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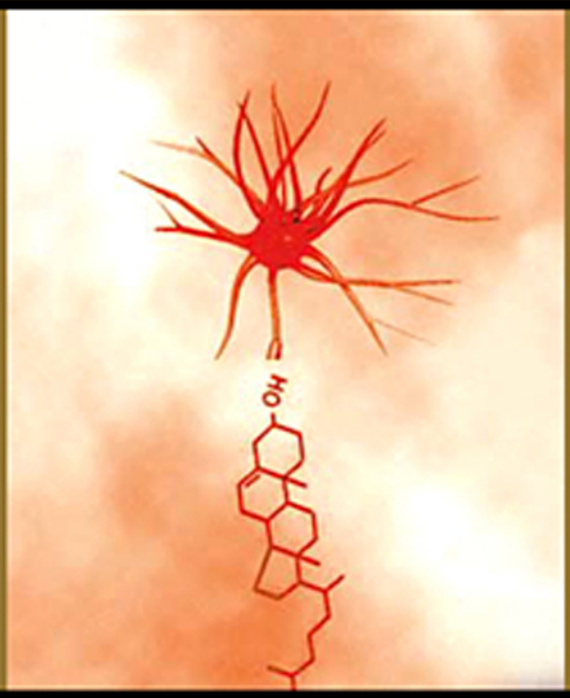
Volume 9



Endocrine Manifestations of Systemic Autoimmune Diseases

Edited by
Sara E. Walker & Luis J. Jara

Handbook of
Systemic Autoimmune Diseases

Volume 9

Endocrine Manifestations of Systemic Autoimmune Diseases

Handbook of Systemic Autoimmune Diseases

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Volume 9	Endocrine Manifestations of Systemic Autoimmune Diseases *Edited by: Sara E. Walker and Luis J. Jara*

Handbook of
Systemic Autoimmune Diseases

Volume 9

Endocrine Manifestations of Systemic Autoimmune Diseases

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Preface

This issue of the *Handbook of Systemic Autoimmune Diseases* addresses endocrine manifestations of systemic autoimmune diseases. Neuroendocrine immunology has been developed in the last years thanks to the activities of different associations such as the International Society for Neuroimmunomodulation (ISNIM), the Study Group on Endocrine Immunology at the American College of Rheumatology (ACR), and the Study Group on Neuroendocrine Immunology of the Rheumatic Diseases (NEIRD) at the European League against Reumatism (EULAR) and others.

The early chapters of the issue will introduce the background of genetic expression, within which reciprocal regulatory networks enhance communication between neuroimmune and endocrine systems, resulting in biologic reactions that lead to clinical syndromes.

The major themes of this volume are clinical and therapeutic approaches to recognizing and treating systemic autoimmune diseases that involve the endocrine system. In particular, immune mechanisms leading to pathological damage and dysfunction of adrenals, gonads, thyroid, pituitary, and pancreas are discussed in well-balanced reviews written by leading authors.

Old and new therapies are presented and evaluated on the light of their more recent effects on the neuroimmune endocrine network, and reviews of the immunologic actions of vitamin D and TNF-α blockers are included.

We hope that readers will appreciate the concept of interplay between different systems. In human autoimmune diseases, available evidence indicates that there is a prolonged preclinical phase, lasting years, during which the above-mentioned systems may play a crucial role before symptomatic disease is evident.

The role of the adrenal glands, gonadal steroids, and pregnancy as modulators of the immune response will represent some of the topics in autoimmunity that are discussed. We are convinced that readers will find new and provocative thoughts in this issue, leading to stimulation of further interesting research in this area.

Finally, we extend our great appreciation to all the authors of this issue for their updated and expert contributions.

Maurizio Cutolo

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Series Editor

Prof. Ronald A. Asherson

Professor Ronald A. Asherson, MD, FACP, MD (Hon) (London), FCP (SA), FACR, Dip O&G (Hon) is Professor of Immunology (Hon), at the School of Pathology, University of the Witwatersrand, as well as being Consultant Rheumatologist at the Rosebank Clinic in Johannesburg, South Africa. He is also a Professor at the Systemic Autoimmune Diseases Unit at the Hospital Clinic, Barcelona, Spain where he regularly visits and coordinates research projects.

Professor Asherson qualified in Medicine at the University of Cape Town in 1957 and, after completing his internship, became H/P to Professor Sir Christopher Booth at the Hammersmith Hospital, London in 1960. In 1961, he accepted a Fellowship at the Columbia Presbyterian Hospital in New York, returning in 1962 to become Registrar and then Senior Registrar till 1964 at Groote Schuur Hospital in Cape Town. After 10 years as a Clinical Tutor in the Department of Medicine, he returned to the United States and was appointed as Assistant Clinical Professor of Medicine at the New York Hospital-Cornell Medical Centre under the late Professor Henry Heinemann. From 1981 to 1986, he was associated with the Rheumatology Department at the Royal Postgraduate Medical School of London. It was at that time that he developed his interest in Connective Tissue Diseases and Antiphospholipid Antibodies.

In 1986, he moved to the Rayne Institute and St. Thomas' Hospital in London, where he was appointed Honorary Consultant Physician and Senior Research Fellow. In 1991, he took a sabbatical at St. Luke's Roosevelt Hospital Center in New York, working with Professor Robert Lahita. In 1992, he returned to South Africa for private practice in Johannesburg.

In 1998, he was elected as Fellow of the American College of Physicians (FACP) as well as a Founding Fellow of the American College of Rheumatology (FACR). From 1988 to 1991, he served on the Council of the Royal Society of Medicine in London. In 1992, he was co-winner of the European League Against Rheumatism (EULAR) Prize and in 1993 was the co-recipient of the International League Against Rheumatism (ILAR) Prize, both for his research on antiphospholipid antibodies. In 1994, he was elected a Fellow of the Royal College of Physicians (FRCP) of London. In 2002, he was awarded an Honorary Doctorate in Medicine from the University of Pleven in Bulgaria.

Professor Asherson has been an invited speaker at many universities and International conferences both in the USA and in Europe. He is the author of more than 300 papers on connective tissue diseases and has contributed to more than 30 textbooks of medicine, rheumatology, and surgery, besides co-edited "Problems in the Rheumatic Diseases", the "Phospholipid Binding Antibodies", and two editions of "The Antiphospholipid Syndrome" and "Vascular Manifestations of the Systemic Autoimmune Diseases". He is currently engaged in research on connective tissue diseases, particularly on the antiphsopholipid syndrome together with colleagues in the USA, Spain, France, and Israel and is in clinical practice in South Africa. In 1999, he was the co-recipient of the Juan Vivancos Prize in Spain and in 2003 was the co-recipient of the Abbott Prize, awarded at the European League Against Rheumatism (EULAR) International Meeting, held in Lisbon, Portugal.

His original description of the "Catastrophic Antiphospholipid Syndrome" and the publishing of more than 40 papers on this new disease was rewarded by the attachment of the eponym "Asherson's Syndrome" to this condition at the November 2002 International Phospholipid Conference held in Sicily. He has established the first International Committee to study survivors of this syndrome.

He is currently editing a series of 12 volumes entitled "The Handbook of Systemic Autoimmune Disease" (Elsevier) and in September of 2003 was Co-Chairman of the First Latin American Congress on Autoimmunity, held in the Galapagos Islands, Ecuador. He co-chaired and participated in a Session at the Milan Conference on "Heart, Rheumatism and Autoimmunity" held in February 2004.

He was awarded an Honorary Fellowship of the Slovakian Rheumatology Association in 2005.

In 2007, he was awarded an Honorary Life Membership of the South African Rheumatology Association. He chaired a combined session of Czech and Slovakian Rheumatologists in Bratislava, Slovakia devoted to the Catastrophic Antiphospholipid Syndrome. In late 2007, he was awarded Honorary Membership and a Diploma as well as the medal of the Russian Society of Obstetrics and Gynaecology for his outstanding contributions to clinical medicine, and the discovery and study of the catastrophic antiphsopholipid syndrome.

Volume Editors

Sara E. Walker

Dr. Sara E. Walker was trained in rheumatology at the Rackham Arthritis Unit at the University of Michigan. She is currently Professor Emeritus of Internal Medicine in the Division of Immunology and Rheumatology at the University of Missouri. She has a longstanding research interest in the effects of peptide hormones on SLE. Dr. Walker is a Master of the American College of Rheumatology and a Master of the American College of Physicians. She was President of the American College of Physicians, from 2002 to 2003.

Luis J. Jara

Dr. Luis J. Jara was born in Perú in 1948. He lived in Mexico since 1982. He received his MD degree from the National University ''Federico Villareal'', Faculty of Medicine in Lima, Perú in 1975. He trained in internal medicine at the National University of San Marcos, Faculty of Medicine ''San Fernando'' from 1975 to 1978. From 1980 to 1982, he obtained a scholarship from the Instituto Mexicano del Seguro Social to be trained in rheumatology at the Hospital de Especialidades Centro Médico La Raza. He was approved by the Rheumatology Mexican Board and granted another scholarship for Hospital Ramón y Cajal, in Madrid, Spain. He went back to Mexico and in 1983 joined the staff of rheumatologists at the Hospital de Especialidades Centro Medico La Raza, Mexico. From 1990 to 1992 he was trained as a research fellow in Rheumatology at the University of South Florida, and the Louisiana State University (LSU). From 1992 till date he belongs to the Mexican National System of Investigators. In 2004 he was appointed as the Head of Research Division. He is currently the Director of Education and Research, Hospital de Especialidades ''Antonio Fraga Mouret'' del Centro Médico Nacional La Raza.

Dr. Jara is a professor of rheumatology at the Universidad Nacional Autónoma de México. He was President of the Mexican College of Rheumatology from 2000 to 2001, and President of the Mexican Board of Rheumatology from 2001 to 2002.

Dr. Jara has written many scientific papers, reviews, and book chapters on different subjects, especially on the role of hormones in systemic lupus erythematosus (SLE) and other aututoimmunne diseases but also on pregnancy in SLE and antiphospholipid syndrome, and on accelerated atherosclerosis in autoimmune diseases. He is married and has three children.

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Contents

Preface v
Series Editor vii
Volume Editors ix
List of Contributors xi

I Pathophysiology

Neuroendocrine Immune Control Mechanisms and their Influence on Autoimmune Disease 3
Silvia Capellino, Rainer H. Straub

Sex Hormones, the Immune System and Autoimmune Diseases 13
Maurizio Cutolo, Silvia Capellino, Rainer H. Straub

Gender Bias in Murine Lupus 21
Elena Peeva, Gisele Zandman-Goddard, Yehuda Shoenfeld

Role of Prolactin in Autoimmune Diseases 29
Annamaria De Bellis, Antonio Bizzarro, Antonio Bellastella

The Pathogenesis of Type 1 Diabetes 45
Jennifer M. Barker

The Genetics of Autoimmune Thyroid Diseases 61
Yaron Tomer

Thyroid Dysfunction and the Immune System 75
Alejandro Ruiz-Argüelles, Mario García-Carrasco

II Clinical Aspects

Autoimmunity and the Pituitary Gland 83
Annamaria Colao, Lídice Bradão Tavares, Rosario Pivonello, Gaetano Lombardi, Diego Ferone

Adrenal Involvement in Systemic Autoimmune Diseases 95
Manuel Ramos-Casals, Pilar Brito-Zeron, Gerard Espinosa, Ricard Cervera

Endometriosis and Autoimmunity 103
Sandra G. Pasoto, Mauricio S. Abrao, Sergio Podgaec, Eloisa Bonfa

Autoimmunity in Turner's, Down's, and Klinefelter's Syndromes 113
Paul E. Belchetz, Carol E. Chu, Ramzi Ajjan

Autoimmune Polyendocrine Syndromes (APS) or Multiple Autoimmune Syndromes (MAS) 135
Corrado Betterle, Fabio Presotto

Endocrine Manifestations of the Antiphospholipid Syndrome 149
Imad Uthman, Munther Khamashta

Autoantibodies and Infertility in Autoimmune Diseases 157
Howard J.A. Carp, Asher Ornoy, Yehuda Shoenfeld

Systemic Lupus Erythematosus and Endocrine Disorders 173
Sara Cortes, Ana Jerónimo, David Isenberg

Pregnancy, Hormones, and Autoimmune Rheumatic Diseases 185
Luis J. Jara, Gabriela Medina, Carmen Navarro, Miguel A. Saavedra, Francisco Blanco-Favela, Luis R. Espinoza

Autoimmune Hypothyroidism and Hyperthyroidism in Systemic Autoimmune Disease 199
R. Hal Scofield

Type 1 Diabetes Mellitus at the Crossroad of Polyautoimmunity 211
Juan-Manuel Anaya, Rodrigo Corena, Verónica Abad

III Hormonal Modulation in the Therapy of Autoimmune Diseases

Estrogen in Systemic Lupus Erythematosus: Lessons from the SELENA Study 223
Sangeeta D. Sule, Michelle Petri

Hormonal Modulation of Autoimmune Diseases: Glucocorticoids 229
Johannes W.J. Bijlsma, Frank Buttgereit, Maurizio Cutolo, José Antonio P. da Silva

Estrogen and Prolactin: Contributions to Autoimmunity in Murine Models of Systemic Lupus Erythematosus 241
Sara E. Walker

Dehydroepiandrosterone 249
Gabriela Schiechl, Rainer H. Straub

Glucocorticoid Therapy for Polymyalgia Rheumatica 257
Roberto Caporali, Carlomaurizio Montecucco

Danazol Therapy 265
Miguel A. Gonzalez-Gay, Ricardo Blanco

IV Novel Therapies

Islet Transplantation for the Treatment of Type I Diabetes 275
Christian Toso, A.M. James Shapiro

The Immunoendocrine Role of Vitamin D in Autoimmunity 293
Yoav Arnson, Howard Amital, Yehuda Shoenfeld

Modulation of Hormone Axes by Anti-TNF Therapy 301
Fabiola Atzeni, Piercarlo Sarzi-Puttini, Maurizio Cutolo, Rainer H. Straub

Subject Index 309

Colour Plate Section

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PART I:

Pathophysiology

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Handbook of Systemic Autoimmune Diseases, Volume 9
Endocrine Manifestations of Systemic Autoimmune Diseases
Sara E. Walker and Luis J. Jara, editors

CHAPTER 1

Neuroendocrine Immune Control Mechanisms and their Influence on Autoimmune Disease

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1. Introduction

The first studies on rheumatoid arthritis (RA) focused on aspects of the immune system and the role of tissue-destructive mesenchymal cells such as fibroblasts and osteoclasts and their factors (Müller-Ladner et al., 1996; Bläß et al., 1999; Walsh and Gravallese, 2004). Since the beginning of the 1980s, it has became evident that patients with RA showed multiple alterations of the endocrine system and the peripheral and even the central nervous system (CNS), and different studies demonstrated that the nervous system can directly alter the immune response (Levine et al., 1985). In order to understand the pathophysiology of RA, it is necessary to consider these different alterations and how they interact with each other. In this review, alterations of the endocrine and nervous systems and new therapeutic strategies are discussed.

