constitutively active mutations in GP130, which encodes a subunit of the IL-6 receptor, has been demonstrated in human inflammatory HCC (Rebouissou et al. 2009). A critical role for STAT3 in inflammation-induced adenocarcinomas was also demonstrated using a transgenic mouse model with a constitutively active GP130 in epithelial cells (Li et al. 2007). Studies in mice with Gp130 mutations demonstrated that an increase in GP130 and STAT3 signaling led to inflammation-associated gastric tumorigenesis (Ernst et al. 2008). Several infectious agents also exert their tumorigenic effects through STAT3 activation and depend on STAT3 for their oncogenic potential (Yu et al. 2009). For instance, infection with *H. pylori*, which is associated with gastric cancer, activates STAT3 through its cytotoxin-associated gene A in host cells (Bronte-Tinkew et al. 2009). In addition, a critical role of STAT3 activation in mediating ultraviolet light-induced skin cancer in a transgenic mouse model and cigarette smoke-associated cancer development has also been demonstrated (Sano et al. 2005; Arredondo et al. 2006).

STAT3 can also act in close liaison with NF-κB to mediate various steps involved in initiation, promotion, and development of cancer (Grivennikov and Karin 2010a). Moreover, NF-κB and STAT3 control both distinct and overlapping groups of genes involved during tumorigenesis (Yu et al. 2009). Global profiling of STAT3-dependent genes in mouse lung cells revealed a large number of genes whose expression is controlled by STAT3, among which a number of typical NF-κB target genes are also present (Dauer et al. 2005). Furthermore, in a recent study it was demonstrated that obesity-promoted hepatocellular carcinoma development was dependent on enhanced production of the tumor-promoting cytokines IL-6 and TNF-α, which cause hepatic inflammation and activation of the STAT3 (Park et al. 2010). Thus, the STAT3 activation pathway is also an important contributor to inflammation-induced cancers, making it an attractive target for treating or preventing inflammation.

13.13 CONCLUSION AND PERSPECTIVES

Overall this chapter reemphasizes the critical link between chronic inflammation and cancer. It is becoming quite evident that chronic inflammation contributes to carcinogenesis at all three stages: initiation, proliferation, and progression. However, while most evidence discussed in this chapter suggests that proinflammatory cytokines, enzymes, oncogenes, and transcription factors play a major role in mediating tumorigenesis, existing literature also suggests that inhibition of proinflammatory pathways is not always beneficial. For example, inhibition of NF-κB has been reported to accelerate HCC development and enhance proliferation of tumor-initiating cells (He et al. 2010). Administration of TNF-α blockers to patients with rheumatoid arthritis has been found to increase the risk for developing lymphomas (Geborek et al. 2005). Moreover, genetic studies in patients with hyper-IgE syndrome identified dominant-negative STAT3 gene mutations as the probable cause of the disease in a few patients (Milner et al. 2008). Thus, elucidation of the underlying mechanisms will help to better understand the interaction between tumor cells and their inflammatory microenvironment, and consequently how to interfere and block

such pro-tumor biomarkers with minimum toxic effects. A number of synthetic and natural compounds specifically inhibiting the above-described inflammatory biomarkers are currently in preclinical and clinical studies. Further strategies that can interfere with the recruitment of bone marrow-derived cells or direct therapy at specific components of the tumor microenvironment may result in novel treatment regimens for inflammation-driven cancers in the near future.

ABBREVIATIONS

NF-κB, nuclear factor-κB; IκB, inhibitory subunit of NF-κB; IL, interleukin; MMP, matrix metalloproteinase, COX, cyclooxygenase, LOX, lipoxygenase; MAPK, mitogen-activated protein kinase; TNF-α, tumor necrosis factor; AML, acute myelogenous leukemia; CLL, chronic lymphocytic leukemia; ALL, acute lymphocytic anemia; EGFR, epidermal growth factor receptor; VCAM-1, vascular cell adhesion molecule-1; ICAM, intracellular adhesion molecule; ELAM, endothelial cell leukocyte adhesion molecule; MMP-2, matrix metalloproteinase; iNOS, inducible nitric oxide synthase; B-CLL, B cell chronic lymphocytic leukemia; HCL, hairy cell leukemia; VEGF, vascular endothelial growth factor; RCC, renal cell carcinoma; STAT3, signal transducer and activator of transcription 3; MUC1, mucin 1; CARD 11, caspase recruitment domain family 11; RAS, rat sarcoma viral oncogene; RAF, murine leukemia viral oncogene; BCR-ABL, break-point cluster-c-abl oncogene; IKK, IκB kinase enzyme complex; Dbl/Dbs, transforming protein isolated from diffuse B cell lymphoma; TEL-Jak2, telomere maintenance–Janus kinase 2; GSK, glycogen synthase kinase; NIK, NF-κB interacting kinase; PDGFR, platelet-derived growth factor receptor; EGFR, epidermal growth factor receptor; HER2, erythroblastic leukemia viral oncogene; FLT3, fms-related tyrosine kinase 3; LT-βR, lymphotoxin β receptor; BAFF, B cell activating factor belonging to the TNF family; BAFFR, B cell activating factor belonging to the TNF family receptor; HTLV-1, human T cell leukemia virus type 1; KSHV, Kaposi's sarcoma-associated herpes virus; HHV8, human herpes virus 8; EBV, Epstein Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; LMP1, latent membrane protein 1; vFLIP, viral FADD-like interleukin-1β-converting enzyme (FLICE)/caspase-8-inhibitory protein; TRAF, TNF receptor-associated factor; IRF, interferon regulatory factor; HDAC, histone deacetylase; GADD, growth arrest and DNA damage inducible; uPA, urokinase plasminogen activator; CXCR, chemokine (C-X-C motif) receptor; MHC, major histocompatibility complex; XIAP, X-linked inhibitor of apoptosis.

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14 Chronic Diseases Caused by Chronic Inflammation Require Chronic Treatment

The Anti-Inflammatory Lifestyle

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CONTENTS

"Let food be thy medicine, and medicine be thy food," or the famous American adage, "You are what you eat."

Hippocrates c. 460 BC–377 BC

14.1 INTRODUCTION

Every second an American has at least one chronic illness, and 60 million Americans suffer from multiple chronic conditions. According to some studies, despite advances in modern medicine, our health is worse today than ever before in our history. In the year 2000, the U.S. population was 276 million, and nearly half of the population—125 million Americans—lived with some type of chronic condition. According to Kenneth Thorpe, chairman of the Department of Health Policy and Management at Emory University, 75% of the country's $2.5 trillion in healthcare expenditures is spent on four increasingly prevalent chronic diseases: obesity, type 2 diabetes, heart disease, and cancer.[1] Most cases of these diseases are preventable because they are caused by behaviors such as poor diet, inadequate exercise, and smoking. Obesity alone threatens to overwhelm the system. A recent study found that if trends continue, annual healthcare costs related to obesity will total $344 billion by 2018, or more than 20% of total healthcare spending.[568] It is estimated that the current health/sickness care industry costs $1.5 trillion.

As the overall human life span is increasing in both developed and developing countries, so is the incidence of chronic diseases. For instance, in the United States persons aged 65 years and older have nearly 10 times the risk of developing cancer than persons under age 65 years (2,196 vs. 223 per 100,000 population).[2–4] Persons over 65 have 16 times higher risk of dying from cancer than those below 65 years of age. Recently it was reported that almost all cancers peak at age around 75, and centenarians are asymptomatic or untargeted by cancer.[5] It appears that almost all chronic diseases are the diseases of old age caused by lifestyle factors. These include cancer, hypertension, blood pressure, atherosclerosis, diabetes, obesity, Alzheimer's disease, Parkinson's disease, multiple sclerosis, depression, anxiety, chronic fatigue, osteoporosis, arthritis, cerebral ischemia, memory loss, loss of cognitive functions, and other autoimmune, neurological, pulmonary, and cardiovascular diseases. Several of these diseases are interlinked.[6] For instance, obesity is a major risk factor for a wide variety of diseases, including cancer, diabetes, and cardiovascular diseases.

According to a 2009 report from the Centers for Disease Control and Prevention (CDC), obesity in the United States continues to increase, and currently 72.5 million, or 26.7% of the population, is obese. If the numbers keep going up, more people will get sick and die from the complications of obesity, such as heart disease, stroke, diabetes, and cancer. The report estimates the medical cost of obesity to be as high as $147 billion a year. Similarly, over 20 million new cases of cancer are predicted in 2025 globally, compared with 12 million in 2008 (WHO). The cost of cancer in the United States alone is over $220 billion, and yet it is a preventable disease. The sale of anticancer drugs alone exceeded $50 billion worldwide in 2009. According to the CDC, as many as 74.5 million Americans have hypertension/high blood pressure; 3.6 million children have hypertension. Why are things going in the wrong

direction? The report indicated too little exercise and too much of the wrong kinds of food, which means not enough fruits and vegetables and too many high-calorie meals full of meat, sugar, and fat, like French fries, soda, and other sweet drinks. Below we discuss various lifestyle factors that lead to these chronic diseases, what their molecular bases are, and what kind of chronic treatment is needed to control these chronic diseases.

14.2 MOST CHRONIC DISEASES ARE LINKED TO CHRONIC INFLAMMATION

Historically, inflammation has been known by different names in different cultures. Ayurveda, a 6,000-year-old science of long life, describes inflammation as *Souz* and attributes it to dysregulation of three different functions called *doshas*. These include *pitta* (bile; energy principle that uses bile to direct digestion and hence metabolism into the venous system), *vata* (wind; impulse principle necessary to mobilize the function of the nervous system), and *kapha* (phlegm; body fluid principle, which relates to mucous, lubrication, and the carrier of nutrients into the arterial system). One of the oldest descriptions of inflammation is that by the Roman physician Cornelius Celsus in the first century CE, who indicated that inflammation consists of heat, pain, redness, and swelling (see Heidland et al.[7]). Rudolf Virchow from Würzburg, Germany, first linked most chronic diseases to inflammation in the nineteenth century. Extensive research within the past two decades, both observational and experimental, has made clear that most chronic diseases are preceded by chronic low-level inflammation that may last as long as two to three decades. Because most chronic diseases manifest at age 50 or later, chronic inflammation must begin between 20 and 30 years of age.[8]

At the molecular level, numerous biomarkers of inflammation have been identified, including transcription factors such as NF-κB and STAT3; inflammatory cytokines and chemokines such as TNF-α, IL-1, IL-6, IL-8, and MCP-1; proinflammatory enzymes such as COX-2, 5-LOX, 12-LOX, and MMPs; and other factors, such as prostate specific antigen (PSA), C-reactive protein (CRP), adhesion molecules, and angiogenesis markers such as vascular endothelial growth factor (VEGF), and the epithelial-mesenchymal transition-inducing factor (TWIST) (Figure 14.1).[9] Transcription factors such as NF-κB have been shown to regulate the expression of more than 500 different proteins, most of which have been linked to inflammation.[10] Most of these inflammatory biomarkers now have also been linked to various chronic diseases. For instance, whereas PSA is routinely used to monitor patients with prostate cancer, CRP is used to monitor patients with cardiovascular diseases. Similarly, levels of MCP-1, IL-6, and CRP have been linked with obesity.[11] As indicated by two recent studies, the requirement for NF-κB signaling pathway mediated through *KRAS* has also been implicated in lung adenocarcinoma.[12,13]

Numerous evidence indicates that cancer is a proinflammatory disease.[14–17] Although acute inflammation is an important component of the immune system, dysregulated chronic inflammation contributes to cancer and other diseases.[18] As shown in Table 14.1, the expression of various proinflammatory biomarkers has been found to be associated with various cancers and with the survival of cancer patients.

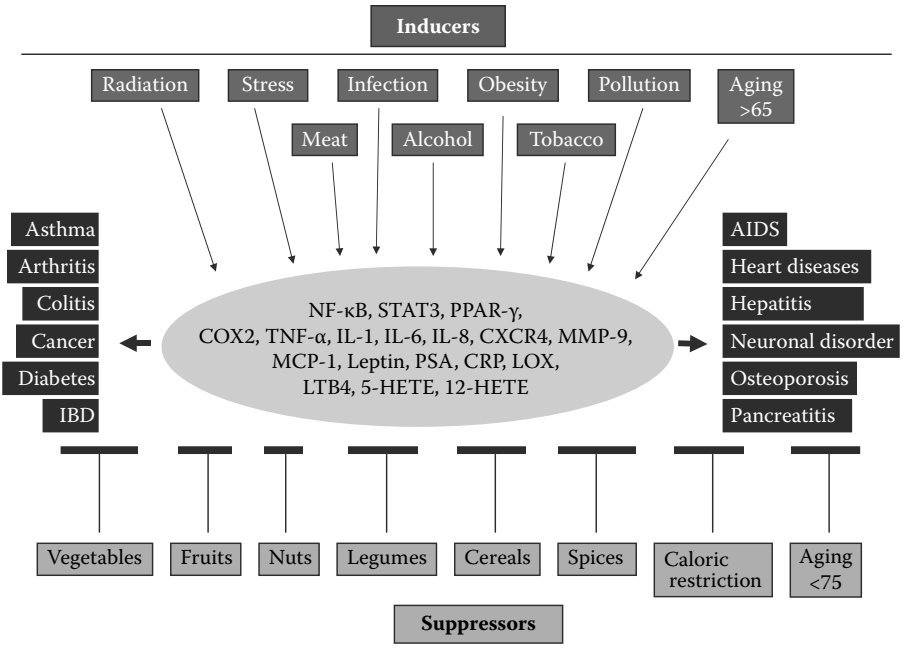


FIGURE 14.1 The link between lifestyle factors, inflammation, chronic diseases, and chronic treatment.

For instance, studies have shown that 82% of late-stage prostate cancers express p-STAT3, and this correlates with increased severity of the disease and shorter survival times.[19–21] The expression of p-STAT3 is due to increased expression of IL-6 in patients.[22,23] Another study showed that persistently activated STAT3 maintains constitutive NF-κB activity in tumors.[24] In addition, COX-2 overexpression has been closely linked to the development of colorectal cancer; thus, inhibitors of COX-2, such as Celebrex, have been approved for the treatment of patients with familial adenomatous polyposis, who are at high risk to develop colorectal cancer.