2. Systemic neuroendocrine alterations

2.1. Decreased responsiveness of the HPA axis to stressful events

It is well known that stressful events can change the activity of RA (Zautra et al., 1997), but the reasons remained unclear for a long time. Normally, stressful/inflammatory conditions activate the immune system and subsequently the hypothalamic–pituitary–adrenal (HPA) axis through peripheral and central production of inflammatory cytokines. Nowadays we know that patients with RA have plasma cortisol levels similar to control subjects even in the presence of high amounts of circulating inflammatory cytokines (see below) (Crofford et al., 1997). It is obvious that there is a deficit in cortisol secretion relative to inflammation (see 2.2.). After experimentally induced physical or psychological stress, RA patients present inappropriately low cortisol levels because of defects in neuroendocrine axes. For example, as a consequence of physical exercise, inducing changes in cortisol release comparable to psychological stress, serum levels of cortisol decreased in patients with RA but increased in healthy controls (Fig. 1) (Pool et al., 2004). These results reveal that stress in combination with a deficient HPA axis activity leads to an unexpected decrease in cortisol. On the contrary, the stress of insulin-induced hypoglycemia leads to a normal cortisol increase in patients with RA (Rovensky et al., 2002). Therefore, we hypothesize that in RA patients, mild psychological or physical stress leads to activation of the immune system, whereas strong, acute stress (hypoglycemia) can have immunosuppressive effects (Straub et al., 2005).

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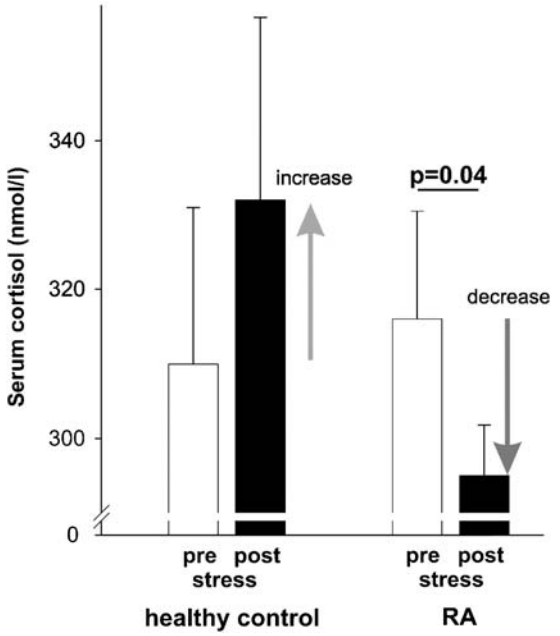

Figure 1. Influence of acute stress on cortisol secretion in patients with rheumatoid arthritis (RA) in relation to healthy controls (Co). Exercise was used as stress paradigm. White bars indicate the serum levels of cortisol before exercise (=stress); dark bars indicate the serum cortisol levels after exercise. (According to Pool et al., 2004.)

2.2. Inadequate cortisol production in relation to systemic inflammation

During the acute phase of RA (first weeks of overt inflammation), the secretion of cortisol, dehydroepiandrosterone (DHEA), and androstenedione increases (acute phase of the pathology). However, after weeks this initial increase of these adrenal hormones normalizes. In contrast, during the course of the chronic disease the secretion of cortisol remains relatively stable (or near normal). This is probably due to adaptation of the hypothalamus, the pituitary gland, and the adrenal gland in the presence of proinflammatory stimuli. However, the production of inflammatory cytokines remains high in the chronic phase of RA. Therefore, in relation to systemic inflammation, the levels of cortisol are inadequately low. It is reported that the serum concentration of cortisol in healthy controls is 230 nmol/l in relation to 1 pg/ml interleukin (IL)-6 (Fig. 2) (Straub et al., 2002). Patients with a milder

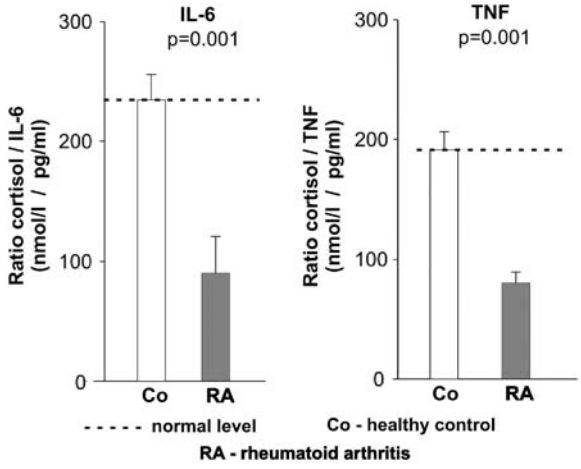

Figure 2. Inadequately low cortisol secretion in relation to serum levels of inflammation markers in rheumatoid arthritis (RA) patients in relation to healthy controls (Co). Abbreviations: IL-6, interleukin-6; TNF, tumor necrosis factor.

form of arthritis (reactive arthritis) demonstrate 130 nmol/l and patients with a highly inflammatory state (RA) have only 90 nmol/l cortisol per 1 pg/ml of IL-6. We can conclude that RA patients in the chronic phase of the disease have only half the amount of anti-inflammatory cortisol compared to the quantity of proinflammatory cytokines, and are therefore not prepared to respond efficiently to inflammation.

2.3. Increase of the sympathetic nervous tone

In patients with RA and other chronic inflammatory diseases, an increased sympathetic nervous tone was reported (Leden et al., 1983; Perry et al., 1989; Kuis et al., 1996; Glück et al., 2000). The reason for high sympathetic activity is probably the inefficient cortisol production in the chronic phase of arthritis. In fact, in order to stabilize blood pressure, systemic circulation, and glucose homeostasis, the sympathetic nervous tone must increase in the presence of relatively low cortisol levels and vasodilatory cytokines such as tumor necrosis factor (TNF). However, the increased sympathetic tonus is not leading to a relevant increase of the anti-inflammatory noradrenaline in inflamed tissue

because achieved concentrations are much too low (an elevated sympathetic tone means a plasma concentration of noradrenaline of 2–5 nmol/l; anti-inflammatory concentrations are in the micromolar range). Therefore, the altered sympathetic activity is not sufficient to compensate for the lack of cortisol.

In contrast, in patients with RA an elevated systemic sympathetic tone possibly leads to increased atherosclerosis and coronary heart disease. It was demonstrated that RA patients have a significantly higher risk of experiencing unrecognized myocardial infarction and cardiovascular diseases such as arterial occlusive events (del Rincon et al., 2001; Maradit-Kremers et al., 2005). The increased risk of cardiovascular diseases cannot be explained using the normal incidence rate in the healthy age-matched population. Therefore, early recognition of RA patients with hyperactivity of the sympathetic nervous system could be important in order to institute measures to prevent cardiovascular diseases. Neuropeptide Y (NPY) is an excellent indicator of sympathetic activity (Morris et al., 1986), and we have demonstrated that serum concentrations of NPY are higher in RA and systemic lupus erythematosus (SLE) patients compared to healthy subjects (Härle et al., 2006). Therefore, NPY could be used as a marker of the sympathetic hyperactivity in patients with chronic rheumatic diseases.

2.4. Alterations of adrenal androgen metabolism

In the inflammatory process typical in RA patients, different physiological systems are altered, but adrenal function undergoes the greatest change. As already mentioned, in the acute phase of RA (first weeks of the disease) the secretion of cortisol, DHEA, and androstenedione increases. However, at the very beginning of the overt inflammatory disease, the level of DHEAS decreases in spite of the constant conversion of pregnenolone to DHEA and progesterone to androstenedione. In this early acute phase, the adrenal gland is able to respond to the increased request for androgens (early acute stress response). During the chronic course of the disease within months and years, the adrenal androgen production decreases, whereas the adrenal cortisol secretion remains relatively stable (Hedman et al., 1992). Hypothalamic and pituitary hormones and systemic circulating cytokines, for example, IL-6 and TNF, are involved in these alterations (Fig. 3). In fact, adrenocorticotropic hormone (ACTH) and IL-6 activate different important enzymes involved in androgen metabolism, whereas the proinflammatory cytokine TNF inhibits different metabolic pathways of androgen conversion (reviewed in Herrmann et al., 2002). In the late phase of the disease, there is a decrease in the production of DHEA, DHEAS, and androstenedione.

2.5. Altered estrogen metabolism

An increased susceptibility of women for most autoimmune diseases, such as RA and SLE, is well known (Lahita et al., 1987; Laivoranta-Nyman et al., 2001). This clinical evidence supports that sex hormones play an important role in regulation of the immune response and cell proliferation (Cutolo et al., 2005). Indeed, several in vitro studies clearly demonstrated that estrogens at low concentrations play a key role in the pathophysiology of RA, as stimulators of the immune response (Cutolo et al., 1996; Capellino et al., 2007), whereas testosterone has an anti-inflammatory effect on immune cells. Although the altered sex hormone metabolism is more evident in the periphery, with significantly higher synovial fluid (SF) levels of estrogens relative to androgens, there is also evidence of unbalanced systemic estrogen metabolism. As already reported, inflammatory cytokines stimulate conversion of androgens into estrogens (Herrmann et al., 2002). The lack of anti-inflammatory androgens and the higher concentration of estrogens might lead to proinflammatory conditions.

In the last years, there has been a growing interest not only in 17β-estradiol and estrone, the two major estrogens, but also in a group of hydroxylated estrogen metabolites, which are important modulators of cell growth (Cutolo et al., 2003) (Fig. 3). Cancer research revealed a mitogenic tumor growth-stimulating role of 16α-hydroxylated estrogens, a finding that supports the potent pro-proliferative activity of these metabolites (Telang et al., 1992). On the contrary, 2-hydroxyestrone seems to

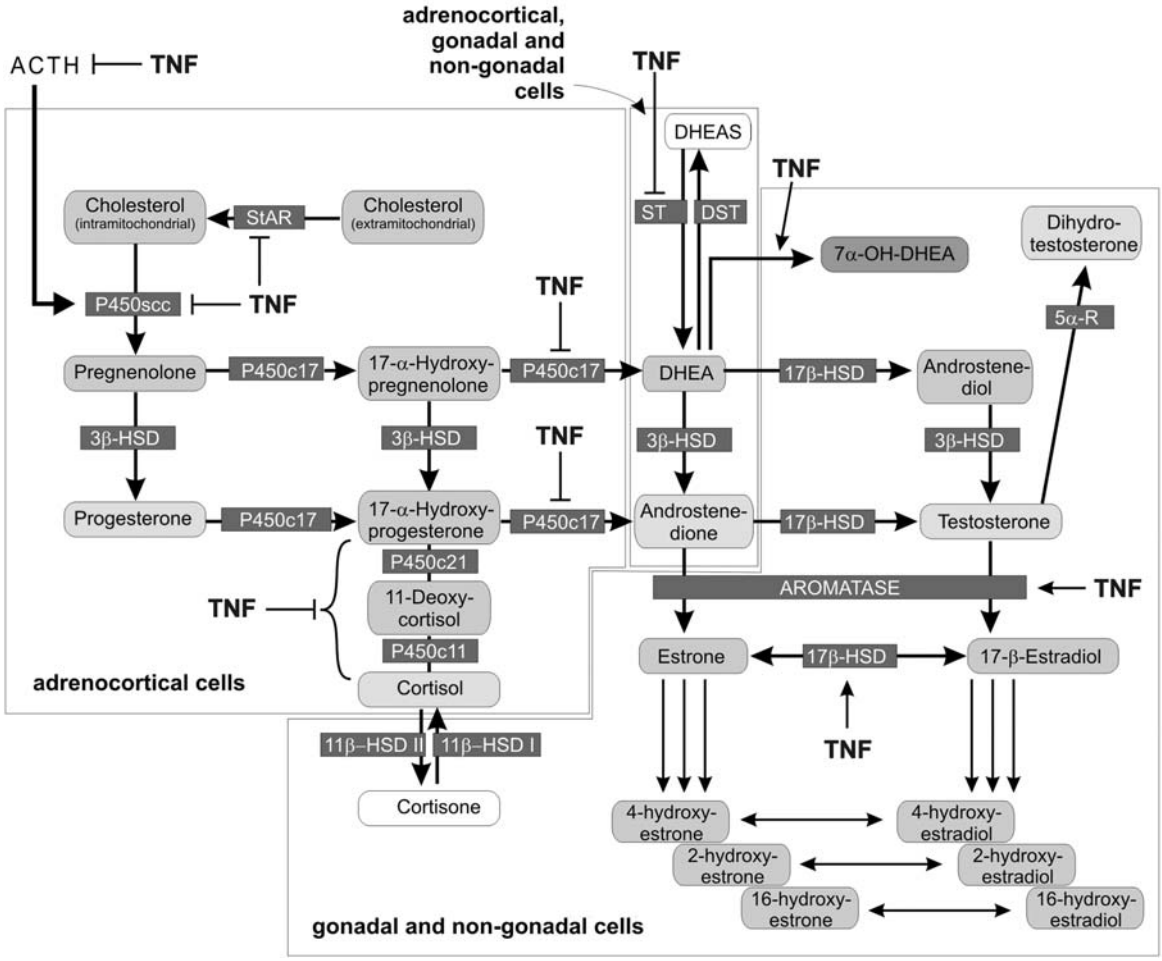

Figure 3. Hormonal conversion in gonadal, non-gonadal, and adrenocortical cells. Red (green) colors indicate proinflammatory (anti-inflammatory) factors. Arrows indicate a stimulatory effect whereas lines with a bar at the end indicate inhibitory effects. Abbreviations: DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; TNF, tumor necrosis factor; ACTH, adrenocorticotropic hormone; StAR, steroidogenic acute regulatory molecule; P450scc, cytochrome P-450-mediated cholesterol side-chain cleavage; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; 11β-HSD, 11β-hydroxysteroid dehydrogenase; 5α-R, 5α-reductase; ST, sulfatase; DST, DHEA sulfotransferase. (See Colour Plate Section.)

exert anti-proliferative effects, at least on breast cancer cells (Schneider et al., 1984; Bradlow et al., 1996). Recently, it has been demonstrated that patients affected by systemic inflammatory diseases such as RA and SLE present an altered balance of several of these metabolites (Castagnetta et al., 2003; Weidler et al., 2004). We found that total urinary loss of 2-hydroxyestrogens was 10 times higher in healthy subjects compared to patients with either SLE or RA irrespective of prior prednisolone treatment or sex, whereas the urinary concentration and loss of 16α-hydroxyestrone did not differ between healthy subjects and patients with RA/SLE (Weidler et al., 2004). The altered estrogen metabolism was also demonstrated in SF of RA patients (see below), but at the moment there are no studies about the role of these estrogen metabolites in inflammatory responses in RA. A better understanding of the roles of downstream metabolites of 17β-estradiol and estrone in the inflammatory process could have important applications to treating inflammatory diseases such as RA.

2.6. Chronic fatigue and depression in RA patients due to elevated circulating cytokines

It is well known that patients affected by RA and other chronic autoimmune diseases show signs of chronic fatigue and depression (Morrow et al., 1994; Dickens and Creed, 2001; Wolfe and Michaud, 2004; Rupp et al., 2004). In recent years, it has been demonstrated that cytokines induce so-called sickness behavior (Dantzer et al., 2002). Also, the injection of lipopolysaccharide into healthy controls led to a significant increase of the depression score (Reichenberg et al., 2001). Further findings demonstrated that sleep and declarative memory deteriorated when cytokine concentrations were elevated (Cohen et al., 2003). Patients with RA under anti-TNF antibody therapy demonstrated a marked reduction in fatigue scores (Wolfe and Michaud, 2004). These findings clearly show that elevated circulating cytokines have an impact on brain function. Furthermore, therapies that block the inflammatory response, such as anti-TNF therapy, could have favorable effects on the CNS.