Studies have indicated that cancer patients experience various symptoms, including fatigue, neuropathic pain, lack of sleep, lack of appetite, depression, and cognitive slowing, and that chemotherapy further enhances these symptoms. Circulating inflammatory cytokine levels have also been linked to symptom severity in cancer patients.[24–26] For instance, it has been shown that overproduction of IL-6 in patients with multiple myeloma (MM) is associated with severity of the disease.[27] The role of cytokines in cancer-related fatigue has been demonstrated.[28] Cancer and symptoms of cancer share common mechanisms.[29,30] Activating the adrenergic and cholinergic pathway can downregulate inflammatory cytokine levels. For instance, ghrelin, a gastric hormone, can downregulate proinflammatory cytokines in sepsis through activation of the vagus nerve. A cholinergic agonist such as anabaseine and a nicotinic receptor antagonist (chlorisondamine diiodide) have been shown to inhibit TNF-α and NF-κB. Thalidomide therapy in patients with MM modulates NF-κB, IL-1, IL-2, and IFN-γ.[31,32] Fatigue associated with cancer and cancer treatment has

TABLE 14.1
Inflammatory Biomarkers in Patients with Various Cancers

Biomarker	Reference
Brain	
COX-2	
• Expression in tumor cells is associated with more aggressive gliomas (>50% of 66 patients) and is a strong predictor of poor survival	147
• Positivity in 44% of 34 tumors; median survival was 37 months for positive lesion and 93 months for negative lesion	148
• Expressed in 7 (43.8%) of 16 ependymoma specimens	149
• Expressed in 79 cases (95%) with an increased expression in grade IV vs. grades II and III; associated with shortened survival	150
NF-κB	
• Estimated median survival time was 354 weeks with anaplastic astrocytomas and 79 ± 10.8 weeks with glioblastomas; 1- and 2-year survival rates were 100 and 100% with anaplastic astrocytomas and 69.2 and 30.8% with glioblastomas	151
• Expressed in all tumor samples of 27 patients with gliomas, correlated with tumor grade	152
• NF-κB and pIκBα expression increased with tumor grade	153
IL-8	
• Highly secreted in the PBMCs of glioma patients; positively correlated with histological grade and tumor necrosis	154
IL-10	
• Selectively expressed within invasive gliomas vs. less malignant, localized glioma group; might have prognostic significance for immunotherapy	155
STAT3	
• p-STAT3 in patients (45) with GBM was 11.8 ± 13.5%, and no correlation between p-STAT3 levels in the tumor and percent of PBMCs displaying p-STAT3	156
Breast	
COX-2	
• Expressed in 118 of 205 (57.6%) patients; patients with tumors that coexpressed both COX-2 and c-erb-B2 had a sign DFS rate than those that did not (60.2 vs. 78.3%)	157
• Expressed in 60% of breast cancer patients	158
• Cytoplasmic COX-2 expressed in 66.9% carcinoma samples but no association between COX-2 expression and patient OS or DFS	159
• Overexpressed in 46.8% of surgical specimens (44 of 94); patients with COX-2-positive tumors had significantly shorter survival time (both DFS and OS) than those with negative tumors	160
• Expression was positive in 58% of 504 cases and is associated with younger age, larger tumor size, worse local control, distant metastasis, and worse overall survival	161
• Expressed in 14 of 54 cases (36%), positively associated with ITA expression but not predictive of survival	162
• Expressed in 49% of 57 patients, median DFS was 37 months, median OS was not reached; no significant difference between patients with normal and overexpression of COX-2	163

continued

TABLE 14.1 (Continued)
Inflammatory Biomarkers in Patients with Various Cancers

Biomarker	Reference
Breast (continued)	
NF-κB	
• Expressed in 55 of 82 (67.1%) cancer specimens, higher than the adjacent normal tissue but significant difference in tumor size, steroid receptors, stages, histological types, and node status	164
• Expressed in 16% of 82 samples; associated with a poor response to doxorubicin-based chemotherapy	165
STAT3	
• Cytoplasmic expression of STAT3 (69.2%) and p-STAT3 (19.6%) had no significant association with survival; nuclear expression of STAT3 (23.1%) had short-term survival at 5 years of follow-up, and p-STAT3	
• (43.5%) had both short-term 5-year survival and long-term 20-year survival	166
• Expressed in 38 of 76 cancers (50%)	167
Cervical	
COX-2	
• Expressed in 64.1% of 167 patients, significantly shorter DFS and cause-specific OS	168
• Expression (100 samples) in large tumor (90.9%) was higher than that in smaller tumor (86.2%)	169
• Expressed in stage IB cervical cancer (49.4% of 89 samples), in cervical adenocarcinoma (86.7%), SCC (40.6%), and lymph node metastasis (100%); no difference in 5-year DFS and OS in positive (81 and 98%) and negative (92 and 95%) expression of COX-2	170
• Expressed in 28 of 40 patients (70%); associated with PALN recurrence	171
• Expressed in 14 of 20 (70%) CSCC patients, associated with poor mid-RT tumor response	172
• Expressed in 60.9% of 23 patients, associated with poor response to treatment and cancer-related death	173
STAT3	
• p-STAT3 expressed significantly higher in CIN 3 (76.92%), in comparison with CIN 1 of 2 (13.33%) among 56 patients	174
• p-STAT3 expressed in 71 of 125 (56.8%) patients, indicating poor prognosis for OS and DFS	175
Colon	
COX-2	
• Expression was found in 48 of 98 (49.0%) cases	176
NF-κB	
• Activation significantly increased in the more progressed cases	177
• NF-κB-RelA was significantly overexpressed and contributes to tumor angiogenesis in CRC	178
• Positive in 46 patients (60%); median TTP and OS were 3 and 9.5 vs. 6.4 and 15.8 months for NF-κB positive vs. negative patients, respectively	179
• Activation was found in 64 of 202 cases (32%), favored the survival of patients	180
• Expressed in 72 of 98 (73.5%) cases	176
• Activation was observed in 40% of CRC tissues and involved in angiogenesis	181

TABLE 14.1 (Continued)
Inflammatory Biomarkers in Patients with Various Cancers

Biomarker	Reference
Colon (continued)	
STAT3	
• Elevated levels of p-STAT3 were correlated with the nodal metastasis and the stage	182
• p-STAT3 expressed in 77 of 139 samples (55.4%); related to carcinogenesis or tumor invasion of CRC	183
• p-STAT3 expressed in 40 of 90 (44.4%) primary CRC tissues; coexpression of nuclear p-STAT3 and beta-catenin was associated with lower patient survival	184
• p-STAT3 was expressed in 57.4% (62 of 108) of tumors and related to tumor invasion and poor prognosis of human colorectal adenocarcinoma	185
• p-STAT3 expression has an important role related to the tumorigenesis and tumor progression of CRAs	186
• CRC cells with high STAT3/p-STAT3 had greater percentage of Bcl2 reactivity (23.05%); STAT3 protects cancerous colorectal epithelial cells from apoptosis	187
Esophagus	
COX-2	
• Expressed significantly in the middle and lower esophagus than in the cervical and upper esophagus; no significant differences in patient prognosis	188
• Overexpression (49%, 47 of 96) was significantly correlated with fewer metastases and less advanced stage, but had no impact on survival	189
• Expressed in 30% of specimens of dysplastic mucosa, and 51.3% of the specimens of SCC; COX-1 expressed significantly higher in normal mucosa (41.9%) than in SCC (18.8%)	190
• Strong expression of COX-2 (42.8%, 12 of 28) is correlated with tumor progression and poor differentiation in ESCC	191
• Expressed significantly higher in well-differentiated tumors (16 of 23, 69.6%) than in moderately (13 of 34, 38.2%) and poorly (2 of 12, 16.7%) differentiated tumors, and correlated with tumor progression	192
• Expressed weakly in 73% and strongly in 27% of tumors; survival was significantly reduced among patients with high, compared with low, COX-2 expression (3 vs. 6 months MOS)	193
• Overexpressed in 82.3% of lymph node–negative and 54.8% of lymph node–positive patients; no correlation with survival during 3 years of follow-up	194
• Tumors with a strong COX-2 expression (14 of 41) were higher than tumors with a weak expression (3 of 14); survival was reduced in patients with strong COX-2 expression, compared to the weak group	195
• Low-level expression of COX-2 (70.7%, 41 of 58) was significantly higher than that of tumors with COX-2 overexpression (42.6%, 23 of 54); significantly associated with poor 3-year overall survival	196
• Expression was correlated with depth of invasion and tumor stage; 5-year survival rate of patients decreased with increased COX-2 expression	197
• Expression was significantly higher in Barrett's (60.0%) and ADC (66.6%), compared to that in GERD, SCC, and normal	198
NF-κB	
• Expressed in 40% of patients with Barrett's epithelium and 61% of resected tumors; 87.5% of NF-κB positive tumors were Stage IIb and III, compared with 12.5% of patients with stage I and IIa disease	199

continued

TABLE 14.1 (Continued)
Inflammatory Biomarkers in Patients with Various Cancers

Biomarker	Reference
Esophagus (continued)	
NF-κB	
• Expressed in 13% (2 of 15) reflux esophagitis, 60% (21 of 35) Barrett's esophagus, and 80% (28 of 35) esophageal adenocarcinoma patients	200
• Expressed in 38% (8 of 21) NF-κB patients who developed metastases, compared to 0 of 22 NF-κB negative patients; 10 of 21 (48%) NF-κB positive patients had 23 months MOS as well as DFS	201
• Nuclear NF-κB was associated with shortened DFS and OS in 123 patients, but also in stage II and III	202
Gall Bladder	
COX-2	
• Expressed in malignant epithelial cells in 38% (17 of 47) of specimens with high-grade urothelial carcinomas	203
• Expressed in mucosal hyperplasia of the gallbladder in 18 of 31 patients with AAPBD	204
• Overexpressed in 62 of 80 patients (78%), associated with disease progression and bladder cancer-specific survival at a median follow-up of 101 months	205
• Overexpressed in 80% of invasive gall bladder cancers, compared to in the tumor central areas	206
• Expression was significantly higher in grade 3 bladder TCC than in grades 1 and 2 bladder TCC; higher in schistosomal-associated TCC than in non-schistosomal-associated TCC	207
Head and Neck	
COX-2	
• Strong COX-2 expression in 3, intermediate expression in 69, weak expression in 24, and absent in 64 specimens; MSTs for the strong, intermediate, and null COX-2 expressors were 1.04, 5.50, and 8.54 years, respectively	208
IL-6	
• Serum IL-6 was significantly increased in patients compared to healthy individuals; correlated with the stage of tumor progression in HNSCC patients	209
NF-κB	
• Nuclear RelA and cytoplasmic pIκBα expression are associated with poorer prognosis in NSCLC patients	210
• Nuclear RelA and cytoplasmic pIcleα expression independently predict overall survival	211
STAT3	
• Nuclear p-STAT3 expressed in 54% of tumors, but no relationship was found between p-STAT3 and prognosis following surgical resection	212
Leukemia	
COX-2	
• Expressed in 76.32% (29 of 38) of CML-CP patients and 75.86% (22 of 29) of CLL patients; correlated with prognosis of CML-CP and CLL	213

TABLE 14.1 (Continued)
Inflammatory Biomarkers in Patients with Various Cancers

Biomarker	Reference
Leukemia (continued)	
NF-κB	
• Elevated in 14 of 30 (47%) cases of AML and in both cases of CML-BC; provides the cells with survival advantages *in vivo*	214
STAT3	
• Constitutive STAT3 was expressed in 28 of 63 (44%) patients; DFS was significantly shorter in patients with than in patients without constitutive STAT3 activity	215
Liver	
COX-2	
• Higher tumor cytosolic COX-2 levels were associated with worse patient survival	216
• COX-2 gene was expressed in 35 of 59 tumors and associated with a poorer disease-free survival rate	217
• High expression in well-differentiated HCC cases (80%) compared with advanced HCC tumors	218
NF-κB	
• NF-κB p50 and p52 subunits were expressed in tumor cell nuclei (40 and 48%)	219
Lung	
COX-2	
• Expressed in 70% of invasive adenocarcinoma, much greater in lymph node metastases than in primary tumors	220
• Expressed in 19 of 21 adenocarcinomas; more expression in well-differentiated adenocarcinomas than poorly differentiated ones	221
• Increased COX-2 expression in 72% (93 of 130) cases; elevated expression is correlated with shortened survival in patients with stage I disease	222
• Overexpressed in 80% of CCH, AAH, BAC, and I-Ad, but not related with clinicopathological factors or survival	223
• Among 160 patients, 3 had strong expression, 69 had intermediate, 24 had weak, and 64 had no detectable COX-2; MSTs for the strong, intermediate or weak, and null COX-2 expressors were 1.04, 5.50, and 8.54 years, respectively	209
IL-6/IL-1β	
• Patients with metastatic disease and tumor progression have increased levels, associated with a worse prognosis	224
STAT3	
• Nuclear p-STAT3 expressed in 54% of 176 NSCLC tumors; correlated with smaller tumors and limited smoking	212
• p-STAT-3 was expressed in 38% of 145 patients, higher in adenocarcinoma (46%) vs. squamous cell (27%); STAT-3 activation status does not provide prognostic information in resected disease	225
• p-STAT3 expressed in 51.2% of 162 samples; 41.7 months median DFS, and 80.2 months median OS	226
• 72% of high-risk women demonstrated STAT3, while 87% of high-risk men demonstrated STAT3	227

continued

TABLE 14.1 (Continued)
Inflammatory Biomarkers in Patients with Various Cancers

Biomarker	Reference
Lung (continued)	
TNF	
• Patients with tumor progression had an increase of cytokines serum levels, associated with worse prognosis	224
• High-risk patients, with the shortest recurrence-free survival, had increased activation of TNF (76%)	227
Lymphoma	
COX-2	
• Expression was found in 49 of 54 (80%) patients	228
NF-κB	
• Detected in 17 of 50 (34%) bone marrow specimens	229
TNF	
• TNF-α was detected in 3 of 43 patients (7%); no correlation with any other clinical parameter	230
Melanoma	
COX-2	
• Positive COX-2 expression of 13.3% (2 of 15) and negative expression of 86.7% (13 of 15) in patients who underwent enucleation after radiotherapy	231
IL-6	
• IL-6 level in the redness group (15 of 27) was significantly higher than in the group without redness (12 of 27); could be a sign of a better prognosis of the melanoma	232
IL-8	
• Detected in all 27 melanoma patients; could be a sign of a better prognosis of the melanoma	232
NF-κB	
• High intensity of NF-κB activation (73.9%; 17 of 23 tumors) correlated with low degree of differentiation of the tumors studied	233
STAT3	
• Relative balance of p-STAT1/p-STAT3 is associated with melanocyte differentiation, modulated by IFN-α	234
TNF	
• High expression of TNF-α in 7 of 21 patients treated with IFN-α in contrast to 13 of 16 in untreated group; patients with low staining score for TNF-α (7 of 13) showed a marked regression of tumors, compared with those with a high staining score (1 of 5)	235
Multiple Myeloma	
COX-2	
• 31 MM samples (54%) expressed COX-2 and correlates with shorter progression-free survival (17 vs. 30 months)	236
IL-1β	
• 49 of 51 patients with active myeloma and 7 of 7 patients with smoldering myeloma expressed IL-1β, and 95% of MM patients but less than 25% of MGUS patients are positive for IL-1β production	237

TABLE 14.1 (Continued)
Inflammatory Biomarkers in Patients with Various Cancers

Biomarker	Reference
Multiple Myeloma (continued)	
NF-κB	
• Expressed in approximately 20% of 155 patients	50
• Expressed the activated forms of NF-κB in all 22 MM patient samples, but to a variable degree	238
Ovary	
COX-2	
• Tumors with high COX-2 expression had a worse prognosis than tumors with low expression	239
• Expressed in a significantly higher percentage of not responding cases (11 of 13; 84.6%) than in patients responding to chemotherapy (9 of 26; 34.6%)	240
• High expression in 85% of carcinomas, had shorter overall survival, but not statistically significant	241
• Expression in EOC was 78.3%; associated with risk factors for recurrence, drug resistance, and poor prognosis	242
• Overexpressed in hyperplasia and endometrial adenocarcinoma cases (30 patients; 60%); not correlated with classical prognostic factors and had no impact on overall survival	243
• Overexpressed in 20% of the tumors (160 patients); MST for COX-2-negative tumors was 21.6 vs. 36 months for COX-2-positive tumors	244
• Expressed in 107 (34.0%) tumor cases; no relation with any histopathologic parameter or survival	245
• Patients with high COX-2-expressed tumors (40 of 44 specimens; 90.1%) had shorter overall survival, but not statistically significant	246
NF-κB	
• p65 expressed higher in epithelial ovarian cancer than in normal; correlated with late clinical stage and poor histological differentiation	247
• p53 expressed in 21 (6.7%) tumor cases; related to tumor grade and stage of disease, and it is an independent prognostic factor in EEC	245
• p65 is frequently expressed in advanced stage serous ovarian carcinoma (72 of 75 effusions; 96%), and its nuclear localization is associated with poor progression-free survival	248
STAT3	
• High nuclear expression of p-STAT3 (>10% of positive-stained cells) was linked with poor overall survival	249
• STAT3 expressed in stages III and IV (96.9%) was significantly higher than that in stages I and II (72.2%); p-STAT3 expression was correlated with disease stage, degree of differentiation, and lymph node metastasis, but negatively correlated with the prognosis of EOC patients	250
TNF	
• Expressed in 4 of 5 ascites and 7 of 16 tissue sections of patients	251
• TNF-α expressed more frequently in M-CSF-positive (52%, 11 of 21) than in M-CSF-negative tumors (11%, 1 of 9); expression was associated with better responses to chemotherapy	252
• Expressed higher in epithelial ovarian cancer patients than in benign ovarian diseases	253

continued

TABLE 14.1 (Continued)
Inflammatory Biomarkers in Patients with Various Cancers

Biomarker	Reference
Pancreas	
NF-κB	
• High cytoplasmic and nuclear expression of RelA/p65 in 42 and 37 carcinomas from 82 patients had negative prognostic impact with 2-year survival rates	254
• Sixteen tumors showed a strong expression for NF-κB among 44 patients	255
COX-2	
• COX-2 protein expression was found in 30 tumors among 44 patients	255
Prostate	
COX-2	
• COX-2 mRNA expression occurs in high-grade tumors (7 of 16 benign samples and 5 of 16 tumors)	256
IL-6	
• Higher serum levels of IL-6 in prostate cancer patients (23 of 46) with bone metastasis than in patients without bone metastasis (23 of 46); related to metastatic burden to osseous tissue but not bone resorption	257
LOX	
• 15-LOX-2 mRNA was detected in 21 of 25 benign samples	256
NF-κB	
• 10 of 17 patients positive for NF-κB had a poor outcome, whereas 2 of 13 patients had a good outcome; 11 of 13 specimens from good outcome were in the low-risk category, and 12 of 17 in the poor outcome group were in the high-risk category	257
STAT3	
• Constitutive STAT3 activation elevated in 82% (37 of 45) of tumors compared with nontumor prostate tissues	20
• Patients with 3+ immunostaining of p-STAT3 (41 of 93) had greater prostate-specific antigen failure rate than those with 2+ or less (<23); contributes to invasiveness and aggressiveness of prostate cancer	19
TNF	
• Increased serum levels of TNF-α in 85% of the 46 patients; related to metastatic burden to osseous tissue but not bone resorption	257
Sarcoma	
STAT3	
• 25 of 49 (51%) tumors were p-STAT3-positive; high-level STAT3 activation correlated with better OS	259
• Overexpression of p-STAT3 is associated with poor prognosis	260

TABLE 14.1 (Continued)
Inflammatory Biomarkers in Patients with Various Cancers

Note: DFS, disease-free survival; OS overall survival; ITA, intratumoral aromatase; SCC, squamous cell carcinoma; CSCC, cervical squamous cell carcinoma; EEC, endometrioid endometrial cancer; PALN, paraaortic lymph node; CIN, cervical intraepithelial neoplasia; CCH, cuboidal cell hyperplasia, AAH, atypical adenomatous hyperplasia, BAC, bronchioloalveolar carcinoma; I-Ad, invasive adenocarcinoma; AML, acute myeloid leukemia; CML-CP, chronic myeloid leukemia in chronic phase; CML-BC, chronic myeloid leukemia in blast crisis; GERD, gastroesophageal reflux disease; ADC, adenocarcinoma; ESCC, esophageal squamous cell carcinoma; MST, median survival time; NSCLC, non-small-cell lung cancer; PBMC, peripheral blood mononuclear cell; TTP, time to progression; CRA, colorectal adenocarcinoma; M-CSF, macrophage colony-stimulating factor; EOC, epithelial ovarian cancer; AAPBD, anomalous arrangement of the pancreaticobiliary duct; TCC, transitional cell carcinoma; CLL, chronic lymphocytic leukemia.

also been shown to be due to increased inflammation.[33] Pain, fatigue, disturbed sleep, lack of appetite, drowsiness, numbness, muscle weakness, and bone aches are commonly associated with treatment in patients with cancer such as MM. Lenalidomide, a 4-amino-glutamyl analogue of thalidomide, is associated with pain and fatigue.

Chronic inflammation has also been linked to the biological aging process.[34] For instance, it has been shown that genetic blockade of NF-κB in the skin of chronologically aged mice reversed the global gene expression program and tissue characteristics to those of young mice, demonstrating that disruption of a single gene is sufficient to reverse features of aging, at least for the short term.[35] Sarcopenia, or muscle loss with aging, is driven by a smoldering inflammatory state induced by elevated IL-6 and CRP levels, and intervention by nutritional supplements, exercise, caloric restriction, anabolic hormones, anti-inflammatory agents, and antioxidants has been proposed.[36]

Inflammation has also been shown to mediate cardiovascular diseases (CVDs).[37,38] Dehghan et al. reported that CRP was better than cholesterol as a biomarker of systemic inflammation in CVD.[39] The CVD is also connected with aging. CVD affects 15% of adults in their late 30s and early 40s, 50% of 55- to 64-year-olds, and 65% of those 65 and older. Strong evidence has emerged that inflammation and stress are closely linked to diabetes, another chronic disease.[40] Chronic inflammation has also been shown to mediate overweight and obesity.[41] Park et al. showed that, whether diet induced or genetic, obesity leads to a chronic inflammatory response that increases the risk for cancers.[42]

14.3 MOST CHRONIC DISEASES ARE COMPLEX AND ARE REGULATED BY MULTIPLE GENES

It is estimated that the human body is made up of around 13 trillion cells, and that most of these turn over within 100 days. In addition, cancer stem cells circulate in the body; when these cells appear in the circulation is not understood. According to some studies, most human subjects carry tumors, even if these remain dormant for

the person's entire life. Only in some individuals do these dormant tumors become malignant, and what transforms a dormant cancer into a malignancy is not understood. Angiogenesis is thought to be one of the switches. However, lifestyle factors must turn on or off angiogenesis specific to the tumor cells.[43] Angiogenesis is also critical for the metastasis of cancer to vital organs, and as many as 90% of patients who die from cancer do so owing to metastasis of the tumor. Inhibition of angiogenesis, while designed to prevent metastasis of cancer, has been found to reduce the delivery of anticancer drugs to all parts of the tumor.

There are around 25,431 human genes, 2,995 of which have been linked with 153 different pathways. About 350 genes have been linked with any given cancer. The progression of a normal cell to a cancer cell requires dozens of mutations; thus, targeting a few gene products is likely to be ineffective for treating cancer.[44–46] For example, up to 12 different pathways can be involved in the survival of a single cancer type.[42] Most biological processes have alternate pathways, and redundancy cannot be bypassed by single, highly targeted agents, such as COX-2 inhibitors. As the signaling pathways that control cellular proliferation and death are being revealed, a large number of targets have emerged as candidates for cancer therapy. For their survival, cancer cells depend on numerous highly activated pathways; inhibition of these pathways has a strong apoptotic effect and can lead to tumor regression. But drugs that exploit this weakness, such as imatinib, have not cured patients: withdrawal of the drug leads to disease recurrence, and sustained treatment leads to the emergence of drug-resistant clones. Thus, it appears that cancer cannot be cured, but has to be controlled as a chronic disease.[47] Some of these genes and their products play a vital role in cellular transformation, the survival and proliferation of a tumor. Several genes connected with the upregulation of inflammation have been identified in most tumors (Table 14.1). A recent genome-wide analysis of basal-like breast cancer has indicated that secondary tumors may arise from a minority of cells within the primary tumor.[48]

Activation of NF-κB, the proinflammatory transcription factor, has been noted in many tumor types, but it has been linked to an underlying genetic mutation.[49] An integrated analysis of high-density oligonucleotide array CGH and gene expression profiling data from 155 MM samples identified a wide array of abnormalities contributing to the dysregulation of NF-κB in approximately 20% of patients. Keats et al. reported that promiscuous mutations activate the noncanonical NF-κB pathway in MM. They found mutations in 10 genes causing the inactivation of *TRAF2*, *TRAF3*, *CYLD*, and *cIAP1/cIAP2*, and activation of *NFKB1*, *NFKB2*, *CD40*, *LTBR*, *TACI*, and *NIK* that result primarily in constitutive activation of the noncanonical NF-κB pathway, with the single most common abnormality being inactivation of *TRAF3*. These results show the importance of the NF-κB pathway in the pathogenesis of MM.[50] Boehm et al. identified *IKBKE*, another proinflammatory gene, as a breast cancer oncogene through integrative genomic approaches.[51] Whole-genome structural analyses revealed that one of these kinases, IKBKE (IKKε), is amplified and overexpressed in breast cancer cell lines and patient-derived tumors. Suppression of IKKε expression in breast cancer cell lines that harbor IKBKE amplifications induces cell death. IKKε activates the NF-κB pathway in breast cancers.[51]

When examined for the landscape of somatic copy-number alteration across human cancers, Beroukhim et al.[52] found a powerful way to discover key genes with causal

roles in oncogenesis through delineation of genomic regions that undergo frequent alteration in human cancers. They did high-resolution analyses of somatic copy-number alterations (SCNAs) from 3,131 cancer specimens belonging to 26 histological types and identified 158 regions of focal SCNAs that are altered at significant frequency across several cancer types, including the BCL2 family of apoptosis regulators and the NF-κB pathway. Bignell et al. examined the signatures of mutation and selection in the cancer genome.[53] They identified 2,428 somatic homozygous deletions in 746 cancer cell lines. Overall, these reports suggest that cancer is due to dysregulation of multiple genes, and that several of these genes activate proinflammatory pathways.

14.4 MOST CHRONIC DISEASES ARE DISEASES OF LIFESTYLE

It is now clear that most chronic diseases are caused by lifestyle factors, such as stress, tobacco, diet, alcohol, infections, environmental pollution, and radiation.[54] For instance, different kinds of stress, whether mechanical, physical, chemical, or psychological, have been associated with cancer. Stress can alter immunological, neurological, and endocrine functions and promote cancer progression.[55] Thaker et al. recently showed that chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma.[56] Moreover, stress hormones such as norepinephrine can increase the expression of IL-6 in cancer cells through Src tyrosine kinase activation, leading to tumor progression.[57] Another recent report indicated that environmental enrichment can suppress tumor growth in mice by stimulating the hypothalamus to produce brain-derived neurotrophic factor, which acts on the sympathetic nervous system to reduce leptin production in white fat tissue.[58] Cao et al. described the mechanism by which the environment can influence tumor proliferation and progression.[59] In addition, socially isolated rats have shown a significantly increased mammary tumor burden.[60] Chronic stress increases adrenal glucocorticoid secretion and tumor progression.[61] This is consistent with a report that social isolation of humans leads to increased mortality.[62] At the molecular level, glucocorticoids and noradrenalin are potential candidates for the connection of stress to cancer.[63] Interestingly, the same pathway controls depression, which is common among cancer patients and is associated with decreased survival. Depression is also associated with social withdrawal and decreased physical activity.[64] Thus, beta-adrenergic receptor blockers are being explored as a therapeutic intervention for the treatment of cancer.[65]

Almost one-third of all cancers in the United States have been linked to tobacco. Tobacco is used by more than 1.3 billion people worldwide.[66] Its use kills 5 million people annually, and the World Health Organization anticipates this to increase to 8 million by 2030. Tobacco is the single biggest cause of cancer globally.[67] Cancers linked to tobacco include esophageal cancer, pancreatic cancer, lung cancer, colon cancer, and others. Tobacco use is also a risk factor for chronic obstructive pulmonary disease, obesity, diabetes, and cardiovascular diseases. Tobacco-mediated addiction and its role in neurological diseases are well recognized.

Another inflammatory lifestyle factor, excessive alcohol consumption, can lead to pancreatitis, hepatitis, and colitis. It is one of the major risk factors for liver cancer, esophageal cancer, and pancreatic cancer,[68] and has also been associated with various neurological diseases, obesity, and diabetes. In addition, radiation exposure is a

well-known risk factor for various skin diseases, including cancer. Environmental pollutants such as diesel, cigarette smoke, and heavy metals are also major risk factors for a number of chronic diseases. In addition, infection by viruses, bacteria, fungi, and yeast can be major risk for certain chronic diseases, particularly human papilloma virus (HPV), human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), *Helicobacter pylori*, and *Schistosoma*, among others. For instance, as many as 5 million Americans are infected with HBV or HCV. Hepatitis attacks the liver and causes cancer, cirrhosis, and other liver problems. The hepatitis virus remains dormant for years with no symptoms. As many as 65% of HBV-infected and 75% of HCV-infected carriers do not know they have the virus. These viruses are spread through bodily fluids through routes such as transfusion, unprotected sex, and injection drug use. The HBV vaccine given to most children in the United States gives lifelong protection, but no vaccine for HCV exists yet.

14.5 CERTAIN LIFESTYLE FACTORS CAN ACTIVATE INFLAMMATION

The lifestyle factors mentioned above, including stress, tobacco, diet, alcohol, infections, environmental pollution, and radiation, have been shown to activate inflammatory pathways. These inflammatory processes have been implicated in the pathogenesis of both depression and cancer, and a relationship between the proinflammatory cytokine IL-6, cortisol, and vegetative depression has been reported in ovarian cancer patients.[69] In addition, some of the evidence that chronic diseases are regulated by lifestyle comes from migration studies. For instance, if a man of Chinese origin remains in China, his chances of being diagnosed with prostate cancer is 2 in 100,000; if he migrates to the United States within the first 5 years of life, his chances increase to 23 in 100,000, compared with 37 in 100,000 for American-born Chinese and 58 in 100,000 for white Americans.[70] These observations implicate the role of lifestyle in chronic diseases such as cancer.