3. Local neuroendocrine alterations in the inflamed synovium

3.1. Loss of sympathetic nerve fibers

It is widely accepted that substance P, a neurotransmitter of sensory afferents, is proinflammatory (Straub and Cutolo, 2001). Its peptidergic co-transmitter calcitonin gene-related peptide (CGRP), however, has anti-inflammatory capabilities.

With respect to the sympathetic nervous system and its transmitters, the situation is not as uniform as with substance P. Norepinephrine (NE) and adenosine, which are colocalized in vesicles of the sympathetic nerve terminal, are ligands of different receptor subtypes with opposing intracellular signal transduction pathways (Spengler et al., 1994; Eickelberg et al.,1999). Therefore, completely different effects may arise depending on local concentrations. In fact, neurotransmitters of the sympathetic nervous system (noradrenaline, adenosine, and endogenous opioids) have anti-inflammatory effects when local concentrations are high (micromolar range: via β-adrenoreceptors, A2 adenosine receptors, and μ-opioid receptors), and these neurotransmitters exert proinflammatory effects at low doses (nanomolar range: via α-adrenoreceptors and A1 adenosine receptors). It was demonstrated that there are fewer sympathetic nerve fibers in inflamed tissue of patients with RA than in osteoarthritis (OA) or trauma patients (Fig. 4) (Pereira da Silva and Carmo-Fonseca, 1990; Miller et al., 2000). In contrast, SP-positive nerve fibers are somewhat elevated in RA as compared to OA (Miller et al., 2000), and CGRP-positive nerve fibers are similarly reduced as sympathetic nerve fibers (Dirmeier et al., 2008). In summary, there is a large preponderance of sensory pain nerve fibers, which produce the proinflammatory SP, in relation to sympathetic nerve fibers and CGRP-positive nerve fibers, which have an anti-inflammatory role. This preponderance most likely contributes to inflammation and continuous pain because the inhibitory influence of sympathetic neurotransmitters and CGRP is lost relative to the proinflammatory influence of SP.

Interestingly, a significantly higher amount of the exclusively sympathetic nerve repellent factor semaphorin 3C was detected in RA patients (Miller et al., 2004), and it was demonstrated that the cells responsible for the semaphorin 3C production are fibroblasts and macrophages. These findings suggest that semaphorin 3C, which is selectively directed against sympathetic nerve fibers, could be one element responsible for reduced sympathetic innervation in RA tissue. The inability of sympathetic nerve fibers to innervate the synovium could contribute to the chronic nature of RA.

3.2. Noradrenalin-producing synovial cells

Despite the loss of sympathetic nerve fibers in RA patients, the spontaneous release of noradrenalin from synovial tissue (measured with a superfusion technique) is similar in RA and OA patients (Miller et al., 2000). Thus, another source of noradrenalin might be present in RA. In fact, we found cells positive for tyrosine hydroxylase (TH), the key enzyme for production of noradrenalin, in synovial

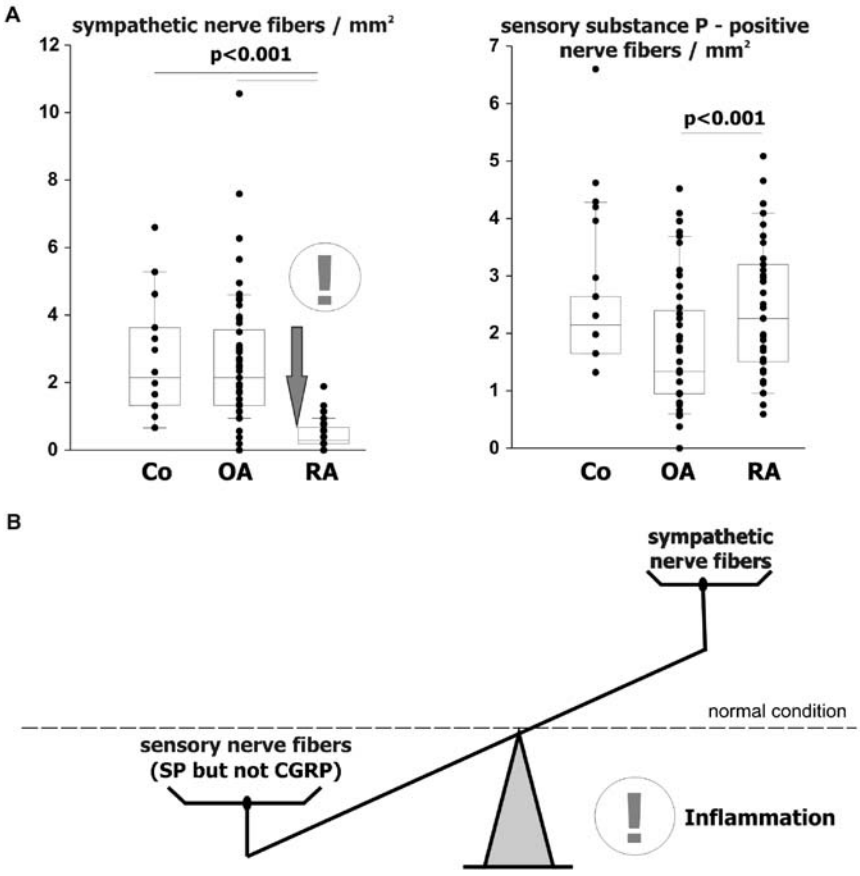

Figure 4. Loss of sympathetic nerve fibers in patients with rheumatoid arthritis (RA). (A) Density of sympathetic (left) and sensory nerve fibers (substance P, right) in synovial tissue of patients with rheumatoid arthritis (RA), osteoarthritis (OA), and healthy controls (Co). (B) The preponderance of substance P-positive nerve fibers (sensory nerve fibers) in relation to sympathetic nerve fibers in patients with RA leads to inflammation. Abbreviations: CGRP, calcitonin gene-related peptide; SP, substance P.

tissue from RA patients. The density of these cells is significantly higher in RA compared to OA patients, and we did not find these cells in tissue from control patients (Fig. 5). These noradrenalin-producing cells seem to replace sympathetic nerve fibers in the synovial tissue. At this time, we do not know whether these cells are also able to secrete NPY, ATP, or endogenous opioids and whether they are really comparable to sympathetic nerve endings. Loss of sympathetic nerve fibers leads to uncoupling of the synovium from the hypothalamus-autonomic nervous system axis. The loss of endogenous sympathetic anti-inflammatory neurotransmitters, together with uncoupling of the synovial tissue, could support the disease process.

3.3. Altered levels of sex hormones in the synovial tissue/fluid

Estrogen metabolism is unbalanced in patients affected by RA. In earlier work, we showed that the SF concentration of free estrogens tends to be higher in RA patients compared to control subjects with traumatic knee injury (Castagnetta et al., 2003). Similarly, the SF estrone is higher in RA patients compared to controls. This elevated production of proinflammatory estrogens does not correlate with a similar elevated production of anti-inflammatory androgens. In fact, the molar ratio of free estrogens/free androgens in SF is significantly higher compared to control subjects. These findings suggest an

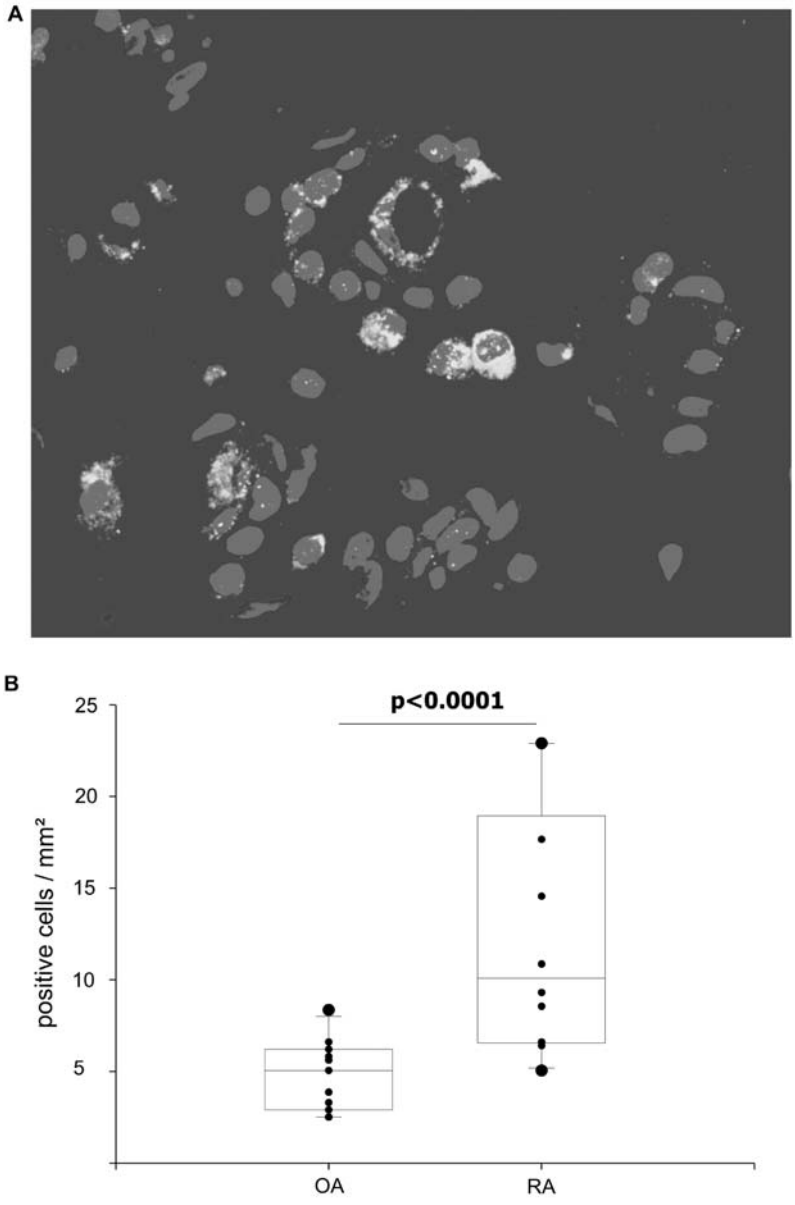

Figure 5. Tyrosine hydroxylase (TH) positive cells in synovial tissue. (A) Immunofluorescence staining of tyrosine hydroxylase (red staining) in synovial tissue from an RA patient, counterstained with DAPI (blue) as unspecific DNA staining. (B) Density of TH-positive cells in synovial tissue from 10 rheumatoid arthritis (RA) and 10 osteoarthritis (OA) patients. The density of TH-positive cells is significantly higher in synovial tissue of RA patients ($p < 0.0001$). (See Colour Plate Section.)

increased activity of aromatase, the enzyme for the conversion of estrogens from the precursor androgens (Fig. 3), which has recently been demonstrated in synovial tissue (Schmidt et al., 2005).

Synovial cells express estrogen receptors, and the high levels of proinflammatory estrogenic hormones could play a crucial role in maintaining the inflammatory response. In fact, in RA patients

the number of cells expressing estrogen receptors correlates positively with the secretion of synovial IL-6 and IL-8 (Capellino et al., 2007). This indicates that inflammatory factors might upregulate expression of estrogen receptors in RA, a finding that was not present in OA. Interestingly, the SF concentration of the biologically active estrogen metabolites 16α-hydroxyestrone and 4-hydroxyestradiol was higher in RA compared to controls (Castagnetta et al., 2003). As the urinary loss of 16-hydroxyestrogens does not differ in RA patients and control subjects (see above), this could suggest only a local imbalance with respect to the mitogenic 16α-hydroxyestrogens in RA.

4. Conclusions

This review summarizes some hormonal and neuronal factors involved in pathophysiology of RA. During the last 15 years, this new scientific approach was largely intensified. It becomes more and more evident that RA pathophysiology can only be explained by taking into account mechanisms outside the immune system. In addition, understanding hormonal and neuronal factors in the pathophysiology of RA probably opens new avenues to new therapeutic targets and genetic markers.

Key points

- In patients with RA one observes a decreased responsiveness of the hypothalamic–pituitary–adrenal axis to stressful events.
- There is inadequate cortisol production in relation to systemic inflammation.
- Patients display an increase of the sympathetic nervous tone.
- Loss of adrenal androgens and local androgens in the joint.
- There is an increase of estrogens in the joint.
- Chronic fatigue and depression in RA patients is very common.

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CHAPTER 2

Sex Hormones, the Immune System and Autoimmune Diseases

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Abstract

Sex hormones interfere with the immune response. Estrogens enhance humoral immunity, and androgens and progesterone are natural immune suppressors. Some physiological, pathological, and therapeutical conditions may change the serum estrogen milieu and/or peripheral conversion rate and these factors include the menstrual cycle, pregnancy, the postpartum period, menopause, old age, chronic stress, altered circadian rhythms, inflammation, the use of corticosteroids, oral contraceptives, and steroid hormone replacements. These factors alter androgen/estrogen ratios and have related effects. Inflammatory cytokines (i.e., TNFα, IL-1, and IL-6) increase aromatase activity, and this action may partially explain abnormal peripheral estrogen synthesis in rheumatoid arthritis (i.e., increased availability of 17β-estradiol and its metabolites in synovial fluids). Similar mechanisms in systemic lupus erythematosus may explain the altered serum sex hormone levels and decreased androgens and DHEAS that have been reported in that disease. The local effects of sex hormones in autoimmune rheumatic diseases seem to consist mainly of altered cell proliferation and cytokine production. Women, who have increased exposure to cell traffic in pregnancy, would be expected to have a higher occurrence of autoimmune conditions.

1. Sex hormones and autoimmune diseases

During the fertile age, women are more often affected by autoimmune rheumatic diseases than men (Kvien et al., 2006). Rheumatic disorders with autoimmune involvement such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are thought to result from the combination of several predisposing factors, including relationships between epitopes of the trigger agent (i.e., virus) and histocompatibility antigens (i.e., HLA), latitude effects, the status of the stress response system, including the hypothalamic–pituitary–adrenocortical axis (HPA), the sympathetic nervous system (SNS), and gonadal hormones (hypothalamic–pituitary–gonadal axis (HPG)) (Bijlsma et al., 2006; Cutolo et al., 2006a, b; Straub and Cutolo, 2006). Pre- and post-menopausal concentrations of circulating sex hormones further influence the occurrence of rheumatic diseases. Obviously, sex hormones play important roles as modulators of disease onset and perpetuation of disease. Like cortisol, sex hormones have circadian rhythms (Cutolo et al., 2005a, b, c).