14.6 MULTITARGETING IS NEEDED

Human protein–protein interactions and functional relations from publicly available databases contain close to 250,000 interactions among 14,503 unique gene products.[71] Thus, multitargeting or promiscuity is becoming a virtue in drug development.[72] Dietary agents are highly multitargeted and ultimately suppress inflammation. For instance, epigenetic changes such as histone modification, which plays a major role in the expression of inflammatory biomarkers, are modulated by dietary components.[73] Autophagy, which plays a major role in cancer, is also modulated by dietary agents.[74] Tumor cell survival, proliferation, invasion, angiogenesis, and metastasis of cancer cells have been found to be modulated by dietary agents.[43,75–77]

14.7 PLANT-BASED VERSUS ANIMAL-BASED DIET

It has been suggested that plants may provide important combination therapies that can simultaneously affect multiple pharmacological targets and provide clinical efficacy beyond the reach of single compound–based drugs.[78] Historically, the

prevention of beriberi by eating unpolished rice, cure of scurvy by eating citrus fruits, and support of fertility by eating leafy vegetables were shown to be related to factors that in 1912 came to be called vitamins (*vita* means "life"). For example, Herbert M. Evans and Katherine S. Bishop found that laboratory rats failed to reproduce when lard was their only source of dietary fat. The researchers found a compound in both wheat germ and lettuce that corrected the problem, and this compound was initially called fertility factor, then later renamed vitamin E or tocopherol. This illustrates the importance of including plant-based foods in one's diet. It is now clear that various vitamins from plants can decrease the risk of various chronic diseases, including cancer. For instance, vitamin D as a food supplement has conclusively been shown to decrease the risk of numerous cancers.[79,80]

Diet can be either plant or animal based. These two diets differ not only in calories, but also in sources of health-promoting components. Doll and Peto, in 1981, were among the first to identify and quantitate the causes of cancer and to point to dietary factors as a major contributor to disease risk. A recent international report from the American Institute of Cancer Research and World Cancer Research Fund made eight major recommendations to reduce cancer through dietary control. In addition to weight control and exercise, the report suggests eating mostly foods of plant origin while limiting the intake of red meats and alcohol and avoiding processed meat. Numerous studies, both observational and experimental, have indicated that meat promotes chronic diseases, including cancer. Various studies have suggested that meat consumption by humans is linked to various cancers, as outlined in Table 14.2. These include breast cancer, colorectal cancer, pancreatic cancer, bladder cancer, oral cancer, esophageal cancer, lung cancer, and others. For instance Wu et al. recently reported that people who frequently eat meat, especially if it is well done or cooked at high temperatures that generate heterocyclic amines, may have a greater chance of developing bladder cancer (personal communication). The evidence that meat-derived components such as 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) and other heterocyclic aromatic amines (HAAs) can induce tumors is also supported by various experimental animal studies (see Nowell et al.[81]).

In contrast, a plant-based diet has been linked with suppression of cancer and various other chronic diseases (see Table 14.3). A plant-based diet can include almost 95 different types of vegetables, 215 types of fruits, 108 types of spices, 30 types of pulses, 11 types of nuts, and 11 types of cereals (see Figure 14.2). Thousands of different nutraceuticals or phytochemicals have been identified in edible plants. These more than 8,000 compounds include folate, chlorophyll, carotenoids, steroids, saponins, alkaloids, flavonoids, isothiocyanates (sulfur-containing glycosides), sulforaphane, triterpenes (structurally related to steroids), and chalcones (see Table 14.4). More than 5,000 different flavonoids have been described in six major subclasses, including flavones (e.g., apigenin and luteolin), flavonols (e.g., quercetin and myricetin), flavanones (e.g., naringenin and hesperidin), catechins (e.g., epicatechin and gallocatechin), anthocyanidins (e.g., cyanidine and pelargonidin), and isoflavones (e.g., genistein and daidezin).[10,78] The bioavailability, metabolism, and safety of these flavonoids, once represented as vitamin P, was described by Szent-Györgyi in 1936.[82,83]

TABLE 14.2
Effect of Meat Consumption on Various Types of Cancer

Cancer Type	Reference
Breast Cancer	
Risk factor is higher for women who had a main meal with meat 5 times/week than those who had such meal 2 times/week	261
Increase in the relative risk (1.87) of breast cancer in women in the upper quintile of meat consumption, compared with the lowest quintile	262
An increased risk was observed for red meat intake (OR 4.2, 95% CL 2.3–7.7) for consumption in the upper quartile	263
Red meat, total meat, beef, and fried meat appear to be strong risk factors in human breast carcinogenesis	264
Women who consumed hamburger, beef steak, and bacon consistently very well done had a 4.62 times higher risk than those who consumed such meat rare or medium done	265
Positive association of breast cancer with the NAT1*11 allele was more evident in those who consumed a high level of red meat or consistently consumed well done red meat	266
Consumption of well-done meat was associated with eightfold elevated breast cancer risk compared with those consuming rare or medium-done meats	267
Elevated risk with increasing doneness level of red meat intake among women with the Arg/Arg or the Arg/His genotype	268
Red meat intake was strongly related to elevated risk of estrogen and progesterone receptor-positive breast cancers	269
Consumption of grilled or barbecued and smoked meats over the life course increases risk of postmenopausal BC	270
Both pre- and postmenopausal women who consumed the total, processed, and red meat had the highest risk of BC	271
NAT 1 and 2 modify the association between meat consumption and risk of BC in genetically susceptible women	272
Higher red meat intake in adolescence may increase the risk of premenopausal breast cancer	273
Red meat, MeIQx, and dietary iron elevated the risk of invasive breast cancer	274
Processed meat, not total, and red meat intakes were associated with a possible increased risk of breast cancer	275
No Effect	
Consumption of meats and other concentrated sources of HAs is not associated with increased breast cancer risk	276
No significant associations between meat intake or cooking method of meat and breast cancer risk	277
No association between NAT2 and breast cancer, and no significant associations with red meat for any doneness	278
No significant association between intake of total meat, red meat, or white meat and breast cancer risk	279
Intake of meat or fish during mid-life and later was not associated with risk of breast cancer	280
No relation between any type of meat consumption (total meat, processed meat, fresh meat, red and white meat) and breast cancer risk; GSTM1 null genotype increases breast cancer risk	281
No significant association between meat, meat cooking methods, and meat mutagen intake and postmenopausal BC in the NIH-AARP diet	282

TABLE 14.2 (Continued)
Effect of Meat Consumption on Various Types of Cancer

Cancer Type	Reference
Breast Cancer (continued)	
No Effect	
No association between red meat intake and overall BC risk, but fried meat intake may increase the risk of ER+/PR-BC	283
No statistically significant associations with BC for individual HCAs or for total estimated mutagenic activity of meat	284
No consistent association between breast cancer risk and the consumption of meat, egg, and dairy product	285
Higher intake of HCAs or MDM was not associated with elevated risk of postmenopausal breast cancer	286
Colorectal	
The ratio of the intake of red meat to that of chicken and fish was strongly associated with an increased incidence of colon cancer	287
Intakes of red meat were weakly positively associated with risk in both males and females	288
Consumption of beef, pork, or lamb as a main dish 5 or more times/week had a relative risk of 3.57 compared to those eating less than 1/month in men	289
Processed meats, not total fresh meat, beef, pork, minced meat, chicken, and fish, were associated with an increased risk in men and women	290
Increased endogenous formation of N-nitroso compounds in colon had positive association between red meat and CC	291
Prevalence of distal colorectal adenomas is associated with the type of meat preparation and frequency of intake	292
Stronger association of red meat intake with cancer risk among NAT rapid acetylators, among men 60 years old or older	293
Consistent trend toward higher risks for cancer with higher intakes of meat in rapid acetylators for NAT1 and 2 genotypes	294
Increased risk of colorectal adenomas with higher intake of well-done/very well-done red meat, and grilled meat	295
Positive association between the risk of colorectal polyps and beef consumption and an increase in the ratio of consumption of the red meat to that of fish/chicken	296
Exposure to carcinogens (presumably HAAs) through consumption of well-done meat increases the risk of CRC	297
Heterocyclic amines produced from meats cooked well done by high temperature elevated the risk of colorectal adenomas	298
Fried, baked, or broiled meat intake of ≥2 servings/week (high), compared with ≤1 serving/week showed twofold increase in risk of adenoma	299
Preference for well-done red meat was associated with a 8.8-fold increased risk of CRC among ever-smokers	300
High intake of processed red meat moderately but significantly increases colorectal cancer risk	301
Higher exposure to HCAs and increased consumption of red meats cooked well done or very well done increased the risk of cancer	302

continued

TABLE 14.2 (Continued)
Effect of Meat Consumption on Various Types of Cancer

Cancer Type	Reference
Colorectal (continued)	
Red meat intake increased colorectal cancer risk among men, whereas poultry and fish decreased risk among women	303
Consumption of red meat and specific preparation methods increased the risk of adenomas in sporadic group, not in HNPCC	304
Strong association between colon cancer and pan-fried, well-done/very well-done meat intake and levels of heterocyclic amines	305
Endogenous N-nitrosation may account for the increased risk associated with red meat consumption in colorectal cancer	306
Increased risk of CRC was found for those consuming relatively large amounts of cold cuts and sausages and bovine viscera	307
Consumption of fresh red meat and processed meat is associated with an increased risk of rectal cancer	308
HCA that forms when meat is cooked, is associated in rectal cancer risk	309
Increased risk of CRC is related to cooking temperature and close contact of the food to the heating source	310
Overall meat and red meat consumption increased risk of CRC, while weak evidence of interaction is found between red meat intake and GSTT1 GSTP1 and NQO1 phenotypes	311
Red meat increases the risk of colorectal cancer, particularly among genetically susceptible individuals	312
Prolonged high consumption of red and processed meat increases the risk of cancer in the distal portion of the large intestine	313
Ten grams of barbecued red meat (a major source of BP) per day was associated with a 29% increased risk of large adenoma	314
Interactions between moderate-high meat consumption and the CD36 gene A52C polymorphism increase the risk of cancer	315
Significant positive association between red meat consumption and risk of distal colon cancer	316
Several types of meat contribute differently to the etiology of colon and rectal cancer, depending on APC mutation status	317
Risk increased among men with the CYP1A1 *1 genotype and high white meat mutagen index, and among women with the CYP1A1 *2 genotype	318
Colorectal cancer risk was positively associated with high intake of red and processed meat	319
Well-done red meat and greater intake of bacon and sausage were associated with increased risk of colorectal adenoma	320
High meat consumption and the Pro/Pro + C/C genotype had a stronger increased than low meat intake and the same genotype	321
High consumption of red meat and processed meat is associated with an increased risk of colorectal cancer	322
Frequent consumption of red meat significantly increased colorectal cancer risk in all NAT2 fast acetylators	323
High consumption of processed meat increased the risk in comparison with low consumption	324
Recurrence of advanced lesions significantly associated with the highest tertile of intake for pan-fried and well-done/very well-done red meat; recurrence of multiple adenomas is associated with processed, pan-fried, and well-done/very well-done red meat	325

TABLE 14.2 (Continued)
Effect of Meat Consumption on Various Types of Cancer

Cancer Type	Reference
Colorectal (continued)	
A higher ratio of total meat to total fruit, berry, and vegetable intake was positively associated with both high- and low-risk adenomas	326
High intake of total meat or red meat and meat carcinogens is associated with hyperplastic polyps	327
Dietary red meat causes greater levels of colonic DNA single-strand and double-strand breaks than white meat	328
Nitrite and nitrate intake from processed meat intake increases the risk of colorectal adenoma	329
Familial CRC cases in the highest meat consumption quartile have decreased overall survival and increased risk of death	330
Well-done red meat intake was associated with an increased risk of CRC regardless of carcinogen-metabolizing genotype	331
Carriers of the variant allele of MDR1 intron 3 were at 1.52-fold higher risk of CRC than homozygous wild type allele carriers	332
Increased risk of CRC for intake of ≥3 servings per week of red meat or high-temperature cooked red meat	333
CRC risk was significantly increased in rapid NAT-1 carriers with high white meat consumption, compared to those carrying the slow NAT-1 genotype with low white meat consumption	334
Hazard ratios comparing the fifth to first quintiles for both red and processed meat intakes indicated an elevated risk for CRC	306
Pickled red meat consumption increased the risk of CRC in a dose-response manner	335
No Effect	
No association between consumption of well-done or medium-cooked beef and colorectal cancer	336
Less significant association between frequency and type of meat consumption and colon cancer risk	337
No evidence of an association between either meat or fat and colorectal cancer incidence	338
Meat consumption is not a risk factor for colorectal cancer	339, 340
No association between red meat consumption or how well red meat was cooked and colorectal carcinogenesis	341
No significant associations between tobacco smoking, consumption of red, processed, and fried meat, and CRC risk	342
No significant associations were observed for intakes of red meat, processed meat, or meat doneness preference	343
Little evidence of association between consumption of red and processed meat and colorectal cancer risk	344
Oral and Esophageal Cancer	
Heavy consumption of salted meat was associated with a significant (4.7%) increased risk of oropharyngeal cancer	345
High intake of salted meat and lamb, and polyunsaturated fat from meat increased the risk of esophageal cancer	346, 347

continued

TABLE 14.2 (Continued)
Effect of Meat Consumption on Various Types of Cancer

Cancer Type	Reference
Intestinal Cancer	
No clear associations for red or processed meat intake and either adenocarcinoma or carcinoid tumors of the small intestine	348
Stomach Cancer	
Barbecuing/grilling and high intake of red meat were associated with increased risks	349
Several types of cured meat were weakly positively associated with stomach cancer risk	350
Total, red, and processed meat intakes increased the risk of gastric noncardia cancer, in *H. pylori* antibody–positive subjects but not with cardia gastric cancer	351
High consumption of processed meat, but not of other meats, significantly increased twofold risk of stomach cancer among women in the top quintile of N-nitrosodimethylamine intake than those in the bottom quintile	352
Increased consumption of processed meat is associated with an increased risk of stomach cancer	353
No Effect	
Intake of fried, barbecued, and salted meat was not associated with risk of gastric cancer	354
Lung Cancer	
A significant increase in risk of lung cancer is associated with red meat, beef, and fried meat	355
Risk increased for total meat consumption, red meat, well-done, and fried red meat	356
Inhalation of carcinogens generated during frying of meat increases the risk of lung cancer	357
Consumption of red meat was associated with an increased risk of lung cancer	358
Exposure to meat aerosols is associated with lung cancer apparent in the highest tertile of exposure	359
The highest vs. the lowest quartile of intake of total meat, red, and processed meat increased the risk of lung cancer	360
Red meat, processed meat, and meat mutagens were independently associated with increased risk of lung cancer	361
High intake of red meat increased risk of lung carcinoma in both men and women, and high intake of processed meat increased the risk only in men	362
No Effect	
Consumption of meat reduces the risk of lung cancer	363
No meat type, cooking method, doneness level, or intake of specific meat mutagens or heme iron is associated with lung cancer risk	364
Laryngeal Cancer	
Consumption of salted meat and fresh meat (beef) increased the risk of laryngeal cancer	354
Red and total meat intakes were associated with strong increases in risk of laryngeal cancer	365
Showed strong direct trends in risk between consumption of processed meat and the various neoplasms	366
Pancreatic Cancer	
Grilled red meat intake is a risk factor for pancreatic cancer	367
Fifth quintile of daily intake of processed meat had a 68% increased risk compared with those in the lowest quintile; intakes of pork and total red meat were both associated with 50% increases in risk	368

TABLE 14.2 (Continued)
Effect of Meat Consumption on Various Types of Cancer

Cancer Type	Reference
Pancreatic Cancer (continued)	
Long-term red meat consumption was positively associated with risk of pancreatic cancer	369
Total, red, and high-temperature cooked meat intakes were positively associated with pancreatic cancer in men only	370
No Effect	
Meat intake is not related to pancreatic cancer risk	371
Intake of fresh meat or other types of meat is not associated with risk of pancreatic cancer	372
Brain Tumor	
Eating hot dogs 1 or >1 times/week was associated with brain tumors in children; the combination of no vitamins and eating meats was associated more strongly with brain cancer than either of them alone	373
Prostate Cancer	
Meat doneness was inconsistently associated with PC risk, but associated with increased risk for well-done beefsteak	374
Only very well-done meat was positively associated with prostate cancer risk	375
High consumption of cooked processed meats contributes to prostate cancer risk only among black men in the United States	376
Consumption of processed meat, but not total meat or red meat had a possible increased risk of prostate cancer	377
Grilled red meat consumption was significantly associated with higher adduct levels in tumor cells	378
Intake of well or very well-done total meat increased 1.26-fold the risk of incident prostate cancer	379
Grilled/barbecued red and processed meat is positively associated with prostate cancer	380
No Effect	
Intake of various meats, and fats from meat, had no association with overall or nonlocalized or high-grade PC	381
Intakes of processed and unprocessed red meat were not associated with prostate cancer recurrence or progression	382
Ovarian Cancer	
Women with the highest intake of processed meat had a significantly increased risk of ovarian cancer	383
Bladder Cancer	
Men and women with a high intake of bacon (≥5 servings/week) had an elevated risk of BC than those who never ate it	384
Bladder cancer risk had significant relationship with meat consumption among subjects with the rapid NAT2 genotype	385
No Effect	
No association between the intake of total or any specific type of meat and the risk of bladder cancer	386

continued

TABLE 14.2 (Continued)
Effect of Meat Consumption on Various Types of Cancer

Cancer Type	Reference
Renal Cell Cancer	
Red meat, barbecued meat, protein, and heterocyclic amine intakes were associated with significant increases in risk of RCC	387
A 20–22% higher risk of RCC among those in the highest relative to the lowest category of processed meat consumption	388
No Effect	
Intakes of red meat, processed meat, poultry, and seafood are not associated with risk of renal cell cancer	389
Red or processed meat intake is not associated with kidney cancer	390

Note: NAT, N-acetyltransferase; HCA, heterocyclic amine; HAA, heterocyclic aromatic amine; GSTM1, glutathione S-transferase M1; MDM, meat-derived mutagenic; ER, estrogen; PR, progesterone receptor; HNPCC, hereditary nonpolyposis colorectal cancer; RCC, renal cell carcinoma.