Sex hormones are involved in the immune response, and estrogens enhance humoral immunity

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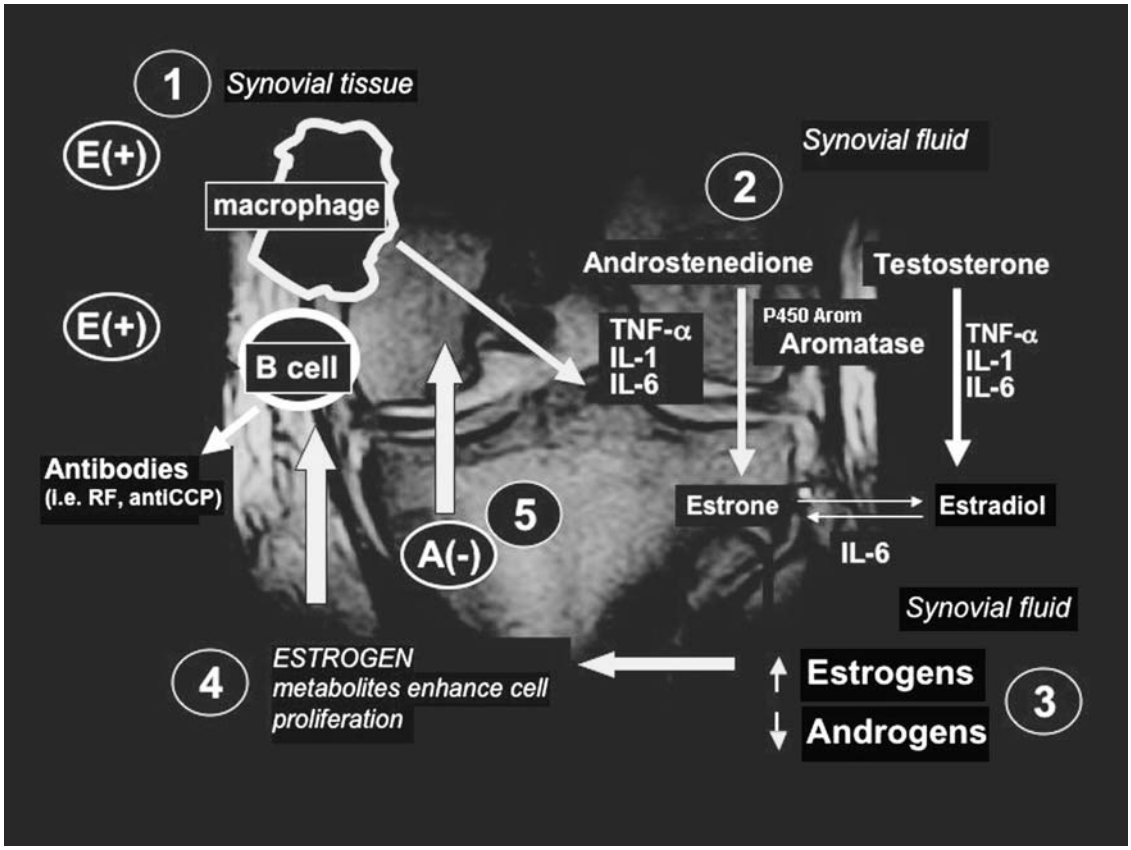

Figure 1. (1) Estrogens enhance the humoral immunity and proliferation of monocytes/macrophages. (2) The pro-inflammatory cytokines (e.g., TNFα, IL-1β, and IL-6) induce aromatases in synovial tissue, thereby accelerating the metabolic conversion of androgens to estrogens. (3) Increased concentrations of estrogens and low androgen concentrations are observed in synovial fluid of RA patients of both sexes. (4) Hydroxylated metabolites of estrogen affect mitogenesis and cell proliferation. (5) Androgens exert apoptotic and anti-proliferative effects. (See Colour Plate Section.)

whereas androgens and progesterone (and glucocorticoids) function as natural immunosuppressors (Cutolo et al., 2005a, b, c; Bijlsma et al., 2006) (Fig. 1(1)). Low concentrations of gonadal and adrenal androgens (testosterone (T), dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA), and its sulfate (DHEAS)), as well as a reduced ratio of androgens to estrogens, have been detected in serum and body fluids (blood, synovial fluid (SF), and saliva) of male and female RA patients, as well as SLE patients. This finding supports a possible pathogenic role for decreased levels of the immunosuppressive androgens (Cutolo et al., 2005a, b, c). In contrast, serum estrogen levels are normal in RA and this lack of a significant change is in strict contrast to the reduced androgen levels typically found in RA patients (Bijlsma et al., 2006).

Physiological, pathological, and therapeutic conditions that can change the serum estrogen milieu and/or peripheral conversion rate include the menstrual cycle, pregnancy, postpartum period, menopause, old age, chronic stress, altered circadian rhythms (i.e., cortisol/melatonin), inflammatory cytokines, use of corticosteroids, oral contraceptives, and steroid hormonal replacement that alter androgen/estrogen ratios (Cutolo et al., 2005a, b, c; Straub et al., 2005). At physiological concentrations, 17β-estradiol and a combination of downstream estrogens stabilized or increased immune stimuli-induced TNF secretion (Janele et al., 2005).

These effects were dependent on the presence of physiological concentrations of cortisol and therefore were related to cortisol's circadian rhythms (Cutolo et al., 2004).

Sex hormones can exert local actions (intracrine) in the tissues in which they are formed or enter the circulation. Both T and 17β-estradiol seem to exert dose and time-dependent effects on cell growth and apoptosis (Bijlsma et al., 2006; Cutolo et al., 2006a, b). These effects, as well as important influences on gene promoters of Th1/Th2 cytokines and the recently discovered increase in SF estrogen concentrations, suggest that estrogens are important in inciting and perpetuating RA (Cutolo et al., 2004; Janele et al., 2005).

Recent data suggest that the sex hormone milieu and B-cell receptor signaling, not antigenic specificity, correlate with the differentiation pathway of B cells. Although both marginal zone and follicular B cells produce anti-DNA antibodies in murine models of SLE, it has been unclear whether these distinct B-cell subsets make identical or different antibodies. Single-cell analysis has demonstrated that the same DNA-reactive B cells can mature to either subset, depending on the hormonal environment. Anti-DNA B cells in estradiol-treated mice become marginal zone cells while identical cells from prolactin-treated mice become follicular cells. Therefore, even the B-cell receptor signaling pathway is influenced by the hormonal environment. These observations have important implications for the pathogenesis and treatment of autoimmune diseases (Venkatesh et al., 2006).

These developments have opened new research avenues. The association between persistent fetal–maternal microchimerism and the development of autoimmune diseases has attracted special interest (Gleicher and Barad, 2007). In analogy to allogeneic organ transplantation, fetal–maternal (and maternal–fetal) microchimerism may play an important role in immunologic tolerance of the fetal semi-allograft. The female preponderance for autoimmune diseases may therefore be understood as a consequence of increased allogeneic cell traffic in females (compared to males) and the increased risk for long-term microchimerism, both of which may lead to abnormal autoimmunity. Under an evolutionary view point, the occurrence of autoimmune diseases, in general, can be seen as the price to be paid for successful reproduction. Women, who are exposed to cell traffic, appear to pay the higher price, reflected in a larger occurrence of autoimmunity.

2. Peripheral sex hormone metabolism in autoimmune diseases

Several findings suggest that conversion of upstream androgen precursors to 17β-estradiol is accelerated in RA and SLE patients. 17β-estradiol, the aromatic product of the gonadal steroid metabolic pathway and the result of peripheral conversion from the adrenal androgen DHEA, has as its upstream precursors hormones such as DHEA, testosterone, and progesterone. In fact, many studies and reviews in the last 20 years have shown reduced serum concentrations of DHEAS, testosterone, and progesterone in both male and female RA and SLE patients (Lahita et al., 1987; Bijlsma et al., 2002). These data strongly support the existence of accelerated peripheral metabolic conversion of upstream androgen precursors to 17β-estradiol.

The discovery of very high estrogen concentrations in SF from RA patients of both sexes can be explained by the results of recent studies showing that inflammatory cytokines (i.e., TNFα, IL-1, and IL-6) are increased in RA synovitis and can markedly stimulate aromatase activity in peripheral tissues (Macdiarmid et al., 1994; Purohit et al., 1995) (Fig. 1(2)). The aromatase enzyme complex is involved in the peripheral conversion of androgens (testosterone and androstenedione) to estrogens (estrone and estradiol, respectively). In tissues rich in macrophages, a significant correlation was found between aromatase activity and IL-6 production. Aromatase has been found also in synoviocytes (Le Bail et al., 2001). Therefore, the increased aromatase activity induced by locally produced inflammatory cytokines (i.e., TNFα, IL-1, and IL-6) might explain the relatively low androgens and high estrogens in synovial RA fluids, as well as the effects of these hormones on synovial cells, as reported from this laboratory (Castagnetta et al., 1999).

The role of local concentrations of sex hormones at the level of inflammatory foci is of great value in explaining the effects exerted by these hormones on the immune-inflammatory reaction. Men with RA have a higher than normal frequency of low testosterone levels. Interestingly, in a recent study, DHEAS and estrone concentrations were lower and estadiol was higher in male RA patients compared with healthy controls (Tengstrand et al., 2003). In this study, estrone did not correlate with any disease variable but estradiol did have strong positive correlation with all measured indices of inflammation. Men with RA had aberrations in all sex hormones analyzed, although only estradiol consistently correlated with inflammation. The low levels of estrone and DHEAS may reflect a shift in adrenal steroidogenesis toward the glucocorticoid pathway, whereas the high estradiol levels seemed to be caused by increased conversion of estrone to estradiol as an effect of 17β-hydroxysteroid dehydrogenase.

In SLE patients, aromatase activity in skin and subcutaneous tissue showed a tendency to be increased compared to control subjects. Aromatase activity in SLE patients varied inversely with disease activity, and the patients had decreased serum levels of androgen and increased levels of estrogen (Folomeev et al., 1992). Therefore, tissue aromatase activity showed significant direct correlation with concentrations of circulating estrogen in SLE patients. These data suggest that abnormal regulation of aromatase activity (i.e., increased activity) could partially explain the abnormalities of peripheral estrogen synthesis (i.e., increased availability of 17β-estradiol and possible metabolites) that have been found in SLE, as well as the altered serum sex hormone levels and ratio, i.e., decreased androgens and DHEAS in SLE (Fig. 1(3)).

Recently, Straub suggested that urinary excretion of hydroxyestrogens (namely, 16α-hydroxyestrone and 2-hydroxyestrogens) reflected production in the tissues, since no respective hydroxylase activity is expected in the urine (Castagnetta et al., 2003; Weidler et al., 2004). On the other hand, as recently reviewed, peripheral estrogen hydroxylation was increased in both men and women with SLE. The estrogenic metabolites have been reported to increase B-cell differentiation and activate T cells (Kanda et al., 1999).

Elevated serum levels of 16α-hydroxyestrone, already described in SLE patients, indicate that men with disease differ from women with disease to the extent that only 16α-hydroxyestrone was elevated in men, whereas women had elevations of both 16α-hydroxyestrone and estriol (Lahita et al., 1979). These data suggest that abnormal patterns of 17β-estradiol metabolism lead to increased estrogenic activity in SLE patients. In the SFs of RA patients, the increased estrogen concentrations observed in both sexes consist mainly of hydroxylated forms, in particular 16α-hydroxyestrone, an endogenous hormone that encourages mitogenesis and cell proliferation (Castagnetta et al., 1999, 2003) (Fig. 1(4)). In these studies, the molar ratio of free estrogens/free androgens was elevated significantly in RA SFs. The serum levels of 17β-estradiol were not typically outside of the physiologic ranges in RA or in SLE patients of both sexes, and the alterations in estrogen metabolism were again observed in both male and female patients (Cutolo et al., 2003; McMurray and May, 2003).

17β-Estradiol is thought to play dual pro- and anti-inflammatory roles in chronic inflammatory diseases, related to low and high concentrations, respectively. Therefore, it is possible that the phenomenon might simply depend on different dose-related rates of peripheral 17β-estradiol conversion to pro- or anti-inflammatory metabolites such as 16α-hydroxyestrone or naturally occurring antagonists (i.e., 2-hydroxyestrogens), respectively (Cutolo, 2004).

3. Immunomodulation by sex hormones

Macrophage release of pro-inflammatory cytokines (TNFα and IL-6) can be modulated by estrogen by different ways. In a recent study, estrogen was found to alter pro-inflammatory cytokine release from activated monocytes and/or macrophages, in particular through modulation of CD16 expression (Kramer et al., 2004). Recent studies showed that 16α-hydroxyestrone was far more potent than 17β-estradiol in exerting an influence on cell proliferative activities (Fig. 1). More recently, we tested the effects of 17β-estradiol and testosterone on differentiation into activated macrophages of

cultured human myeloid monocytic cells (THP-1) in order to evaluate the influence of both hormones on cell proliferation and apoptosis (Cutolo et al., 2005a, b, c). Effects were evaluated using activity of NFk-B, a complex of molecules that affects cellular activation. Testosterone was found to exert proapoptotic effects and reduce macrophage proliferation, whereas 17β-estradiol induced the opposite effects by interfering with NFk-B activities (Fig. 1(5)). These results supported the hypothesis that sex hormones modulate cell growth and apoptosis.

In another investigation, 17β-estradiol was found to increase IgG and IgM production by peripheral blood mononuclear cells (PBMC) from SLE patients, resulting in elevated levels of polyclonal IgG (including IgG anti-dsDNA) by enhancing B-cell activity via interleukin-10 (IL-10) (Folomeev et al., 1992). It would be of great interest to replicate these results in the presence of 16α-hydroxyestrone as well as naturally occurring 2-hydroxylated anti-estrogen.

It was reported recently that disease activity in SLE patients had negative correlation with urinary concentration of 2-hydroxylated estrogens (Weidler et al., 2004). In addition, interesting changes of serum estrogens that correlated with cytokine variations have been found during pregnancy in SLE patients (Doria et al., 2002, 2004). The major hormonal alteration observed during SLE pregnancies was an unexpected lack of an increase in serum estrogen levels and, to a lesser extent, increased serum progesterone levels, during the second trimester and especially during the third trimester of gestation. The failure of the hormones to increase was likely due to placental compromise. In addition, a lower than expected increase of IL-6 in the third trimester of gestation and persistently high levels of IL-10 during pregnancy seem to be the major alterations of the cytokine milieu in the peripheral circulation of pregnant SLE patients. In conclusion, these variations of steroid hormones and cytokines may result in suppressed activation of humoral immune responses, probably due to a change in the estrogen/androgen balance. In turn, the disordered balance of hormones could account for the increased immunosuppressive effect exerted by cytokines on disease activity during the third trimester.

A recent study evaluated whether patients with SLE experience a decrease in disease activity after natural menopause (Sanchez-Guerrero et al., 2001). Differences in disease activity scores and the number of visits to a rheumatologist's office were only significant when the fourth year before menopause was compared with the fourth year after menopause (Doria et al., 2002). Disease activity was mild during the pre-menopausal and post-menopausal periods in women with SLE and a modest decrease, noted especially in maximum disease activity, was observed after natural menopause (Sanchez-Guerrero et al., 2001).