All of these agents have been found to exhibit antioxidant, anti-inflammatory, antimutagenic, antiproliferative, and anti-angiogenic activities.[84] How bioactive food constituents can induce cancer cell death and thus prevent cancer has been reviewed.[74] For instance, a higher intake of green leafy vegetables, which are rich sources of folates and chlorophyll, has been suggested to reduce the risk of various chronic diseases, including cancer. Methyl group availability has been suggested to be the underlying mechanism of folates' effect on chronic diseases. In support of this, deficiency of folates has been shown to cause DNA damage comparable to that caused by high-dose radiation.[85] In addition, a plant-based diet is a rich source of dietary fiber (plant cell wall), which also has been shown to be protective against a variety of chronic diseases.[86]

The diversity achievable with a plant-based diet is not possible with an animal-based diet. Another aspect of plant-based diets that is highly favorable with respect to chronic diseases is calories. High-calorie diets have been shown to promote chronic diseases through upregulation of inflammation, whereas low-calorie diets have been shown to promote a healthy lifestyle. Caloric restriction has been linked to a decreased incidence of cancer and other chronic diseases.[87] Dietary restriction has also been linked with a slowing of the aging process through suppression of inflammation.[88] It has been proposed that an increase in inflammation in obesity promotes energy expenditure for the purpose of reducing an energy surplus,[89] whereas decreased inflammation under caloric restriction contributes to energy saving. Inflammation is a mechanism for energy balance in the body, and inflammation resistance can lead to obesity. A plant-based diet is usually (but not always) thought to be low in calories, and thus preferred. In addition, a plant-based diet contains a wide variety of vitamins, minerals, and anti-inflammatory agents that can control various chronic diseases. Some of these plant-derived anti-inflammatory agents are flavonoids, flavones, triterpenes, anthocyanins, and chalcones (see Table 14.4).

TABLE 14.3
Effect of Vegetable and Fruit Consumption in Patients with Various Cancers

Cancer Type	Reference
Bladder Cancer	
Consumption of green-yellow vegetables and fruit was protectively associated with risk (CS, 14 years)[a]	391
Fruit and vegetable consumption, combined or separately, does not have association with BC risk (EPICS, 8.7 years)	392
Breast Cancer	
No association between breast cancer risk and total vegetable intake, except dark yellow-orange vegetables; intake of fruits was inversely associated with breast cancer risk (PCCS)	393
Consumption of individual vegetable/fruit groups was inversely and significantly related with BC risk (HCCS, 5 years)	394
Colon Cancer	
No strong and consistent differences between colon cancer subsites for vegetable/fruit associations (CCS, 2 years)	395
Vegetable and fruit intake was inversely associated with the risk for adenomas (CCS, 3 years)	396
Consumption of both fruits and vegetables was found to protect against CRC, as evidenced by interaction between GSTT1 gene expression and vegetable consumption	311
Head and Neck Cancer	
Total fruit and vegetable intake was inversely associated with H&N cancer risk; vegetables were more strongly associated with risk than the fruits (NDHCS, 5 years)	397
Lung Cancer	
Fruit consumption was inversely associated with lung cancer mortality among smokers; risk was significant in a Dutch cohort, not significant in Finnish men, and there was no association in Italian men; vegetable consumption was not related to lung cancer risk in smokers (CS, 25 years from 1970)	398
Increased fruit and vegetable intake is associated with a modest reduction in lung cancer (CS, 6–16 years)	399
Risk for those who seldom consumed vegetables/fruits was twice that of those who consumed frequently, among nonsmokers, smokers, and former smokers	400
Non-Hodgkin's Lymphoma (NHL)	
Greater intake of total fruits and vegetables was associated with lower NHL risk, especially with FL, and was weaker or not apparent for DLBCL (CS, 19 years)	401
Esophageal Cancer	
Total fruits were more protective than vegetables, and citrus fruits were strongly associated with reduced risk (CCS, 19 years)	402
Total fruit and vegetable intake has significant inverse association with ESCC risk but not EAC risk; significant inverse association was found between EAC and spinach intake (NDHCS-USA, 1 year)	403
Increase in consumption of total fruit and vegetables, especially cruciferous vegetables by 100 g/day decreases the incidence of esophageal SCC by 11% (PPCS)	404

continued

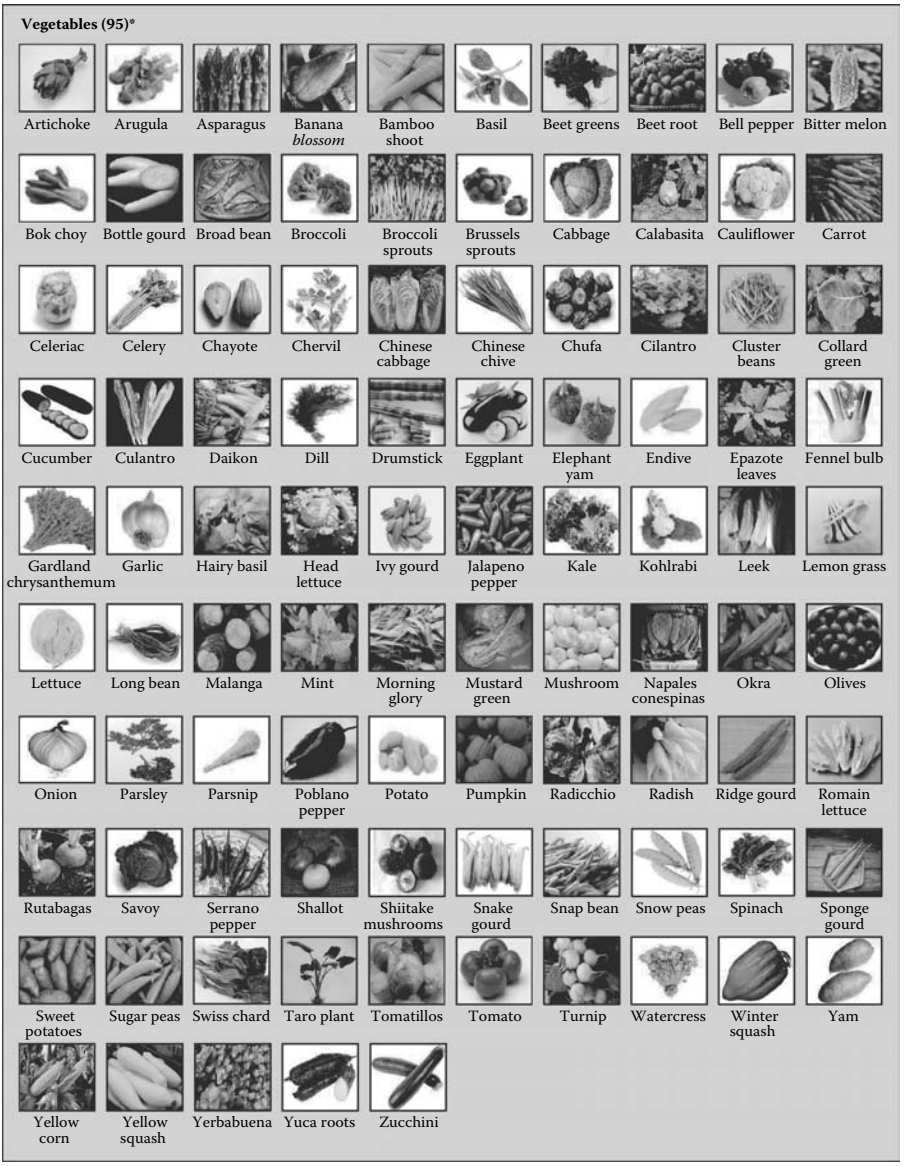
TABLE 14.3 (Continued)
Effect of Vegetable and Fruit Consumption in Patients with Various Cancers

Cancer Type	Reference
Pancreatic Cancer	
Risk is not related to total consumption of fruit and vegetables, combined or separately or as subgroups of vegetables and fruits (EPICS, 8.9 years)	392
Renal Cell Carcinoma	
Consuming 5 or more servings of fruits and vegetables daily had less RR in comparison to consumption less than once daily (PPCS, 13.4 years)	406
Total vegetable and fruit consumption, and vegetable and fruit consumption were not associated with RCC risk (CS, 9.3 years)	407
No association between fruit, vegetables or antioxidant nutrients and RCC risk (ATBC, 19 years)	408
Stomach Cancer	
Stomach cancer risk was inversely related to fruit and vegetable consumption; relative risk among subjects with lowest compared to those with the highest intake was 5.5 (CS, 25 years)	409
Vegetable and fruit intake, even in low amounts, is inversely associated with lower risk of GC, and it is higher in differentiated rather than in undifferentiated types (CS, 10 years)	410
Multiple Cancer	
Frequent vegetable intake is associated with a substantial reduction in several epithelial cancers, while fruit intake has a favorable effect, especially on upper digestive cancers (CCS, 7 years)	411
Vegetable intake consistently protected from all epithelial cancers but not nonepithelial neoplasms; strong protection by fruits was observed for upper digestive tract cancers, not for other epithelial neoplasms (CCS, 14 years)	412

[a] Information in parentheses indicates type of study with follow-up period.

Note: CCS, case-control study; CS, cohort study; CRC, colorectal cancer; FL, follicular lymphoma; DLBCL, diffuse large B-cell lymphoma; RCC, renal cell carcinoma; ATBCS, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; EPICs, European Prospective Investigation into Cancer and Nutrition study; NDHCS, NIH-AARP Diet and Health Cohort Study; ESCC, esophageal squamous cell carcinoma; EAC, esophageal adenocarcinoma; PPCS, population-based prospective cohort study; PCCS, population-based case-control study; HCCS, hospital-based case-control study; RR, relative risk.

Although statins are usually prescribed as drugs for treatment of hypercholesteremia, dietary agents are preferred chronically due to lack of side effects. For instance, it was recently reported that eating peanuts may benefit hypercholesterolemic men.[90] In a randomized crossover study involving 54 hypercholesterolemic men, results indicated that daily consumption of peanuts improved the lipid profile, atherogenic index of plasma (AIP), and coronary heart disease (CHD) risk. In that study, the subjects consumed about 77 g of peanuts daily along with their habitual diet for a period of 4 weeks. Subjects in the peanut group showed significantly reduced total cholesterol/high-density lipoprotein (TC/HDL) cholesterol (HDL-C) ratios and low-density lipoprotein (LDL) cholesterol levels. Additionally, peanut consumption was associated with a significant reduction in AIP and CHD estimated risk over 10 years. Thus, short-term peanut consumption might improve lipid profiles and the AIP and CHD risk in free-living hypercholesterolemic men.[90]



* Indicates total number of vegetables, fruits, spices, pulses, or nuts.

FIGURE 14.2 (See color insert.) Plant-based diets for healthy life.

Another study showed that antioxidant-rich spice blends could decrease the formation of potentially harmful oxidative compounds in cooked hamburger.[91] One of these, malondialdehyde (MDA), is an oxidative by-product of high heat processing of foods that contain unsaturated fatty acids, especially arachidonic acid. These compounds have been linked to unhealthy inflammation. Given the magnitude of

FIGURE 14.2 (Continued) (See color insert.)

FIGURE 14.2 (Continued) (See color insert.)

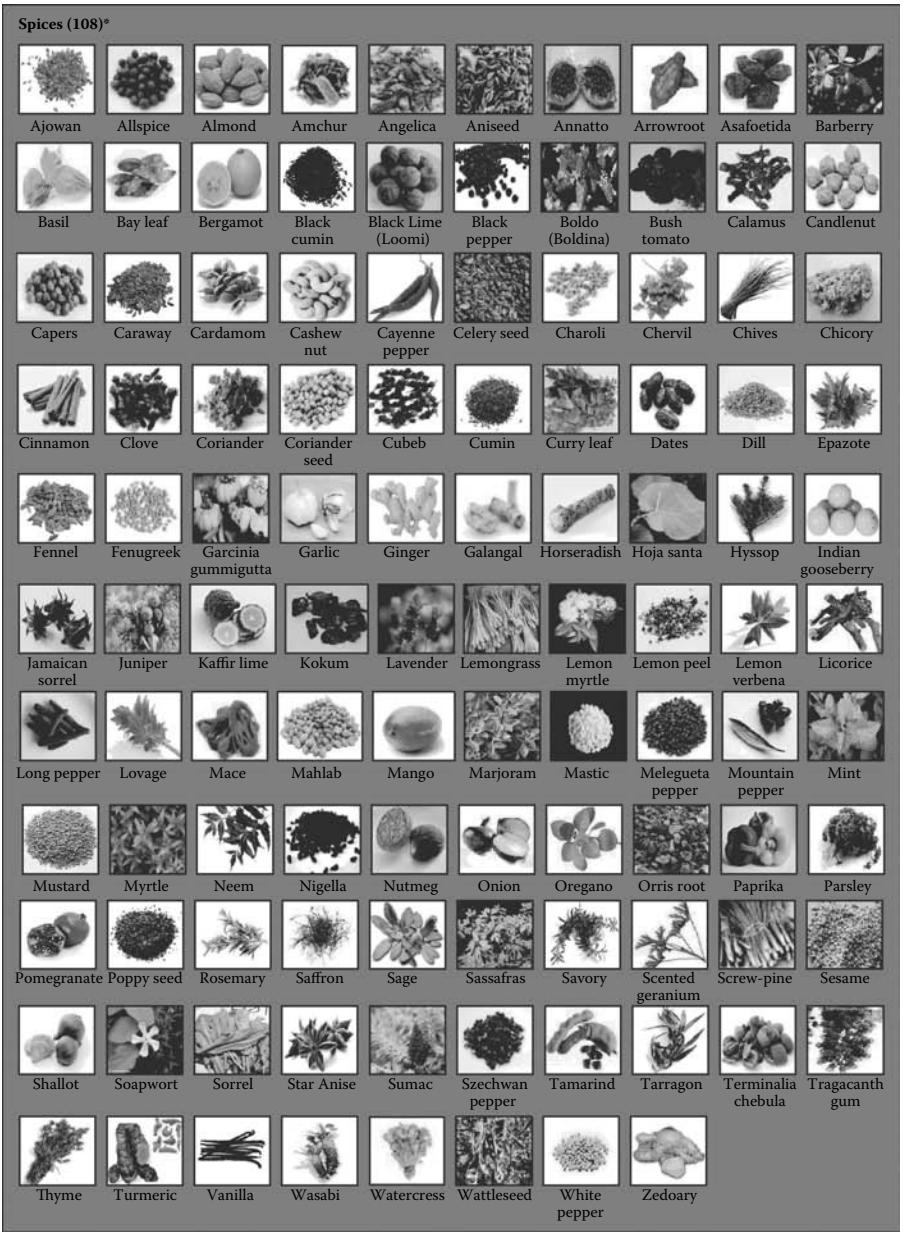


FIGURE 14.2 (Continued) (See color insert.)