4. Conclusions

Sex hormones can exert local actions (intracrine) in the tissues in which they are formed and an accelerated peripheral metabolic conversion of upstream androgen precursors to 17β-estradiol and even conversion to more estrogenic metabolites is observed in RA and SLE patients. The circadian rhythms of cortisol and melatonin circadian rhythms are altered, at least in RA, and these alterations also partially involve circadian synthesis and levels of sex hormones (Cutolo et al., 2006a, b). Local effects of sex hormones in autoimmune rheumatic diseases seem to consist mainly of modulation of cell proliferation and cytokine production and may also affect the development and activation of specific mature B-cell subsets (Peeva and Zouali, 2005). In this respect, it is interesting that male patients with RA seem to profit more from anti-TNFα treatment strategies than do female patients (Straub et al., 2006). In fact, blockade of TNF-induced upregulation of aromatase would particularly increase the level of androgens in male as compared with female patients with RA, and this can lead to the better clinical outcome that has already been reported in male patients. Certainly, endogenous and exogenous hormones have great potential to affect the immune system and can change activity of autoimmune diseases, and it is worthwhile to continue to seek novel and improved applications of hormonal or/and anti-hormonal immunotherapy (i.e., anti-estrogens, receptor modulators, antagonist metabolites, and

androgenic compounds) to treating this family of diseases. In conclusion, sex hormones play a role in the genesis of autoimmunity and future research may provide a therapeutic approach that is capable of altering disease pathogenesis, rather than targeting disease sequelae (Ackerman, 2006).

Key points

- Estrogens enhance humoral immunity, and androgens and progesterone are natural endogenous immune suppressors.
- Inflammatory cytokines (i.e., TNFα, IL-1, and IL-6) increase aromatase activity, and this action may partially explain abnormal peripheral estrogen synthesis in rheumatoid arthritis (i.e., increased availability of 17β-estradiol and its metabolites in synovial fluids).
- The local effects of sex hormones in autoimmune rheumatic diseases seem to consist mainly of altered cell proliferation (i.e. estrogens enhance) and cytokine production.

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CHAPTER 3

Gender Bias in Murine Lupus ☆

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Abstract

Murine experimental studies demonstrate that the gender bias in the prototypical autoimmune disease systemic lupus erythematosus (SLE) may be influenced by sex hormones and genetic factors. The female hormone estrogen, as well as prolactin, act as immunostimulators that accelerate the onset of disease and cause early mortality in the NZB/W F1 murine model of lupus. Both estrogen and prolactin induce lupus in mice which are not spontaneously autoimmune by impairing the deletion of autoreactive B cells and skewing their maturation toward marginal zone phenotype and follicular phenotype, respectively. Blockade of estrogen and inhibition of pituitary prolactin secretion with selective estrogen receptor modulators and bromocriptine, respectively, provide a therapeutic effect in lupus-prone mice, as well as in mice with estrogen or prolactin-induced lupus. The effects of the hormones are genetically determined, and it has been established that certain genetic factors such as the lupus susceptibility locus *Sle3* synergize with prolactin to allow the development of lupus. In contrast to female sex hormones, androgens have an ameliorative effect on disease activity in lupus-prone mice.

Alterations of specific gender-associated genetic factors are linked to murine lupus. The Yaa mutation on the Y chromosome of lupus-prone BXSB male mice contains a duplication of a set of genes from the X chromosome. One of these duplicated genes encodes for TLR7, and the extra copy of this gene leads to a doubling of the RNA receptor and makes the BXSB mice predisposed to an autoimmune response to the body's own RNA.

Our review of the impact of gender via sex hormones and explicit genetic factors in the pathogenesis of murine lupus suggests that a better understanding of mechanisms underlying sexual dimorphism of the immune system may lead to the development of novel therapeutic approaches to lupus.

1. Introduction

The sexually dimorphic prevalence of autoimmune diseases remains one of the most intriguing clinical observations among this group of diseases. A wealth

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of clinical and laboratory data generated over the past 20 years demonstrate that sex hormones affect the immune system by modulating multiple immune functions including lymphocyte maturation and activation, synthesis of autoantibodies, and cytokines.

The most solid data on the effects of female sex hormones on the immune system have been derived from experimental work in mice. Murine studies evaluating the effects of sex hormones on immune cells and on generation, survival, and activation of autoreactive cells have contributed greatly to our understanding of the pathogenesis of lupus and the female predominance of this disease. This chapter will review studies on gender bias in murine lupus and will focus on hormonal and sex chromosome–associated factors that are linked with lupus.

2. The female sex hormones in the pathogenesis of lupus

Female sex hormones can initiate or accelerate an autoimmune process and thereby contribute to gender-biased autoimmune disorders. Estrogen and prolactin are both considered immunomodulating hormones that are implicated in autoimmunity. Not only endogenous estrogens, but also environmental estrogens may act in conjunction with other factors to override immune tolerance to self-antigens (Peeva and Zouali, 2005). There is much evidence for the role of sex hormones in the pathogenesis of systemic lupus erythematosus (SLE) based on molecular studies, experimental animal studies, and clinical observations. In the B-cell compartment, both prolactin and estrogen affect maturation and selection of autoreactive B cells and autoantibody secretion, while progesterone is an immunosuppressor. The impact of prolactin and estrogen may be based on their capacity to allow autoreactive B cells to escape the normal mechanisms of tolerance and mature to fully functional antibody-secreting B cells that can cause clinically apparent lupus. Studies in mice expressing a transgene for a pathogenic anti-DNA antibody have shown that both hormones impair tolerance induction by interfering with the negative selection of autoreactive B cells during the transitional stage of B-cell development in the spleen. Normally, the transitional T1 B cell subset is bigger than the transitional T2 B cell subset and the smaller number of transitional T2 B cells reflects the fact that many T1 B cells undergo BCR-mediated deletion because of their autoreactivity. Thus, the T1–T2 interface is an important checkpoint for tolerance induction. Both estrogen and prolactin have the capacity to impair negative selection of autoreactive B cells by inverting the T1 to T2 ratio. Interestingly, these hormones skew further B-cell maturation in different directions. Estrogen leads to the survival and activation of autoreactive B cells with a marginal zone phenotype (Grimaldi et al., 2001), whereas prolactin induces self-reactive B cells with a follicular phenotype (Peeva et al., 2003).

In murine models of lupus, female NZBXNZW F1 lupus-prone mice develop the disease earlier and have shorter life spans than males (Steward and Hay, 1976). In female NZB/W F1 mice, ovariectomy significantly reduces the development of autoantibodies (Roubinian et al., 1978). Treatment with estrogen or prolactin exacerbates disease activity and causes early mortality (Peeva and Zouali, 2005). These observations lead to the idea that agents modulating estrogen activity or blocking estrogen synthesis may have a therapeutic effect in lupus. Treatment with the selective estrogen receptor modulator (SERM) tamoxifen, a widely used agent in the therapy of breast cancer, ameliorated lupus activity in NZB/W F1 female mice and the beneficial effects of the agent were associated with a specific reduction in IgG3 autoantibody titer (Sthoeger et al., 2003). In addition, tamoxifen prevented the development of estrogen-induced murine lupus by deterring DNA-reactive B cells from becoming marginal zone B cells (Peeva et al., 2005), which are known to harbor autoreactivity in the estrogen-induced model of lupus (Grimaldi et al., 2001, 2005). In addition, NZB/W F1 mice treated with the anti-estrogen agent, nafoxidine, displayed reduced serum anti-DNA antibody levels, decreased proteinuria, and increased survival (Duvic et al., 1978). Treatment with raloxifene, a SERM used extensively in the therapy of osteoporosis, increased survival in MRL/lpr lupus-prone mice by mitigating the progression of lupus nephritis (Apelgren et al., 1996).

Recent studies showed that raloxifene in NZB/W F1 mice can postpone the onset of lupus and delay the development of nephritis (Peeva, unpublished data). In addition, blockade of estrogen synthesis with the aromatase inhibitor, 4-hydroxyandrostendione, improved the activity of lupus nephritis in MRL/lpr mice (Greenstein et al., 1993), and the treatment with letrozol, a newer aromatase inhibitor, postponed the onset of lupus in ovariectomized NZB/W F1 mice (Peeva, unpublished data).

A small clinical trial involving 11 lupus patients yielded no evidence that tamoxifen had an ameliorative effect on the clinical activity or laboratory indices of SLE (Sturgess et al., 1984). Also, a clinical trial of 16 SLE patients assigned to receive raloxifene, and 17 controls demonstrated no effect of the SERM on the disease activity measured by SLEDAI (Mok et al., 2005). The reasons for the discrepant results between the effects of the SERMs in murine studies and these small trials in humans are not clear.

Twenty to thirty percent of patients with SLE exhibit mild-to-moderate hyperprolactinemia (Szyper-Kravitz et al., 2005), and in a significant number of these patients, lupus activity or specific organ involvement correlates with the serum prolactin levels (Munoz et al., 1994; Mc Murray et al., 1995; Miranda et al., 1998). Unstimulated peripheral blood monocytes (PBMs) from SLE patients tend to produce more prolactin than the PBMs from healthy individuals, and the hormone induces the production of anti-dsDNA antibodies by PBMs (Gutierrez et al., 1995). In addition, prolactin leads to the production of IFNγ, an important mediator in the pathogenesis of SLE (Golbus et al., 1988) and especially lupus nephritis (Chen et al., 2003). Hyperprolactinemia accelerates disease activity in NZB/W F1 lupus-prone mice (McMurray et al., 1993, 1994a, b). In female NZB/W F1 mice, persistently increased serum prolactin levels induced elevated serum IgG and circulating immune complexes, early proteinuria, and accelerated mortality (McMurray et al., 1993). In male *NZB/W F1* mice, in which the disease develops later and has a milder course, hyperprolactinemia induced early onset of lupus and premature mortality. Thus, a sustained mild-to-moderate increase in serum prolactin levels was sufficient to accelerate disease activity and decrease longevity in NZB/W F1 mice. However, no linear correlation between the degree of hyperprolactinemia and disease activity has been observed, since both mild to moderately increased serum prolactin levels as well as very high serum prolactin levels have similar effects on lupus activity (Walker et al., 1992).

Sustained increases in serum prolactin levels also induce a lupus-like syndrome in mice that are not genetically predisposed to the disease (Peeva et al., 2003). Moderately increased serum prolactin levels break tolerance by impairing negative selection of autoreactive B cells and allowing for their maturation into fully functional B cells with a follicular phenotype (Peeva et al., 2003). Follicular B cells are T cell–dependent, and therefore the effects of prolactin are highly reliant on T cell–B cell interactions and the activation of the CD40-CD40L costimulatory pathway (Noelle and Erickson, 2005).

Indirect support for the role of prolactin in the pathogenesis of lupus has been provided by studies that investigated the therapeutic effects of inhibiting prolactin secretion with the dopaminergic agent, bromocriptine. Treatment with bromocriptine led to lower anti-dsDNA antibody titers and improved survival of NZB/W F1 mice compared with their untreated littermates (McMurray et al., 1991). Bromocriptine also suppressed the development of anti-DNA antibodies in MIV-7 monoclonal antibody-induced lupus in *BALB/c* mice. These murine studies led to clinical trials of bromocriptine in SLE patients (Alvarez-Nemegyei et al., 1998). Bromocriptine was beneficial in a small study of SLE patients with mild-to-moderately active disease, leading to decreased serum immunoglobulin and anti-DNA antibody levels. Discontinuation of bromocriptine was followed by a flare of disease activity (McMurray et al., 1995). Another small clinical trial reported that the therapeutic effect of bromocriptine was comparable to that of hydroxychloroquine, a well-accepted treatment for cutaneous and articular manifestations of SLE (Walker et al., 1998).

Not only the independent immunomodulatory effects of estrogen and prolactin, but also the interplay between these hormones may influence immune cells and their effects. Estrogen increases prolactin secretion, whereas elevated serum

prolactin levels suppress the production of estrogen. The fact that prolactin induces autoreactive B cells with follicular phenotype and estrogen induces autoreactive B cells with marginal zone phenotype indicates that there is hormone-specific mechanism of action, but several experimental studies suggest that baseline prolactin levels are still needed for the effects of estrogen to take place. In NZB/W F1 mice manipulated to create combinations of low and high concentrations of serum estrogen and prolactin, hyperprolactinemia accompanied with either low or high serum estrogen levels led to accelerated nephritis and early mortality. The mice with high estrogen/high prolactin levels also had more anti-DNA antibodies compared to the mice in the low estrogen/low prolactin and the high estrogen/low prolactin groups (Elbourne et al., 1998). In addition, treatment with bromocriptine prevented estrogen-induced lupus in mice transgenic for a pathogenic anti-DNA antibody (Peeva et al., 2000). These studies imply that a baseline prolactin level is necessary for the development of estrogen-induced lupus, as well as for acceleration of disease activity in lupus-prone mice.

3. Male sex hormones in the pathogenesis of lupus

Male androgenic hormones also affect functions of the immune cells and therefore seem to be implicated in the pathogenesis of lupus. Male NZB/W F1 mice develop lupus later than their female counterparts. In contrast, castration of male NZB/W F1 mice leads to early disease onset and a lifespan shorter than that of their intact male littermates. A decreased androgen/estrogen ratio has been associated with lupus, and treatment with androgens showed beneficial effects on disease activity in lupus-prone mice (Steinberg et al., 1979). Therapy with the mild androgen dehydroisoandrosterone prevented the production of autoantibodies and prolonged survival in NZB/W F1 mice (Lucas et al., 1985). Dehydroepiandrosterone (DHEA), an intermediate compound in testosterone metabolism, modified the expression of CD4-related cytokines in NZB/W F1 mice. Mice receiving DHEA displayed delay in the expression of IL-10 and IL-6 and early expression of IL-12 transcripts as well as increased production of IL-2 (Yang et al., 1998). Immunomodulatory effects of DHEA were characterized by an increased production of Th1 cytokines, and human studies demonstrated that female SLE patients had accelerated oxidation of testosterone and defective DHEA activity (Suzuki et al., 1996). These observations led to clinical trials of DHEA in patients with SLE. Five controlled clinical trials performed during the past decade suggested that 200 mg/day of DHEA for 7–12 months decreased the requirement for corticosteroid and reduced the frequency of disease flares in female lupus patients (van Vollenhoven, 2002).

4. Gender-associated genetic factors and lupus

Specific genetic factors located on the X chromosome may be implicated in the development of lupus. This thesis is supported by the observation that the X chromosome includes genes that are crucial in determining sex-hormone levels and maintaining tolerance. For example, the gene encoding the costimulatory molecule CD40L is located on the X chromosome. The CD40-CD40L costimulatory pathway, a critical player in T-cell and B-cell activation, is upregulated in lupus (Grewal and Flavell, 1997). A recent study found that epigenetic dysregulation, such as gene-specific DNA demethylation of CD40L on the inactive X chromosome, occurs in female SLE patients and causes overexpression of CD40L exclusively in women (Li et al., 2006). Whether this same mechanism operates in the murine models of lupus has not been examined.

Duplication of specific genes from the X chromosome plays a role in the development of lupus in certain strains of lupus-prone mice. BXSB mice, a murine model of male lupus, were recently found to have a duplication of genes from the X chromosome on the Y chromosome. Y-linked autoimmune acceleration (Yaa), a major genetic feature of the BXSB mouse (Merino et al., 1992), contains duplicated copies of several X chromosome genes, one of which is the gene encoding for TLR7, a receptor for RNA. This extra copy of the *TLR7*

gene leads to doubling of the RNA receptor, thereby making BXSB mice more likely to mount an autoimmune response to the body's own RNA (Pisitkun et al., 2006). Yaa is not sufficient to induce frank autoimmunity in non-autoimmune B6 mice, but the addition of the lupus susceptibility locus *Sle1* to B6.yaa mice led to the occurrence of fatal lupus (Subramanian et al., 2006). These studies demonstrated the complexity of the genetic factors and genetic interactions implicated in the pathogenesis of lupus, and indicate that specific loci on the sex chromosomes may require the presence of loci from other chromosomes in order to induce breakdown of tolerance and the development of lupus.

5. Genetic factors and the responsiveness to sex hormones in lupus

Murine studies in prolactin-induced lupus have demonstrated that immunomodulatory effects of the hormone are genetically determined. Treatment with prolactin induced breakdown of B-cell tolerance and the appearance of a lupus-like syndrome in mice with the BALB/c genetic background. The lupus syndrome was not induced in mice with the C57Bl/6 background (Peeva et al., 2003). Interestingly, the lupus susceptibility interval *Sle3/5* that was derived from NZM2410 lupus-prone mice conferred B-cell responsiveness to prolactin in C57Bl/6 mice. These animals developed anti-dsDNA antibodies and immune complex–mediated glomerulonephritis (Peeva et al., 2006). The effects of prolactin in this murine model are mediated, at least in part, by upregulation of the CD40-CD40L costimulatory pathway. Nevertheless, the specific genes from the *Sle3/5* interval that confer responsiveness to prolactin, either directly or via epistatic interactions, and thereby allow hormone-mediated breakdown of B-cell tolerance, remain to be elucidated.

6. Conclusions

Female predominance in autoimmunity, specifically lupus, is well established and may be related to sex hormones and/or sex-related genes. As suggested by animal models, estrogen and prolactin may break tolerance and induce lupus, whereas androgens ameliorate lupus activity. The X chromosome and its alterations, including duplication of certain genes, seems to be involved in the pathogenesis of the disease. A better understanding of the gender-related hormonal and genetic factors that are implicated in the development and progression of murine lupus may point the way to novel targets for investigations that lead to new therapies for SLE.

Key points

- Sex hormones modulate disease activity in murine models of lupus.
- Immunomodulatory effects of prolactin, and possibly estrogen, are genetically determined.
- Alterations of specific gender-associated genetic factors, such as the Yaa mutation in lupus-prone BXSB mice, are linked to murine lupus.
- Better understanding of the impact of gender via sex hormones and specific genetic factors in the pathogenesis of murine lupus may lead to the development of novel approaches for treatment of SLE.

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CHAPTER 4

Role of Prolactin in Autoimmune Diseases

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1. Introduction

Prolactin (PRL) is a pituitary hormone whose main role is the induction of lactation. However, more than 300 biological activities have been attributed to PRL, which can be subdivided into 5 categories: reproduction, endocrinology and metabolism, control of water and electrolyte balance, growth and development of brain and behavior, and finally, immune regulation and protection (Bole-Feysot et al., 1998; Goffin et al., 2002). In particular, PRL regulates the differentiation of secretory glands including the mammary gland, ovary, prostate, submaxillary and lacrimal glands, pancreas, and liver (Ben-Jonathan et al., 1996). PRL also regulates proliferation in different cell types, including immune cells (Chikanza, 1999; Li-Yuan, 2002). These multiple actions suggest that PRL has actually many other targets than the mammary glands in which it modulates many physiological processes. PRL, released not only by anterior pituitary gland but also by other extrapituitary sites, exerts these actions by its binding to PRL receptors (PRL-Rs) (Bazan, 1989; Matera, 1997). Over the past two decades many clinical, animal, and in vitro studies have supported the immunostimulatory role of PRL (Dorshkind and Horseman, 2000). In particular, the role of PRL in the interrelationship between the endocrine and the immune system has a biphasic character: under basal conditions the function of immune system does not require PRL action, while under stress condition it does; PRL exerts its immune activity in the context of stress, trauma, injury, inflammation, infection, and various autoimmune diseases (Dorshkind and Horseman, 2001; Kelley et al., 2007). In fact, genetic modified animal models with PRL and PRL-R deficiencies showed marked deficit in mammary gland development and in reproductive function but not alterations of the immune system (Horseman et al., 1997). Instead, immune defects have been observed only in animals under stressful environment (Bouchard et al., 1999).

2. PRL and the neuroendocrine-immune network

The neuroendocrine and the immune system are linked through a regulatory loop that allows a bidirectional communication between them (Besedovsky and del Rey, 1996). These interactions are mediated by hormones produced by hypothalamus and pituitary gland acting on the immune cells and by cytokines produced by the hemopoietic tissues that exert regulatory influences on the hypothalamic–pituitary axis (Chikanza and Grossman, 2000). Such interactions are necessary for the maintenance of organism homeoastasis during stress, infections, and autoimmune diseases (Jara et al., 2006). These conditions are known to evoke a neuroendocrine

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response termed 'general adaptive syndrome' in part characterized by stimulation of hypothalamic–pituitary–adrenal (HPA) axis resulting in an increased production of glucocorticoids (Selye, 1998; Nithya and Buckley, 2004) which induces thymic involution, decrease of CD4+/CD8+ thymocytes and apoptosis (Zoumakis et al., 2004). In addition, stress also induces an increase of PRL and GH, which have been shown to counteract the effect of glucocorticoids (Chikanza, 1999). This interpretation is supported by in vitro studies showing PRL-protective effect in preventing glucocorticoid-induced lymphocyte-cell death (apoptosis) (La Voie and Witorsch, 1995; Buckley, 2001). At last, PRL and GH seem to act as stress-adaptation molecules important in maintaining immune system homeostasis (Richards and Murphy, 2000; Fig. 3).

3. Prolactin receptors

PRL is a four-helix bundle polypeptide with a strong homology to GH and placental lactogen and a weak homology to some cytokines such as interleukin 6 (IL6). In the human, mouse, and rat genome a single gene found on chromosome 6, encodes for PRL mapping in humans closely to the MHC (on chromosome 6: 6p22.2–p21.3) (Matera and Mori, 2000). *PRL* gene is 10 kb in size and it is composed of five exons and four introns. It has been demonstrated that the immune cells may produce PRL (paracrine/autocrine secretion) (Hooghe et al., 2004). The expression of *PRL* gene (messenger RNA, protein) has been evidenced in human T cells, in monocytes, and in B cells (Pellegrini et al., 1992). Moreover, it has been suggested that PRL gene expression in lymphoid cells is regulated independently from the pituitary transcription factor Pit-1 due to the presence of a five non-coding exon (exon1a); the immune cell transcript of the *PRL* gene is 150 nucleotides longer than its pituitary counterpart: the PRL peptide produced from this mRNA is, however, not different from that of pituitary origin (Gellersen et al., 1994).

Three principal forms of PRL of 24, 21, and 11 kDa are synthesized and released by the immune cells. Moreover, a portion of secreted PRL is phosphorylated; since phosphorylated PRL is reported to act as a partial agonist, the ratio between unphosphorylated and phosphorylated PRL may be physiologically relevant. As a result, PRL may be considered not only as an immune-stimulatory endocrine factor but also as an autocrine or paracrine immune-regulatory cytokine (Gutierrez et al., 1995). Moreover, in human immune cells, PRL can be regulated by cytokines and other immune-specific agonists (Gerlo et al., 2005).

The PRL activities are mediated by the PRL-R, a member of cytokine receptor superfamily that includes receptors for growth hormone, many cytokines, and some growth factors (Bazan, 1989; Matera, 1997).

The *PRL-R* gene is localized to chromosome 5p13, and is composed by 10 exons (Gearing et al., 1993). The PRL-R is characterized by a single hydrophobic transmembrane domain which divides the receptor into an extracellular ligand binding domain and an intracellular domain homologous to the GH receptor (Horseman, 2002). In particular, the PRL-R is activated after the dimerization, which occurs after the ligand binding. Activated PRL-R evokes the activation of Janus kinase/signal transducer (JAK) which leads to tyrosine phosphorylation of PRL-R followed also by tyrosine phosphorylation of latent signal transducer and activators of transcription (STAT) molecules (Dorshkind and Horseman, 2000). In particular, STAT-5 transactivation is required for transcription of several genes, including interferon regulatory factors-1 (IRF-1) and suppression of cytokine signaling (SOCS) genes (Fig. 1). At last, pituitary PRL or immune cell–derived PRL activates the JAK 2/STAT pathway in immune cells to stimulate the expression of IRF-1 which subsequently stimulates the transcription of γ-interferon (γIFN) (Hooghe et al., 2004). PRL exerts a regulating effect on IRF-1, an important immune factor in mediating anti-virus and anti-bacterial responses, T-helper 1 (Th1) immune responses, macrophage and dendritic cell (DC) function, natural killer (NK) differentiation, cell-cycle progression, and apoptosis.

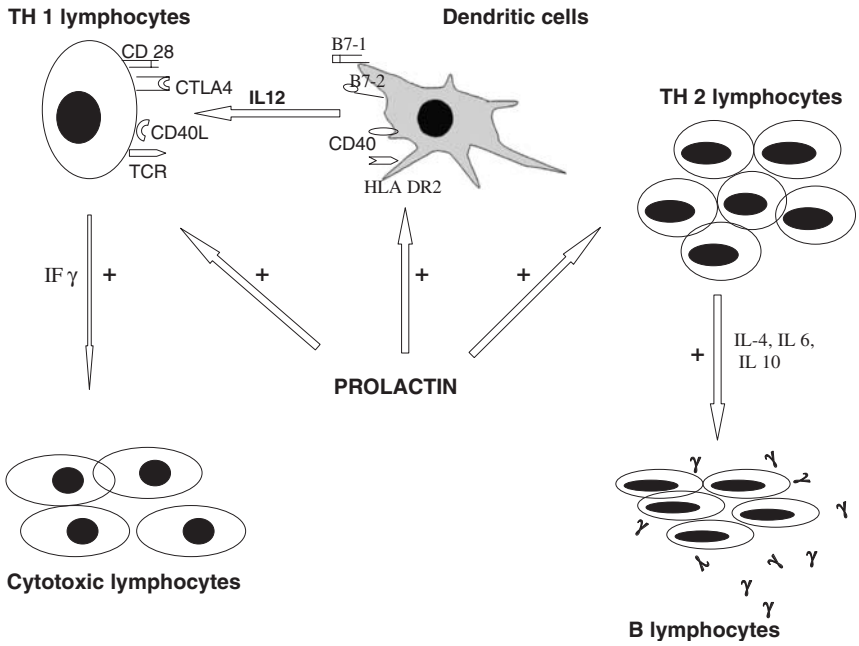

Figure 1. Relationship between PRL and the immune system. Prolactin induces activation of dendritic cells (DC), by increasing the expression of the antigen-presenting MHC class II molecules and costimulatory molecules B7-1, B7-2, and CD40 then increasing the release of IL-12, which activates Th1 cells. PRL can also directly activate of Th1 cells and could also upregulate Th2 cells.

4. PRL and immune system

Exogenous PRL added to concanavaline A has been shown to induce an increase of lymphocyte proliferation; the addition of antibodies to PRL on lymphocyte culture produces a profound inhibition of cell proliferation (Hartmann et al., 1989). PRL has also been shown to stimulate macrophages, DC, T cells, B cells, and NK cells (Kooijman et al., 1996). Two research groups demonstrated a potent effect of PRL on the differentiation and maturation of DC, the most powerful antigen-presenting cells (APC) (Matera et al., 2000a, 2001; Richards and Murphy, 2000). The initial event in the immune process is the presentation of antigen which is processed by DC into a peptide epitope and then binds to the polymorphic region of the major histocompatibility complex (MHC) class 2. To stimulate T cells a subsequent interaction with costimulatory molecules B7-1, B7-2 on DC; and CD28 and CTLA-4 on T lymphocytes is required. Moreover, the interaction between CD40-l expressed on Th cells and CD40 induces DC differentiation which is accompanied by an increased synthesis of IL-12. IL-12/DC increase drives the development of Th1 cells from native T0 cells. Physiological concentrations of PRL added in vitro to GM-CSF are able to drive monocytes into the DC differentiation pathway; instead supraphysiological concentrations can alone increase the differentiation of monocytes into immature DC and subsequently into mature DC (Matera et al., 2000b). PRL also increases the expression of MHC class 2 and then costimulatory molecules B7-1 and B7-2 by DC leading to a more efficient antigen presentation. PRL alone also increases the expression of CD40 by DC and then the release of IL12 favoring a shift of Th response toward Th1 (Matera et al., 2000b; Vera-Lastra et al., 2002). In subsequent stages of the immune process, PRL directly stimulates γIFN release by IL12 (Matera and Mori, 2000). PRL could upregulate not only Th1 but also Th2 cytokines (IL4, IL6, IL10) (Fig. 1). PRL affects B-cell development and maturation causing a

decrease of immature B cells and an increase of all mature ones (Morales et al., 1999). In fact, the proliferation and plasmacytic differentiation of human B cells and IgG production are increased by adding human PRL (10 or 100 ng/ml). At the molecular level PRL causes upregulation of the costimulatory molecule CD40 and the anti-apoptotic protein bcl-2 in B cells. Thus, upregulation of CD40 expression may represent a mechanism by which PRL enhances autoantibody production. Therefore, the upregulation of Bcl-2 expression mediated by PRL may represent a crucial mechanism for the decreased immature B cell apoptosis contributing to the increased number of the mature B cells (Lahat et al., 1993; Krumenacker et al., 1998; Kochendoerfer et al., 2003; Peeva et al., 2004).

5. PRL on Th1/Th2 balance in autoimmune diseases

Several clinical and experimental findings suggest that autoimmune diseases may be the result of a shift in the balance between Th1 and Th2 cytokine responses and then PRL could play a pivotal role in this process (De Bellis et al., 2005; Jara et al., 2006). It has been shown that the prevalent action of either Th1 or Th2 could determine not only the development of a particular autoimmune response but also the progression or less toward a clinical stage of the disease. In particular, when PRL stimulates prevalently DC/Th1 cells the activity of the corresponding autoimmune diseases may be exacerbated; instead, when PRL stimulates predominantly Th2 cells, a possible activity remission of the autoimmune disease can occur through the shift from Th1 to Th2 cytokines (McMurray, 2001a). Conversely, in Th2 autoimmune diseases PRL may increase the activity of the disease through its direct effect on Th2-related autoantibody production (Fig. 2). Interestingly, an example of the role of PRL in the shift in the balance between Th1/Th2 cytokine response is the behavior of disease activity in some autoimmune diseases, in pregnancy, and in post-partum period (McMurray, 2001b). Estrogens stimulate pituitary PRL production and release through suppression of hypothalamic dopaminergic inhibition. Estrogen-stimulated PRL secretion could also explain the higher mean serum PRL levels in women of childbearing age than in men, and the consequent higher autoimmune susceptibility in female sex.