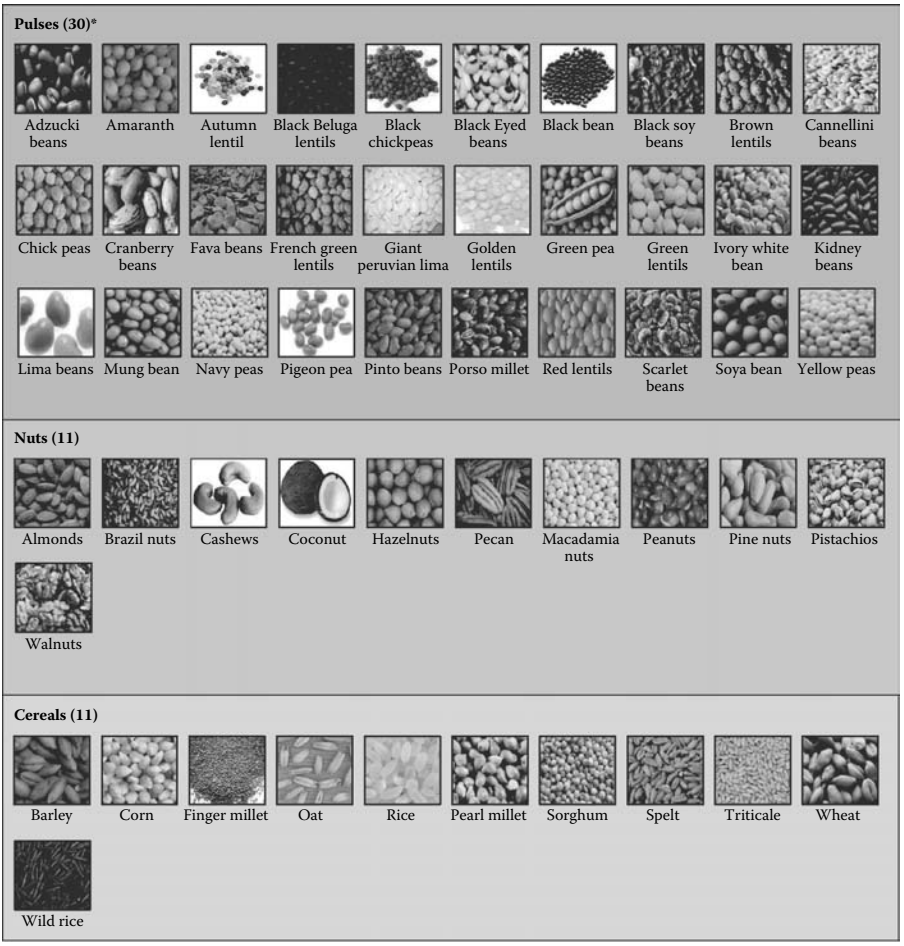


FIGURE 14.2 (Continued) (See color insert.)

decrease in MDA, the researchers concluded that "spices that are rich in antioxidants may be useful when cooking meat products."[569]

Another study showed that white kidney bean extract supplementation may lower the glycemic index of white bread.[92] In an open-label, six-arm, crossover trial including 13 randomized subjects, supplementation with white kidney bean extract (3,000 mg in powder form) was found to significantly reduce the glycemic index of white bread (by 20.23 or 34.11%), an otherwise high glycemic-index food. *In vitro* studies have also found white kidney bean extract to inhibit alpha-amylase, an enzyme involved in digestion of complex carbohydrates, which may explain the glycemic index lowering effect of the extract. Lower doses (1,500 and 2,000 mg) of the extract in powder form were found to induce insignificant reductions in glycemic index, as did 3,000 mg in a capsule form.[92] Thus, white bean extract appears to be a novel and potentially effective method for reducing the glycemic index of existing foods without modifying their ingredient profile.

TABLE 14.4
List of Active Components from Vegetables, Pulses, Cereals, Nuts, Spices, and Fruits

Name	Botanical Name	Active Component	Reference
Vegetables			
Artichoke	*Cynara scolymus*	Cynaropicrin	413
Asparagus	*Asparagus officinalis*	Asparanin A	414
Avocado	*Persea americana*	Quercetin	415
Bell pepper	*Capsicum annuum*	Capsaicin	416, 417
Bitter melon	*Momordica charantia*	Conjugated linolenic acid	418
Broccoli sprouts	*Brassica oleracea*	Carotenoids	419
Calabasita	*Cucurbita moschata*	α-Spinasterol	420
Carrot	*Daucus carota*	β-Carotene	421
Celery	*Apium graveolens*	Falcarinol	422
Chrysanthemum	*Leucanthemum coronarium*	Quercetin	423
Cilantro	*Coriandrum sativum*	Petroselinic acid	424
Culantro	*Eryngium foetidum*	Stigmasterol	425
Fennel bulb	*Foeniculum vulgare*	Falcarinol	422
Garlic	*Allium sativum*	Allicin	426
Hairy basil	*Ocimum americanum*	Ursolic acid	427
Holy basil	*Ocimum tenuiflorum*	Ursolic acid	427
Leek	*Allium ampeloprasum*	Kaempferol aglycone	428
Lettuce	*Lactuca sativa*	Chicoric acid	429
Lemon grass	*Cymbopogon citratus*	Citronellol	430
Loose beets	*Beta vulgaris*	Betalains	431
Malanga	*Xanthosoma sagittifolium*	Carotenoids	432
Mustard green	*Brassica nigra*	Fatty acids	433
Olives	*Olea europaea*	Maslinic acid	434
Onion	*Allium cepa*	Quercetin glycosides	435
Parsley	*Apium petroselinum*	Methoxsalen	436
Radicchio	*Cichorium intybus*	Inulin	437
Radish	*Raphanus sativus*	Flavonoids	438
Rutabagas	*Brassica napus*	Brassinolide	439
Shallot	*Allium ascalonicum*	Isoliquiritigenin	440
Shiitake mushrooms	*Lentinus edodes*	Lentinan	441
Spinach	*Spinacia oleracea*	Glycolipids	442
Sponge gourd	*Luffa aegyptiaca*	Luffin	443
Snap bean	*Phaseolus vulgaris*	Hemagglutinin	444
Snow peas	*Pisum sativum*	Flavonoids	445
Straw mushroom	*Volvariella volvacea*	β-D-Glucan	446
Sugar peas	*Pisum sativum* var.	Pisumin macrocarpon	447
Sweet potato	*Ipomoea batatas*	Polyphenolics	448
Tomatillos	*Physalis philadelphica*	Ixocarpalactone	449
Tomato	*Lycopersicon esculentum*	Lycopene	450

TABLE 14.4 (Continued)
List of Active Components from Vegetables, Pulses, Cereals, Nuts, Spices, and Fruits

Name	Botanical Name	Active Component	Reference
Vegetables (continued)			
Watercress	*Nasturtium officinal*	β-Phenylethyl, 8-methylsulphinyloctyl isothiocyanates	451
Yam	*Dioscorea villosa*	Diosgenin	452
Yard long bean	*Vigna unguiculata*	Trypsin, chymotrypsin inhibitor (BTCI)	453
Yuca roots	*Yucca schidigera*	Yuccaols, resveratrol	454
Pulses			
Cranberry bean	*Phaseolus vulgaris*	Genistein, folacin, linoleic acid	455
Navy bean	*Phaseolus vulgaris*	Genistein, folacin, linoleic acid	455
Lima bean	*Phaseolus lunatus*	Genistin, daidzin, genistein	456
Chick pea	*Cicer arietinum*	Daidzein, Genistein	457
Soy bean	*Glycine max*	Genistein, Daidzein	458
Adzuki bean	*Paseolus angularis*	Catechin, quercetin, myricetin	459
Black Beluga lentil	*Lens culinaris*	Saponins, folacin	460
Green lentil	*Lens culinaris*	Saponins, folacin	460
Giant Peruvian lima bean	*Phaseolus limensis*	Genistin, daidzin, genistein	456
Pigeon pea	*Cajanus cajan*	Genistein, folacin, linoleic acid	461
Green pea	*Pisum sativum*	Kaempferol, coumestrol, cryptoxanthin	462
Yellow pea	*Pisum sativum*	Kaempferol, coumestrol, cryptoxanthin	462
Scarlet runner bean	*Phaseolus coccineus*	Saponins, genistein, folacin	463
Mung bean	*Vigna radiata*	Vitexin, isovitexin	459
Nuts			
Pine nuts	*Pinus pinea* L	Pinolenic acid, linoleic acid	464
Peanuts	*Arachis hypogaea*	Proanthocyanidin-A, D-(+)-catechin	465
Cashew	*Anacardium occidentale*	Anacardic acids, anacardol	466
Walnuts	*Juglans regia*	Ellagitannins	467
Brazil nuts	*Bertholetia excelssa*	Aflatoxin	468
Pistachia	*Pistachia vera*	α-Pinene, β-myrcene	469
Almonds	*Prunus amygdalus*	Sphingolipid, β-sitosterol	470
Filberts (hazelnuts)	*Corylus avellana*	Linoleic, palmitic, stearic acids	471
Coconut	*Cocos nucifera*	4-Hydroxy-4-methylpentan-2-one, n-propyl ethanoate	472

continued

TABLE 14.4 (Continued)
List of Active Components from Vegetables, Pulses, Cereals, Nuts, Spices, and Fruits

Name	Botanical Name	Active Component	Reference
Cereals			
Rice	*Oryza sativa*	Tocotrienol, tocopherol, oryzanol	473
Barley	*Hordeum vulgare*	Tocotrienol, tocopherol, cinnamic acid	474
Corn	*Zea mays*	Tocopherol, phytic acid, cinnamic acid	475
Pearl millet (Bajra)	*Pennisetum glaucum*	Phytic acid, cinnamic acid	476
Oat	*Avena sativa*	Tocotrienol, tocopherol, caffeic acid	477
Sorghum (Jowar)	*Sorghum bicolor*	Phytic acid, cinnamic acid	478
Wheat	*Triticum aestivum*	Tocotrienol, tocopherol, cinnamic acid	474
Finger millet (Ragi)	*Eleusine coracana*	Phytic acid, cinnamic acid	479
Rye	*Secale cereal*	Tocotrienol, tocopherol	480
Spices			
Ajowan	*Trachyspermum copticum*	p-Cymene, thymol	481
Allspice	*Pimenta dioica*	Eugenol, myrcene	482
Almond	*Prunus dulcis*	Oleanolic acid, ursolic acid	483
Angelica	*Angelica archangelica*	Imperatorin, xanthotoxin	484
Anise	*Pimpinella anisum*	Anethole, bergapten	485
Annatto	*Bixa orellana* L.	Bixin	486
Asafoetida	*Ferula assafoetida*	Farnesiferoles	487
Barberry	*Berberis vulgaris*	Berberine	488
Basil	*Ocimum basilicum*	Eugenol	489
Bay leaf	*Laurus nobilis*	Eugenol, methyl eugenol	490
Bee balm (Bergamot)	*Monarda didyma*	Thymol, rutin	[491]
Black cumin	*Bunium persicum*	Caryophyllene, γ-terpinene	492
Black pepper	*Piper nigrum*	Piperine, β-caryophyllene	493
Boldo (Boldina)	*Peumus boldus*	Boldine	494
Cashew nut	*Anacardium occidentale*	Anacardic acid	495
Cassia	*Cinnamomum cassia*	Cinnamaldehyde	496
Chervil	*Anthriscus cerefolium*	Estragole	497
Chili	Genus *Capsicum*	Capsaicin	498
Cinnamon	*Cinnamomum verum*	Cinnamaldehyde, cinnamic acid	499
Clove	*Syzygium aromaticum*	Eugenol, ellagic acid	500
Cubeb	*Piper cubeba*	(–)-Hinokinin, (–)-cubebin	501, 502
Cumin	*Cuminum cyminum*	Cuminaldehyde, b-pinene	493
Curry leaf	*Murraya koenigii*	Mahanimbine, murrayanol	503
Dill	*Anethum graveolens*	Carvones, limonene	504
Fennel	*Foeniculum vulgare*	Eugenol	490

TABLE 14.4 (Continued)
List of Active Components from Vegetables, Pulses, Cereals, Nuts, Spices, and Fruits

Name	Botanical Name	Active Component	Reference
Spices (continued)			
Fenugreek	*Trigonella foenum-graecum*	Diosgenin	505
Galangal	*Alpinia galangal*	1′-Acetocychavicol-acetate	506
Gambooge	*Garcinia gummi-gutta*	Garcinol	507
Garlic	*Allium sativum*	Allicin, S-allylcysteine	508
Ginger	*Zingiber officinale*	[6]-Gingerol, [6]-shogaol	509, 510
Hoja santa	*Piper auritum*	Safrole	511
Horseradish	*Armoracia rusticana*	Allyl isothiocyanate, sinigrin	512
Indian gooseberry	*Emblica officinalis*	Pyrogallol	513
Jamaican sorrel	*Hibiscus sabdariffa*	Delphinidin 3-sambubioside	514
Kalonji	*Nigella sativa*	Thymoquinone	515
Kokum	*Garcinia cambojia*	Gambogic acid	84
Lavender	*Lavandula angustifolia*	β-Sitosterol	516
Lemon grass	*Cymbopogon citratus*	Luteolin	517
Lemon verbena	*Lippia triphylla*	Citral	518
Licorice	*Glycyrrhiza glabra*	Eugenol, gingerol	519
Long pepper	*Piper longum*	Piperine	520
Mace	*Myristica fragrans*	Eugenol	521
Mango	*Mangifera indica*	Geraniol, quercetin	522
Marjoram	*Origanum majorana*	Thymol	523
Mountain pepper	*Tasmannia lanceolata*	Polygodial	524
Mustard	*Brassica indica*	Ferulic acid	525
Myrtle	*Myrtus communis*	Pinene	526
Neem	*Azadirachta indica*	Nimbolide	527
Nutmeg	*Myristica fragrans*	Eugenol	521
Onion	*Allium cepa*	Quercetin	528
Oregano	*Origanum vulgare*	Thymol	529
Orris root	*Iris germanica*	Isopenol	530
Paprika	*Capsicum annuum*	Capsaicin	531
Parsley	*Petroselinum crispum*	Apigenin, caffeic acid	532
Pepper	*Piper nigrum*	Piperine	533
Pomegranate	*Punica granatum*	Betulinic acid, ursolic acid	534
Rosemary	*Rosmarinus officinalis*	Apigenin, betulinic acid	535
Saffron	*Crocus sativus*	Kaempferol, quercetin	536
Screw-pine	*Pandanus utilis*	Carotenoid	537
Sesame	*Sesamum indicum*	β-Carotene, ferulic acid	538
Shallot	*Allium oschaninii*	Isoliquiritigenin	539
Sorrel	*Rumex acetosa*	Anthraquinone	540
Star Anise	*Illicium verum*	α-Pinene	541

continued

TABLE 14.4 (Continued)
List of Active Components from Vegetables, Pulses, Cereals, Nuts, Spices, and Fruits