Pregnancy is characterized by progressive rise in serum estrogens, progesterone, and PRL concentrations as well as in a number of placental hormones. Following the delivery estrogens and progesterone secretion drops, while PRL secretion remains high, particularly in nursing females. Multiple changes in the immune system occur during pregnancy which have been globally characterized by suppression of cell-mediated immunity and stimulation of humoral immunity (Formby, 1995). These changes are consistent with mediated DC/Th1 cell immunosuppressive effect of estrogens and their stimulatory effect of Th2/B cell function directly or through PRL stimulation. It has been observed that rheumatoid arthritis (RA) and multiple sclerosis (MS), both Th1-mediated autoimmune diseases, ameliorated during the progression of pregnancy and exacerbated in post-partum period (Nelson and Ostensen, 1997; Confavreux et al., 1998). The improvement of these diseases in pregnancy could be probably due to the estrogen-mediated suppression on Th1 function, while the post-partum exacerbation could be due to the removal of estrogenic suppression with prevalence of stimulatory role of PRL. Conversely, systemic lupus erythematosus (SLE) belonging to Th2 autoimmune diseases appears to worsen during and even after pregnancy probably due to the combined estrogens and PRL upregulation on Th2 cells during the pregnancy and the effect of elevated PRL concentrations alone in post-partum period (Khamashta et al., 1997).

6. Prolactin and autoimmune diseases

Many studies on animal models demonstrated a clear evidence of PRL involvement in autoimmunity. Increased levels of PRL have been described during acute allograft rejection. This increase plays a role in the development of an efficient Th1

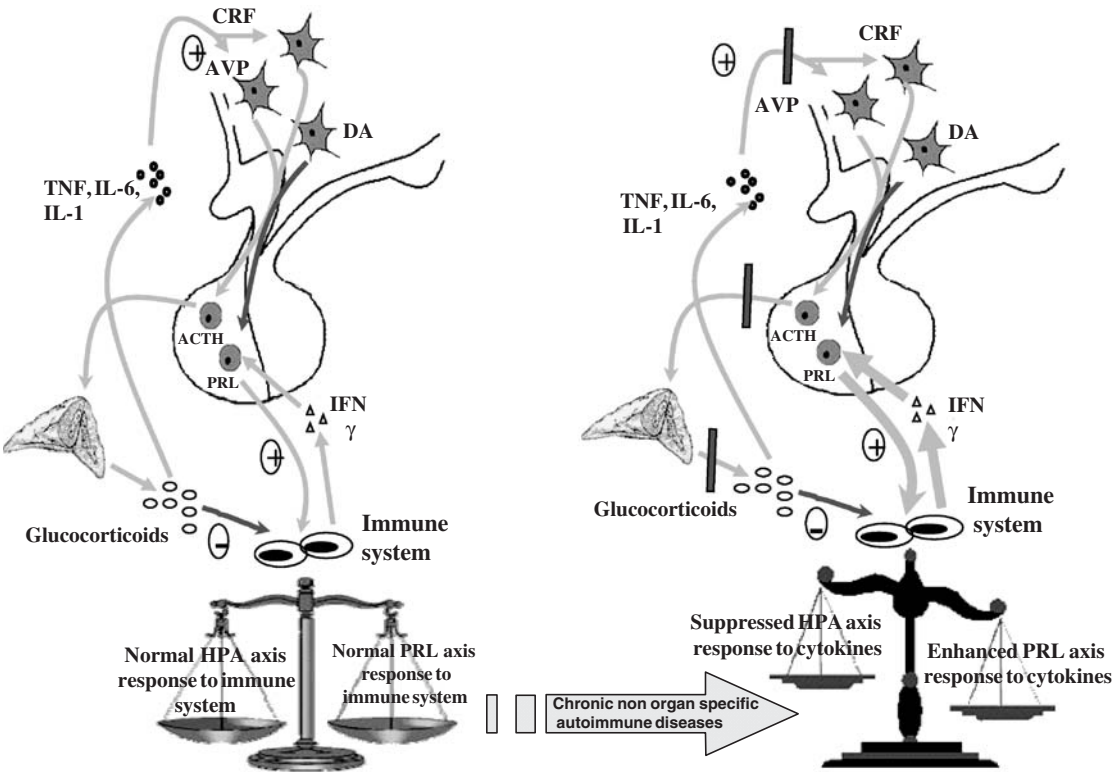

Figure 2. Role of PRL to influence the shift in the balance between Th1/Th2 cytokine response in autoimmune diseases.

cellular response, since rejection is prevented by bromocriptine-induced PRL-level reduction (Carrier et al., 1987). Moreover, experimental allergic encephalomyelitis (EAE), one of the best study models of autoimmune diseases, is characterized by neural-specific Th1 autoantigens (Wekerle et al., 1994). PRL levels have been found to be elevated before the onset and during the disease, while treatment with bromocriptine induces reduction of PRL levels and amelioration of the neurological signs of EAE (Esquifino et al., 2006). High PRL levels have been evidenced in NZB/W1F1 lupus rats correlated to the disease severity, and bromocriptine treatment improves disease features and delays lupus-related death. In fact, in this murine model of SLE, bromocriptine has been shown to suppress immunoglobulin levels, autoantibodies, and immune complex–related glomerulonephritis resulting in improvement of survival rate (McMurray et al., 1994; McMurray, 2001b). The relationship between PRL and autoimmune diseases could also be explained taking into account that the human *PRL* gene is located, as previously described in this chapter, on the short arm of the chromosome 6 close to HLA region. It is well known that some antigens of the HLA complex are correlated to higher frequency of many autoimmune diseases (Matera and Rapaport, 2002). Hyperprolactinemia has been often observed in many non-organ-specific autoimmune diseases as SLE, RA, systemic sclerosis (SSc), and Sjögren syndrome (De Bellis et al., 2005). Moreover, it has also been described in some organ-specific autoimmune diseases as type-1 diabetes mellitus (DM), Graves' diseases (GD), Hashimoto's thyroiditis (HT), Addison's disease (AD), lymphocytic hypophysitis (LYH), celiac disease (CD), and MS (Chuang and Molitch, 2007) (Table 1). In some of these diseases (RA, type-1 DM, HT, MS, and CD) Th1 dominance (γIFN, IL-2, TNFα) is present even if T lymphocytes with Th2 profile (IL-4, IL-5, IL-6, IL-10) are also activated (Vera-Lastra et al., 2002). In fact, it

Table 1
Autoimmune diseases associated with hyperprolactinemia

Non-organ-specific		Organ-specific	
Th1-related	Th2-related	Th1-related	Th2-related
Rheumatoid arthritis Sjögren's syndrome	Systemic lupus erythematosus Systemic sclerosis	Type-1 diabetes mellitus Hashimoto's thyroiditis Celiac disease Multiple sclerosis Addison's disease Lymphocytic adenohypophysitis	Graves' disease

has been shown that many organ-specific antibodies as islet-cell antibodies (ICA) and GAD Ab, tyroglobulin (Tg Ab) and thyroperoxidase (TPO Ab) antibodies, adrenocortical antibodies (ACA) and 21-OH antibodies, and transglutaminase antibodies (tTGAb), are considered good markers of type-1 DM, HT, AD, and CD, respectively (De Bellis et al., 2005). On the other hand, in all these organ-specific autoimmune diseases only T lymphocytes with Th1 profile play a pathogenetic role. In clinical phases of these autoimmune diseases with high titers of organ-specific antibodies, elevated PRL levels have been frequently found (Lever and McKerron, 1984; Legakis et al., 2001; Kapur et al., 2004). Moreover, detection of these antibodies without clinical phase of the corresponding autoimmune organ-specific disease is a frequent finding in hyperprolactinemic patients with other clinical autoimmune diseases such as SLE, RA, primary Sjögren syndrome, and SSc (Goh and Wang, 1986; Kramer et al., 2005). On the other hand, in patients with hyperprolactinemia of various etiologies an increased rate of antibodies including anti-thyroid antibodies, anti-dsDNA, anti-roSSA, anti-cardiolipine, and anti-nuclear antibodies without clinical evidence of autoimmune disease has been described (Ishibashi et al., 1991).

7. Hyperprolactinemia in non-organ-specific autoimmune diseases

7.1. Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is considered to be the major essentially Th2 mediated non-organ-specific autoimmune disease (Vera-Lastra et al., 2002). In this disease, PRL is able to enhance in vitro IgG and in vivo anti-dsDNA antibody production in peripheral mononuclear cells from affected patients (Jacobi et al., 2001). Moreover, it has been shown that lymphocyte-derived PRL is increased in patients with SLE compared with normal subjects (Larrea et al., 1997). An important finding is that physiological PRL concentrations (<20 ng/ml) are more effective than higher PRL ones in inducing IgG production by SLE lymphocytes (Jacobi et al., 2001). To explain the relationship between PRL and SLE a linkage disequilibrium between *HLA-DRBi* (alleles associated with SLE and RA susceptibility) and *PRL* genes on the short arm of the chromosome 6 has been stressed (Stevens et al., 2001a, b). However, subsequently, other authors, in analyzing *PRL* and *PRL-R* genes evidenced that polymorphism in SLE did not find statistically significant differences in the allele distribution (Mellai et al., 2003). Anyway, hyperprolactinemia has been found in 20–30% of patients with SLE suggesting a correlation with clinical disease activity as well as ANA and anti-dsDNA titers (Jara et al., 1992; Walker et al., 1995). Whether hyperprolactinemia is associated with active SLE is still debated (Buskila et al., 1996; Jimena et al., 1998). Several studies demonstrated the occurrence of hyperprolactinemia in patients with SLE but just a few suggested a positive correlation with the disease activity (Jara et al., 2006). These discrepant findings may be explained taking into account different factors interfering with the results such as: statistical power of these studies (Blanco-Favela et al., 1999), heterogeneous groups of patients, variability of the

SLE activity, different treatments, abnormal circadian PRL rhythm, and presence of different isoforms of PRL and anti-PRL antibodies (Leanos et al., 1998; Jacobi et al., 2001; Jara et al., 2001; Leanos-Miranda et al., 2001a; Pacilio et al., 2001). In particular, patients with SLE show an increased PRL production with different molecular weight forms: 23 kDa (monomeric PRL), 60 kDa (big PRL), and 150–170 kDa (big big PRL, called macro-PRL). Lymphocytes in patients with active SLE show an increased production of 60 kDa PRL, while macro-PRL is usually associated with inactive SLE (Cruz et al., 2001). Since studies indicate that big big PRL is mostly constituted by anti-PRL antibodies—monomeric PRL complex, it is possible that anti-PRL antibodies attenuate the biological activity of PRL interfering with PRL-Rs (Leanos-Miranda et al., 2001b).

At last, some authors showed that hyperprolactinemia is strongly associated with disease activity using the systemic lupus activity measure (SLAM) (Rezaieyazdi and Hesamifard, 2006).

Conventional immunosuppressive therapy in SLE patients including glucocorticoids and hydroxycloroquine has been shown to reduce PRL levels and this reduction is directly correlated with decreased SLE activity (Vera-Lastra et al., 2003). A direct assessment of the causal relationship between hyperprolactinemia and SLE is provided by remission of active SLE in patients treated with bromocriptine. In particular, a double-blind placebo-controlled study with low-dose bromocriptine therapy in 36 SLE patients (vs. 30 patients with placebo) treated for a mean of 12.5 months showed a significant decrease in PRL levels associated with a significant decrease in disease activity (Alvarez-Nemegyei et al., 1998). In another double-blind study it has been evidenced that bromocriptine is as efficacious as hydroxycloroquine for the treatment of active SLE (Walker, 2001); bromocriptine-induced remission of activity has been observed also in some patients with PRL levels within the normal range (Chuang and Molitch, 2007). Recently, some authors showed that dopamine influences directly the immune system by binding DA-receptors in human T lymphocytes. Sarkar et al. (2006) demonstrated that the stimulation of dopamine receptors in human T cells induces T-cell quiescence through the inhibition of intracellular signaling pathways. These results suggest that dopamine agonists may play a therapeutic role in SLE patients with or without high PRL levels.

7.2. Rheumatoid arthritis

Rheumatoid arthritis (RA) is considered a Th1-related autoimmune disease. Some studies showed that the serum levels of PRL and the chemochine Mip1a increased in relation to the duration and the severity of RA. Even if some studies demonstrated normal PRL levels in patients with RA (Kullich and Klein, 1998; Ram et al., 2004), Nagafuchi et al. (1999) evidenced that T cells and fibroblasts of patients with RA could produce PRL. In these cells bromocriptine reduced the production of PRL, IL6, TNFα, and matrix metalloproteinase, suggesting an inhibiting effect of bromocriptine on extra-pituitary PRL production; instead, PRL addition restored the IL-6 secretion to control levels. PRL-R is expressed in fibroblast-like cells and in lymphocytes inducing the rapid translocation of STAT-5 from the cytoplasm into the nucleus and enhancing the proliferation of the fibroblasts. Some authors compared bromocriptine with penicillamine therapy in patients with RA; bromocriptine showed clinical improvement overlapping that of penicillamine (Chuang and Molitch, 2007).

8. Other non-organ-specific autoimmune diseases

8.1. Systemic sclerosis

Mild hyperprolactinemia has also been reported in SSc. Basal and TRH stimulated PRL levels significantly higher in childbearing age SSc patients than in controls have been also described. The authors by regression analysis showed that these basal and stimulated PRL concentrations were well correlated with severity of skin sclerosis, peripheral vascular and lung involvement in this

disease, suggesting that PRL may have an immunostimulating role on the activity of SSc (La Montagna et al., 2001).

8.2. Sjögren syndrome

Some studies also showed an association between hyperprolactinemia and Sjögren syndrome; PRL levels were also found to correlate with the index of internal organ disease in this syndrome (Haga and Rygh, 1999).

8.3. Prolactin and neuroendocrine immune network in non-organ-specific autoimmune diseases

A coordinated bidirectional network between the neuroendocrine and immune system is necessary for achievement and maintenance of immune balance. Non-organ-specific autoimmune diseases are caused by a failure of immune-cell tolerance; moreover, an abnormal response of neuroendocrine immune network may participate in the break of tolerance. During chronic inflammatory stimuli, the interaction of HPA axis and PRL secretion with the immune system is abnormal in patients with SLE and RA, particularly during their active disease (Fig. 3). The available data suggest that HPA axis activity is reduced while PRL secretion is increased in these patients (Jara et al., 2006).

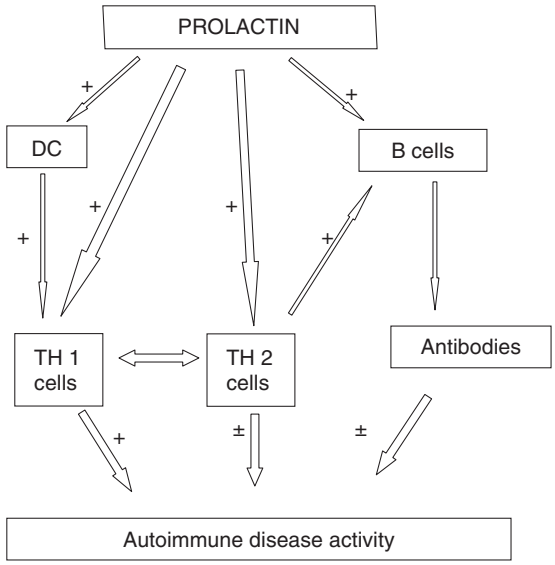

Figure 3. Interrelationship between neuroendocrine and immune system. During inflammatory process active immune system releases proinflammatory cytokines, which stimulate hypothalamic neurons with consequent activation of HPA axis. The release of glucocorticoids suppresses the inflammatory response. On the other hand, inflammatory cytokines induce release of pituitary PRL which enhances inflammatory response. Left: Coordinated activation of HPA axis and PRL axis in acute inflammatory stimuli. Right: Uncoordinated activation of HPA axis and PRL axis during chronic inflammatory stimuli in organ-specific autoimmune diseases.