Name	Botanical Name	Active Component	Reference
Spices (continued)			
Szechwan pepper	*Zanthoxylum* sp.	Berberine	542
Tamarind	*Tamarindus indica*	β-Carotene, cinnamaldehyde	543
Thyme	*Thymus vulgaris*	Thymol	544
Turmeric	*Curcuma longa*	Curcumin	545
Watercress	*Ipomea aquatica*	Indol-3-carbinol	546
Zedoary	*Curcuma zedoaria*	Curcumin	547
Fruits			
Apple	*Malus domestica*	Quercetin, catechin, phloridzin, chlorogenic acid	548
Apricot	*Prunus armeniaca*	β-Carotene, caffeic acid, quercetin	549
Avocado	*Persea americana*	Lutein and carotenoids	550
Acai	*Euterpe oleracea*	Cyandin 3-glucosidecyanidin 3-rutinosidehomoorientin, orientin, isovitexin, scoparin, taxifolin deoxyhexose	551
Banana	*Musa acuminata*	Campsterol, delphinidin, shikimic acid, stigmasterol	552
Blackberry	*Rubus villosus*	Gallic acid, ferulic acid, sitosterol, stigmasterol, ursolic acid, resveratrol	553
Betel nut	*Areca catechu*	Arecoline	554
Cherry	*Prunus iaurocerasus*	Apigenin, quercetin, ursolic acid	75
Fig	*Ficus carica*	Lupeol	555
Grapes	*Vitis vinifera*	Resveratrol	556
Guava	*Psidium guajava*	Gallic acid	557
Jack fruit	*Artocarpus heterophyllus*	Betulinic acid, morin, ursolic acid	558
Lemon	*Citrus limon*	Limonene	75
Mango	*Mangifera indica*	Mangiferin, geraniol, mangiferine, quercetin	559
Mangosteen	*Garcinia indica*	Garcinol	560
Mulberry	*Broussonetia papyrifera*	Lignana	561
Mulberry	*Morus alba*	Anthocyanins	562
Oranges	*Citrus aurantium*	Hesperidine	563
Pears	*Pyrus communis*	Ursolic acid	75
Pineapple	*Ananas comosus*	Ascorbic acid	564
Pomegranate	*Punica granatum*	Ellagic acid	565
Red berry	*Ilex opaca*	Ellagic acid	566
Watermelon	*Citrullus lanatus*	Lycopene	567

Udani and coworkers also examined the effect of mangosteen juice on inflammation in obese adults.[93] In a randomized, double-blind, placebo-controlled study involving 40 overweight and obese subjects, supplementation with a proprietary mangosteen juice blend (9 ounces, twice/day) for a period of 8 weeks was found to significantly reduce levels of high-sensitivity C-reactive protein (hs-CRP), a marker of systemic inflammation. Lower doses of mangosteen juice (3 and 6 ounces, twice a day) did not yield these beneficial results. However, a trend toward a decrease in body mass index was found in all three groups that consumed the juice. No significant changes in inflammatory cytokines or lipid peroxidation were found. Together, these studies indicate that many different plant compounds act against inflammation and provide evidence of the benefits of a plant-based diet.

14.8 ONE SIZE DOES NOT FIT ALL

Observations about genotype versus phenotype have suggested that one size does not fit all.[70] For instance, it remains unknown why breast cancer incidence is highest among native Hawaiians and Japanese, whereas prostate cancer is highest among African Americans. Some evidence has suggested that variations in coding and regulatory regions of genes contribute to the susceptibility to many chronic diseases, despite the fact that >95% of all genetic variation is shared across populations, with <10% being specific to any population.[94] The most common genetic variation is the single-nucleotide polymorphism, a site at which two alternative alleles are observed, each with a frequency of >1%. These genetic variations have led to the concept of personalized nutrition. For instance, cytochrome P450, which mediates oxidative processes, and glutathione S-transferase, which is involved in conjugation and the nucleophilic trapping process, plays an important role in the metabolism of xenobiotics. Different individuals express polymorphism of these enzymes, and this may affect the extent to which diet modulates the risk of chronic diseases.[95,96] Genotypes of metabolizing genes influence the circulating levels of nutraceuticals.[97] For example, high intake of vegetables reduces the risk of breast cancer for carriers of alleles of the CA repeats in the *EGFR* gene.[98] The protective effect of cruciferous vegetables has also been found to be stronger in individuals null for *GSTM1* or *GST1*.[99–103] Tea polyphenols have been found to modify the effect of CYP19A1 and COMT polymorphisms on cancer risk[104,105] and phytoestrogens to interact with polymorphism in *ESR1* and *NR112* genes to modulate estrogen levels.[106] In another example, selenium, an essential micronutrient, is deficient in some parts of China and New Zealand. Half of the population of Auckland men showed serum selenium levels so low as to enhance DNA damage.[107] Vegetable intake in Europe and North America is 30–50% of average levels in Japan and China.[108] Total isoflavone uptake (mostly from soybeans) in Europe is 0.44 mg/day, compared with 33.5 and 46.5 mg/day in China and Japan, respectively.[109] All these factors indicate the complexity of interactions between diets, different cultures in different countries, and genotype.

14.9 DRUGS VERSUS DIET

With human genome and proteome in hand, the National Institutes of Health (NIH), with a budget of more than $30 billion, and drug companies spend more money than ever before on research and development, so the market should be flooded with new drugs. However, the number of drugs approved annually by the Food and Drug Administration (FDA) has steadily declined. This may be because it has become incredibly expensive to bring a new drug to market these days, and because it is possible that we are running out of biological targets for new medicines. There are about 4,000 approved and experimental drugs out there. But all those drugs, many of which are minor variants of one another, actually only target about 400 gene pathways and other biological areas of interest. According to one estimate, although there are about 30,000 human genes, only about 3,000 of those are "druggable," that is, genes that can be reached by drugs. Additionally, only about 3,000 human genes are believed to influence disease activity in the body. Because a drug can only be effective if it targets a genetic pathway involved in disease, and it can only be effective if it can actually reach and act upon that pathway, there may indeed be a limited number of biological targets in the body. So what are scientists doing to get around this apparent plateau in new drug discovery? One approach is to look for new uses for old drugs. Anti-inflammatory agents currently in use include steroids, COX-2 inhibitors (such as celecoxib), NSAIDs (such as aspirin and Tylenol), and TNF blockers (such as Remicade, Humira, and Enbrel). Most of these drugs exhibit numerous adverse effects and thus cannot be consumed chronically.[110]

One major problem is that prevention and treatment of chronic diseases is being sought not in diet, but in drugs developed by pharmaceutical companies. For instance, to prevent cancer, several chemopreventive agents have been approved by the FDA, including tamoxifen, raloxifene, finasteride, and celecoxib, to prevent breast cancer, prostate cancer, and colon cancer. However, notable toxicity is associated with these drugs. For instance, among 1,000 women, 19 would be expected to develop breast cancer over the next 5 years. If those women all took tamoxifen, however, 9 of them would avoid breast cancer. Yet tamoxifen would cause 21 additional cases of endometrial cancer, a cancer of the uterine lining that is typically treatable when caught early, 21 would develop blood clots, 31 would develop cataracts, and 12 would develop sexual problems. More than half of the 1,000 women would naturally develop hormonal symptoms, such as hot flashes, changes in vaginal discharge, or irregular periods, but tamoxifen would cause those symptoms in about an additional 120 women. Raloxifene has been shown to significantly reduce breast cancer risk, but with fewer adverse effects.

Similarly, there are several drugs approved for obesity, including orlistat, sibutramine, rimonabant, metformin, exenatide, and pramlintide. However, all of these drugs are associated with adverse effects.

Structural genomics will play an important role in guiding the development of specific anticancer drugs. Protein kinases currently constitute the largest target family for cancer drug discovery. Ten small-molecule kinase inhibitors have been approved for cancer treatment, but only a few are reasonably specific.

The others inhibit multiple pathways, resulting in off-target effects that relegate them to end-of-life treatments. A commentary in *Nature Chemical Biology* by Stefan Knapp and colleagues from the Structural Genomics Consortium Oxford explained the challenges—and possible solutions—in obtaining highly specific kinase inhibitors, and the important role played by structural genomics.[111] Most kinase inhibitors perform relatively well in phase I clinical trials, with an attrition rate of 53%, which is respectable in comparison with the overall antitumor drug failure rate of 82% at this stage. The challenge now is to improve this attrition rate by producing inhibitors with higher specificity and preventing cross-reactivity. However, both the publication record and the patents resulting from research in this area are skewed toward the 25% of human kinases that are reasonably well understood. Of the remainder, 25% have a completely unknown function, and the other 50% have been only minimally characterized.

Yet it is known that these unstudied or barely studied kinases are frequently mutated in cancer and have been identified in kinome-wide RNA interference knockdown studies, suggesting that these targets have a pivotal role in disease development. Many of these uncharacterized kinases have entered specificity screening, and the results so far indicate that they are strongly inhibited by current clinical or preclinical inhibitors, indicating a cross-reaction. In addition, common kinase inhibitors used as chemical probes in cell biology studies have been found to target several proteins at once, calling into doubt some of the conclusions of those studies. This strong inhibition of distantly related kinases by a single drug is puzzling at first glance. Most of the inhibitors target the ATP-binding site, but many of the kinases that are inhibited by the same compound have only 20% or less sequence similarity at this site. But from the structures that have been solved so far, it is clear that a few conserved interactions are mediating the binding of most inhibitors.

The key features of the kinase active site include a hydrophobic purine-binding cavity, a hinge segment that connects the two lobes of the kinase, and a backbone hydrogen that anchors ATP-mimetic ligands. The active state of kinases is rigid and well conserved within the kinase family, leading to a high degree of cross-reactivity.

By contrast, the inactive state has a much wider range of conformations and is more promising for achieving high specificity—the anticancer drug Lapatinib targets a unique inactive conformation, for example. It is likely that many inactive conformations exist.

High-resolution crystal structures would greatly help the rational design of selective kinase inhibitors. So far, 138 structures of human kinases are available, and high-throughput structural genomics methods are contributing significantly to this drive. The structural biology approach goes hand-in-hand with developments in high-throughput sequencing focused on a comprehensive characterization of the genetic mutations that occur in cancer. This combination of high-throughput approaches should boost kinase drug discovery and cancer treatment.[111,112] Most dietary agents, in comparison to these approaches, are known to target multiple kinases, though with low affinity, which is preferable.

Even though research and development spending by major pharmaceutical companies has roughly doubled in the decade since the Human Genome Project was

largely completed, reaching $46 billion in 2009, the number of new drugs approved each year has stayed about the same. There were 25 in 2009.

One recently approved drug, Crestor, was approved by the FDA for a new category of customers, as a preventive measure for millions of people who do not have cholesterol problems.[113] Statins, of which Crestor is one, raise a person's risk of developing type 2 diabetes by 9% and cause muscle aches. Despite this, Crestor, which is made by AstraZeneca, is the nation's second-best-selling statin, behind Lipitor by Pfizer. An estimated 6.5 million people in this country who have no cholesterol problems and no sign of heart problems will be deemed candidates for statins. That is in addition to the 80 million who already meet the current guidelines for high cholesterol—about half of whom now take statins. A recent clinical study showed a small but measurable reduction of strokes, heart attacks, and other cardiovascular events among people taking Crestor, compared with those taking a placebo. The clinical trial based on which the FDA approved the new preventive use for Crestor was a global study of nearly 18,000 people. It examined only patients with low cholesterol and an elevated level of inflammation as indicated by increased CRP level.

Numerous dietary agents have been identified that can substitute for these anticancer and antiobesity drugs, including vegetables, fruits, spices, pulses, nuts, and cereals. The nutraceuticals derived from these plant-based dietary agents have huge potential. The global nutraceuticals market was estimated in 2007 to be $120 billion and growing at a rate of 7% per year, whereas the global pharmaceutical market is estimated at $600 billion. More than 63% of anticancer drugs introduced over the past 25 years have been natural products or can be traced back to a natural product source.[114] Butler showed that 79 natural products or natural product analogues entered clinical trials as anticancer agents between 2005 and 2007.[115] That nutritional agents can regulate inflammatory processes offers tremendous opportunities.[36,116–118]

14.10 LET FOOD BE THY MEDICINE AND MEDICINE BE THY FOOD

Perhaps one of the best solutions for the treatment of most chronic diseases was set down by Hippocrates almost 25 centuries ago, "Let food be thy medicine, medicine be thy food." Numerous dietary flavonoids have been found that can directly bind to disease-causing proteins or enzymes and inactivate them, as is seen with some protein kinases, such as Akt/protein kinase B (Akt/PKB), Fyn, Janus kinase 1 (JAK1), mitogen-activated protein kinase kinase 1 (MEKK1), phosphoinositide 3-kinase (PI3K), mitogen-activated protein (MAP) kinase kinase 4 (MKK4), Raf1, and chain-associated 70 kDa protein (ZAP-70) kinase.[119] For instance, the flavonoid myricetin (3,3′,4′,5,5′,7-hexahydroxyflavone) has been shown to inhibit the phosphorylation of STAT3 at Tyr705 and Ser727. *Ex vivo* and *in vitro* pull-down assays have shown that myricetin bound to JAK1 and STAT3.[120] Another study showed that myricetin bound to Akt directly by competing with ATP. Molecular modeling has suggested that myricetin easily docks to the ATP-binding site of Akt with hydrogen bonds.[121] Betulinic acid, which is found on the bark of some trees, binds to a G-protein-coupled receptor (TGR5) known to be activated by bile acid.[122] This

receptor has been linked with the prevention of obesity and diabetes and mediates an increase in energy expenditure in brown adipose tissue. Lack of TGR5 decreases energy expenditure and promotes obesity. Another study showed that equol, a metabolite of daidzein derived from soybeans, directly binds to MEKK1 and inhibits its activity.[123] Genistein and daidzein constitute 100–300 mg/100g of soybean. Genistein is a PTK inhibitor. After ingestion, daidzein is converted to equol, which has a longer half-life in blood.[124–126]

In addition, signaling pathways regulated by direct binding of flavonoid-protein kinase include PI3K-Akt signaling, Raf-MEKK1-MAPK signaling, and JAK-STAT3 signaling. The binding sites on protein kinase of flavonoids may vary for the sites of flavonoids binding to protein kinases, affinity of flavonoids binding to protein kinases, and multiple targets vs. selectivity. Numerous studies on direct binding and molecular modeling of flavonoid–protein kinase interaction have been performed. For example, myricetin has been shown to bind to JAK1, Akt, MEKK1, Fyn, MKK4, and PI3K; delphinidin, an anthocyanidin found in banana, binds to Raf1, MEKK1, and Fyn; EGCG, a catechin found primarily in tea, has been shown to bind to Fyn and ZAP-70; the flavonoid quercetin interacts with MEKK1 and PI3K; caffeic acid, a building block of plant lignins, binds and inhibits Fyn; equol binds and inhibits MEKK1; resveratrol, an antimicrobial produced by plants, binds to MEKK1; and procyanidin flavonols bind to MKK4 and MEKK1. Curcumin, a component of the spice turmeric, has been shown to bind to Src, GSK-3β, xanthine oxidase, phosphorylase kinase 1, thioredoxin reductase, and others.[127] These indicate that dietary agents can negatively regulate numerous proinflammatory pathways.

14.11 YOU ARE WHAT YOU EAT!

A famous American adage is "you are what you eat." It is clear that diet plays a very important role in development of most chronic diseases. In contrast to drugs, changing one's diet is a very safe way to prevent and treat chronic diseases. Besides diet, dietary supplements (some of which are outlined in Table 14.4), functional food (calcium-fortified orange juice), and organic food are becoming increasingly popular. According to the *Nutrition Business Journal*, U.S. sales of supplements, natural or organic foods, and functional foods grew to $97.5 billion in 2008 from $47.9 billion in 2000.

14.12 MORE VARIETY, THE BETTER

Considering that there are at least 95 different types of vegetables, 215 types of fruits, 108 types of spices, 30 types of pulses, 11 types of nuts, and 11 types of cereals, and that all these contain several thousand nutraceuticals, plant-based treatments provide an opportunity to target the dysregulation of multiple genes safely and inexpensively in various chronic diseases, which is not possible through the pharmaceutical approach. Chronic intake of these dietary agents is unlikely to be toxic. However, because these nutraceuticals exhibit overlapping and nonoverlapping activities, it is important to mix and match and to use as much variety as possible, rather than more

of the same. Increasing evidence suggests that fruits and vegetables can decrease cancer risk. For instance, onions protect against stomach cancer, and garlic protects against colorectal cancer. The highest consumption of cruciferous vegetables, which contain isothiocyanates and indoles, in the United States reaches 30–40% of consumption in Japan and China.[128] Total isoflavone uptake in Europe is ~0.44 mg/day, compared with 33.5 mg/day in China and 46.5 mg/day in Japan[109] (Table 14.4).

Consuming a balanced diet is the key to taking advantage of the protective and anti-inflammatory effects of a plant-based diet. Eating too much of one kind of food can cause problems, as noted by Waters et al.[129] They conducted a randomized feeding trial in which beagle dogs were given different levels of dietary selenium over a 7-month period, then selenium intake was studied with regard to the level of DNA damage in the prostate. They found a U-shaped dose-response curve for optimum selenium intake to reduce DNA damage. Thus, consuming too much of one thing could be as damaging as consuming too little. Similarly, a high dose of beta carotene was found to increase rather than decrease the incidences of both cardiovascular diseases and cancer.[130]

14.13 DIAL-UP VERSUS DIAL-DOWN CONCEPT

It seems that most chronic diseases are caused by a "dialing up" of multiple genes. If so, "dialing down" should prevent and treat the disease. Modulation of various biomarkers of the disease is beginning to be investigated. For instance, tea polyphenols have been shown to decrease serum levels of PSA, hepatocyte growth factor (HGF), and VEGF in patients with prostate cancer.[131–135] One study found that before radical prostatectomy in 26 patients, daily administration of polyphenon E (1.3 g/daily; 800 mg EGCG) for 34.5 days decreased levels of inflammatory biomarkers. In another study, drinking black tea was shown to decrease levels of inflammatory biomarkers in patients with colon cancer.[136] The efficacy of tea polyphenols has been shown to decrease the risk of gastric cancer,[137] breast cancer,[138] lung cancer,[139] and prostate cancer.[140,141] Similarly, a benefit of pomegranate juice among patients with prostate cancer has been reported.[142]

14.14 CONCLUSION

There are three types of prevention for most chronic diseases: primary prevention, which involves normal healthy individuals; secondary prevention, which involves people who are either at high risk to develop a chronic disease or are already being treated for the disease; and tertiary prevention, which involves individuals who have already been treated and for whom nothing more is available for treatment, usually survivors from the disease, such as cancer or heart attack. For instance, at present there are 12 million cancer survivors in the United States alone. This number has tripled in the past 30 years, and 25% of them will develop multiple cancers, not recurrence. This may be connected to treatment. Oncologists do not use the word *cured* anymore—instead they use *NED* (no evidence of disease). Patients will not go back to normal life after cancer. They will feel fatigue, unchecked inflammation pain, headaches, neuropathy (nerve damage), cognitive impairment (loss of memory

and an inability to concentrate, or "chemo brain"), infertility and sexual dysfunction, depression, and anxiety. There are no agents currently available that can be administered chronically to control or prevent these lingering effects. For treating such long-term problems, dietary agents provide the best solution. However, right kinds of agents are important. Again, diversity of diet is highly preferable to achieve the most benefits from natural variety. We also need more clinical trials to identify biomarkers of disease and their modulation by dietary agents. Most cancer prevention trials that have studied dietary agents, however, showed little or no therapeutic advantage, such as a trial of vitamin E and beta carotene for lung cancer,[143] the beta carotene and retinol trial (CARET) for lung cancer,[144] a vitamin E and selenium trial for prostate cancer,[145] a trial of n-3 fatty acid for cancer cachexia,[146] and others. The lack of benefit shown by these trials probably suggests the problems with study protocols. These dietary agents are not drugs, but they are being viewed as such. Thus, clinical trials that are suitable for dietary agents need to be designed, to prevent overdosing and to study the benefits of the whole food, not simply a single component.

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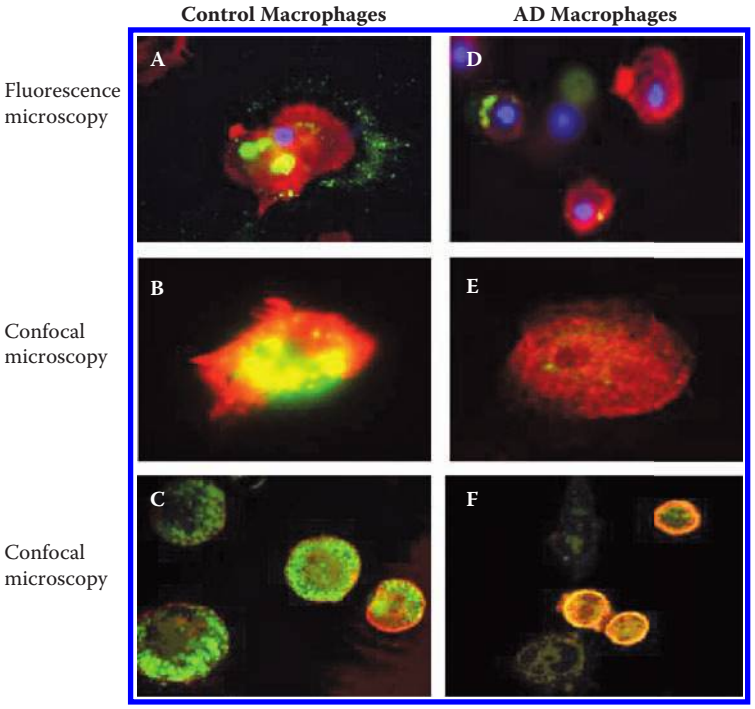

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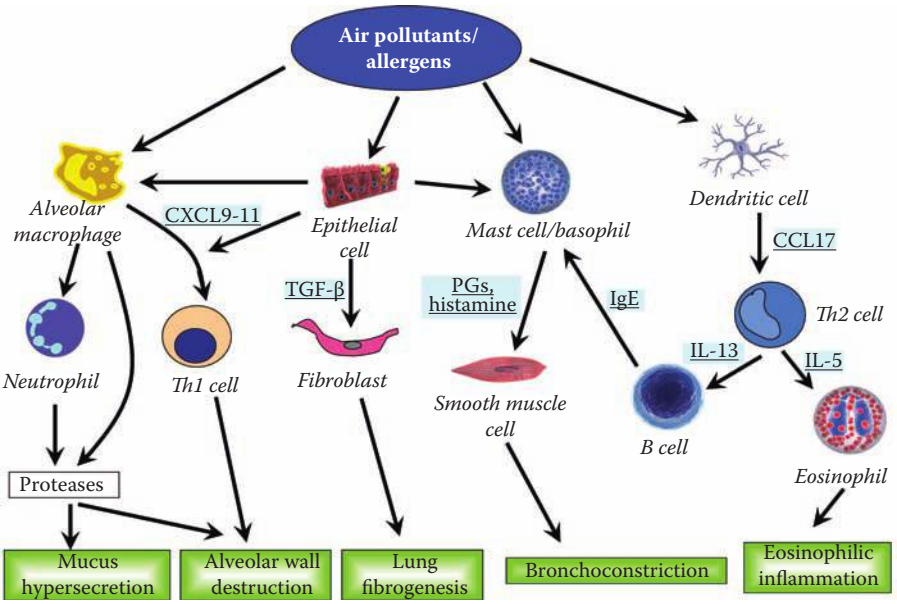

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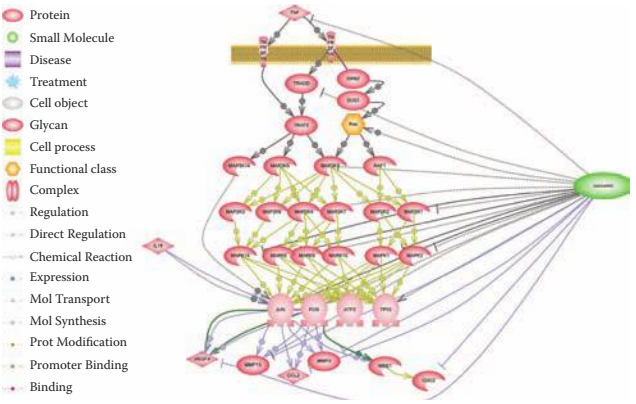

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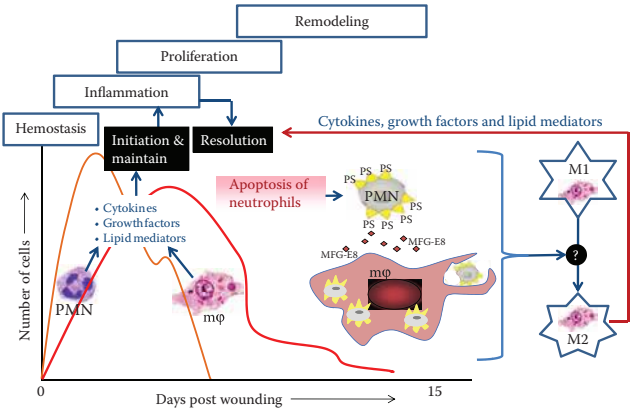

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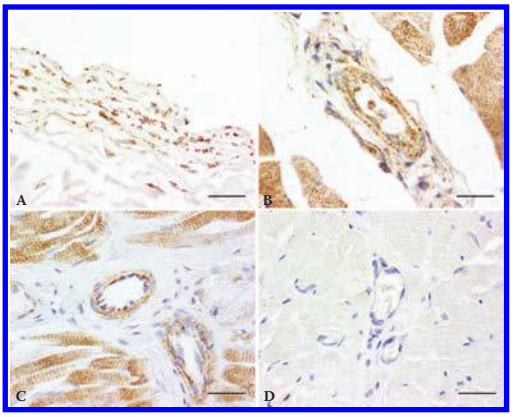

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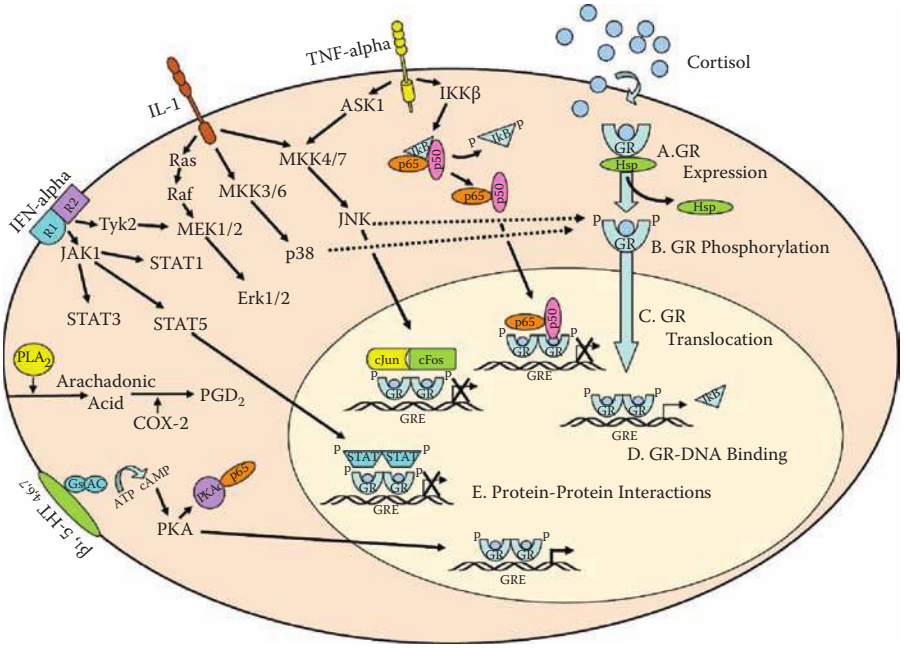

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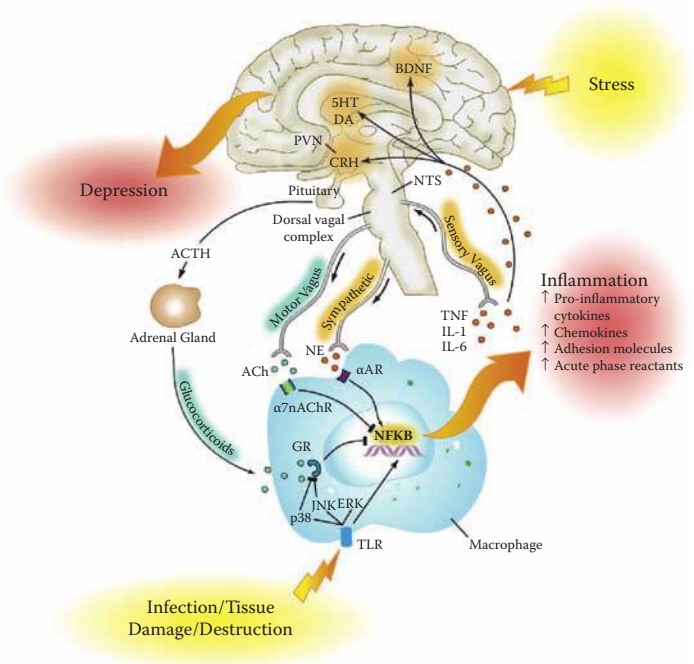

FIGURE 10.3

FIGURE 14.2

FIGURE 14.2 (Continued)

FIGURE 14.2 (Continued)

FIGURE 14.2 (Continued)

FIGURE 14.2 (Continued)