8.4. Mechanisms inducing hyperprolactinemia in non-organ-specific autoimmune diseases

Many patients with non-organ-specific autoimmune diseases have apparently idiopathic hyperprolactinemia remaining without evidence of pituitary adenoma for many years (Walker, 2006). In these cases, non-coordinated bidirectional communication between the neuroendocrine and the immune system could be a putative mechanism inducing PRL secretion increase by pituitary gland and by immune cells (De Bellis et al., 2005). In particular, inflammatory cytokines as γIFN released by immune cells infiltrating the site of the immune process could be able to induce increase of PRL both by pituitary gland and immune cells. In some patients with active phase of SLE, RA, and SS, PRL is able to activate Th1 and Th2 cells even at values in the normal range (Gutierrez et al., 1995). The secretion of pituitary PRL is under the inhibitory hypothalamic dopamine effect. Pituitary and immune cell-derived PRL act via PRL-R present on the hypothalamic dopaminergic neurons stimulating DA synthesis, which inhibits PRL release through D2 receptors present on the pituitary lactotrophs. When the PRL release by immune cells is higher than that by pituitary, it binds to hypothalamic PRL-R,

determining false normal/low levels of pituitary PRL by feedback mechanisms as evidenced in some patients with active phase of non-organ-specific autoimmune diseases (Mendez et al., 2004).

In some patients with non-organ-specific diseases, hyperprolactinemia due to pituitary microadenoma was observed (Jara et al., 2001). Concerning this, prolactinoma has been found in a large cohort of adult patients and in 13-year-old girls suggesting that a non-cyclic secretion of abnormally high PRL concentrations can stimulate autoimmune responses contributing to the pathogenesis of SLE (Reichlin, 1992; Reuman, 2004; Li et al., 2006). Surgical and medical bromocriptine therapy were independently associated with decreased PRL levels and remission of clinical and serological SLE manifestations. Moreover, hyperprolactinemia secondary to microadenoma has been also described in a patient with urticarial vasculitis, Jaccoud's arthropathy, SLE, and Sjögren syndrome (Anaya and Shoenfeld, 2005). Finally a high prevalence of hyperprolactinemia with increased central dopaminergic tone and microadenoma has been described in a group of patients with SSc (Vera-Lastra et al., 2006).

9. Hyperprolactinemia in organ-specific autoimmune diseases

Hyperprolactinemia may also be present in many patients with organ-specific autoimmune diseases (De Bellis et al., 2005). These diseases are characterized by the association in the same patient of various autoimmune diseases. More frequently, in patients with an isolated autoimmune disease a complete autoantibody screening can evidence other organ-specific antibodies without clinical manifestations of the corresponding disease (potential/subclinical autoimmune disease). The coexistence of two or more organ-specific and non-organ-specific autoimmune disease indicates a complete polyendocrine autoimmune syndrome (APS) (Betterle et al., 1996). Previous antibody screening in a large cohort of patients with isolated clinical autoimmune disease or with APS showed apparently idiopathic hyperprolactinemia without finding of prolactinoma in some of these patients (unpublished personal data). Our recent study, looking for organ-specific antibodies in patients with apparently idiopathic hyperprolactinemia and in patients with prolactinoma evidenced a higher prevalence of some of these antibodies (ICA, GAD Ab, TgAb, TPO Ab, APGA, APA, tTGAb) in those with idiopathic hyperprolactinemia with respect to those with prolactinoma (De Bellis et al., 2007).

9.1. Celiac disease

Kapur et al. (2004) recently showed increased PRL levels in many patients with active celiac disease but not in those with non-active celiac disease under gluten-free diet.

9.2. Multiple sclerosis

The link of MS and PRL, if any, is indeed very tenuous (Hooghe et al., 2004). Serum PRL are normal but TRH-induced secretion is higher in MS patients than in normal controls (Azar and Yamout, 1999).

Hyperprolactinemia may, however, be one of the characteristic features of Asian MS patients with preferential involvement of the optic nerve, also being significantly associated with acute relapse of the disease (Yamasaki et al., 2000). However, these findings are not be confirmed by others (Heesen et al., 2002). Some studies showed that there were no significant differences in serum PRL levels between MS patients and control groups (Harirchian et al., 2006). However, further studies in more homogeneous subgroups of MS patients are needed to clarify these aspects.

9.3. Type-1 diabetes mellitus

Although the pathogenesis of type-1 DM is far from clear, Th1 cells are considered pathogenetic and Th2 cells protective in type-1 DM. PRL exerts

an hyperglycemic activity and this complicates the interpretation of studies addressing its possible role in the pathogenesis of diabetes (Freemark et al., 2002). Moreover, SOCS 1 and 3 (induced by many cytokines including PRL) inhibit signal transduction by insulin (Rui et al., 2002). The incidence of DM is significantly lower in female mice receiving bromocriptine (Hawkins et al., 1994), but a more recent study indicates a strong protective effects by pregnancy hormones and a partially protective effect by PRL administration on the development of DM (Atwater et al., 2002).

9.4. Autoimmune thyroid diseases

Also in thyroid autoimmunity PRL plays a potential stimulating role. Thyrotropin-releasing hormone is a PRL-releasing factor and this accounts for the rise in PRL in patients with hypothyroidism. For instance, patients with Hashimoto's thyroiditis show PRL values significantly higher than normal controls (Notsu et al., 1997). Hypothyroidism is frequent in Hashimoto's disease; however, recent experimental studies suggest not only PRL is the result of hypothyroidism but it may also contribute to the progression of the disease (You et al., 1999). In fact, thyrocytes from normal subjects exposed in vitro to PRL expressed higher levels of ICAM-1, B7-1, and TPO suggesting that PRL could play a role in the development of autoimmune thyroid diseases. Hyperprolactinemia has also been observed in patients with other non-organ- and organ-specific autoimmune diseases and high prevalence of anti-thyroid antibodies. Finally, a reduction of anti-thyroid antibodies has been described in hyperprolactinemic patients treated with dopamine agonists (Kramer et al., 2005).

9.5. Lymphocytic hypophysitis

Hyperprolactinemia is present in many patients with lymphocytic hypophysitis (LYH). In this disease endocrine and paracrine/autocrine PRL secretion occurs in the same site of pituitary gland. For this reason, patients with LYH can have higher prevalence of hyperprolactinemia with respect to those with other autoimmune diseases (personal data). High PRL levels have been frequently observed in patients with LYH associated with an enlargement of the pituitary gland on magnetic resonance imaging (MRI). A multifactorial etiology has been suggested for the hyperprolactinemia in such cases: in particular, a decrease in the dopamine delivery to the anteropituitary due to stalk compression by pituitary suprasellary inflammatory mass, alteration of dopamine receptors, lactotroph hyperplasia due to stimulating effect of anti-pituitary antibodies (APA), or escape PRL into the circulation secondary to the massive cellular destruction (Bottazzo et al., 1975; Thodou et al., 1995; Bellastella et al., 2003; Caturegli et al., 2005).

Other patients with LYH may present normal characteristics on MRI with varying degrees of pituitary failure and presence of APA (De Bellis et al., 2005). As previously described in this chapter, in our recent study we performed a screening of organ-specific antibodies in patients with idiopathic hyperprolactinemia and in patients with microprolactinoma (De Bellis et al., 2007). APA were detected at high titers in 24.7% patients with idiopathic hyperprolactinemia, but not in those with prolactinoma. Moreover, APA-positive patients showed normal pituitary function or partial pituitary impairment. We suggested that evaluation of APA in patients with apparently idiopathic hyperprolactinemia could be useful to disclose cases of autoimmune pituitary disease with pituitary function still normal (potential LYH) or with partial deficiency of other pituitary hormones (subclinical LYH). The diffuse lymphocyte infiltration of the anteropituitary gland could determine the increase of PRL release by both inflammated lactotrophs and immune cells infiltrating the gland, taking into account the relationship between the immune system and PRL secretion. Anyway, the PRL increase in LYH could be secondary to inflammatory process of pituitary gland; on the other hand, high levels of PRL could have a role in perpetuating the immune process in LYH. In a study, which is still in progress, some APA-positive and -negative

patients with apparently idiopathic hyperprolactinemia were submitted to cabergoline therapy for 2 years and free of therapy for another year. All APA-positive patients (with potential/subclinical LYH) showed normalization of PRL levels, APA disappearance, and recovery of pituitary function, when initially impaired, during cabergoline treatment, which persisted 1 year after drug withdrawal suggesting an immunosuppressive effect of dopamine agonist. Thus, cabergoline therapy seems to be able to induce progressive disappearance of APA with recovery of a normal pituitary function probably interrupting the pituitary cell damage through normalization of PRL levels or for a direct immunosuppressive effect of the drug.

Key points

- PRL is secreted not only by anterior pituitary gland but also by many extrapituitary sites including the immune system. The endocrine/paracrine PRL has been shown to stimulate the immune cells by binding to PRL receptors.
- The role of PRL in the interrelationship between the endocrine and the immune system has a biphasic character. Prolactin exerts its immune activity in the context of stress, trauma, injury, inflammation, infection, and various autoimmune diseases but not in basal conditions.
- Prolactin induces activation of dendritic cells, T cells, B cells, and NK cells.
- Clinical and experimental findings suggest that autoimmune diseases may be the results of a shift in the balance between Th1 and Th2 cytokine responses to inducing factors and then PRL could play a pivotal role in this process. The prevalent action of either Th1 or Th2 could determine not only the development of a particular autoimmune response but also the progression or less toward a clinical stage of the disease.
- Some studies showed that hyperprolactinemia is strongly associated with disease activity in systemic autoimmune diseases such as SLE, SSc, and Sjögren's syndrome.
- The causal relationship between hyperprolactinemia and these systemic autoimmune diseases is also suggested by remission of disease activity in patients treated with bromocriptine.
- Hyperprolactinemia has been described in organ-specific autoimmune diseases such as type 1 diabetes mellitus, Graves' diseases, Hashimoto's thyroiditis, Addison's disease, lymphocytic hypophysitis, celiac disease, and multiple sclerosis, often associated with the presence of respective autoantibodies.
- High levels of PRL have been also frequently detected in patients with LYH. Several mechanisms have been invoked to explain the hyperprolactinemia in LYH. The PRL increase could be secondary to the inflammatory process of the pituitary gland but, on the other hand, this increase could have a role in enhancing and perpetuating the immune process.
- Moreover, the detection of antipituitary antibodies targeting cells secreting PRL and other pituitary hormones in some patients with idiopathic hyperprolactinemia suggests the occurrence of a possible potential/subclinical LYH in these patients.
- Finally, the role of antiprolactinemic drugs to interrupt the course of the immune process in LYH is still discussed.

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CHAPTER 5

The Pathogenesis of Type 1 Diabetes

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1. Introduction

Type 1 diabetes (T1D) is caused by the autoimmune destruction of the insulin producing β-cell of the pancreatic islet. T1D is the most common cause of diabetes in childhood and is increasingly recognized as a cause of diabetes initially presenting in adulthood. T1D is the cause of 5–10% of diabetes. The origin of the autoimmunity is thought to be multi-factorial and has been attributed to genetic and environmental factors. A clear understanding of factors leading to the development of T1D is of paramount importance to the development of prevention and treatment strategies. In this chapter, we will explore a proposed model for the development of T1D, discuss factors that have been implicated in its development and briefly touch on intervention trials to prevent the development of T1D in groups at risk.

2. Classification of diabetes

Diabetes is diagnosed by the presence of fasting and/or postprandial hyperglycemia. In order to meet the formal definition of diabetes, fasting blood glucose must be ≥ 126 mg/dL or random blood glucose must be ≥ 200 mg/dL. Without symptoms, results must be confirmed in duplicate. In the presence of symptoms of hyperglycemia (the classic triad of polyuria, polydipsia, and weight loss), a single blood glucose ≥ 200 mg/dL is diagnostic of diabetes. Diabetes can also be diagnosed on oral glucose tolerance testing (OGTT) with 2-h glucose after a glucose load of ≥ 200 mg/dL consistent with diabetes. Again, without symptoms, results must be confirmed in duplicate (American Diabetes Association, 2007).

The most common causes of diabetes are autoimmune T1D and type 2 diabetes (T2D), which is characterized by insulin resistance and impaired insulin secretion. T1D has been further divided to type 1A diabetes, which is defined as autoimmune diabetes, and type 1B diabetes, which is termed idiopathic diabetes and does not have a known etiology, although there appears to be a strong genetic contribution (American Diabetes Association, 2007).

While it is fairly simple to diagnose diabetes, classifying diabetes may be more difficult. The most common cause of diabetes in childhood is T1D. However, older obese children may develop T2D and adults may develop T1D. Additionally, obese children are at risk for the development of T1D and cannot be assumed to have T2D on the basis of the body habitus alone. The appropriate treatment of diabetes depends on its accurate classification, which may require laboratory testing and careful follow-up over time.

3. Models for the development of T1D

The natural history of the prediabetic period has been the subject of intense research. Several

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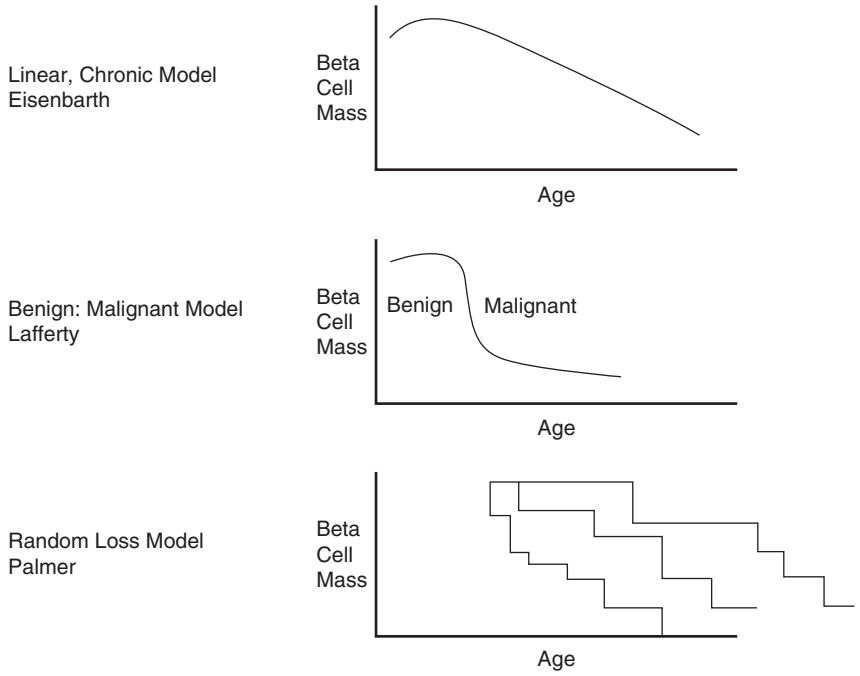

Figure 1. Models for the natural history of the prediabetic period. In each model β-cell mass is on the y-axis and time is on the x-axis. The chronic linear model hypothesizes that β-cell mass declines in a linear way over time. The benign:malignant model hypothesizes that autoimmunity initiates as a benign process that ultimately can be triggered to a malignant destruction of β-cells over time. The random loss model describes a random decline in β-cell mass over time. (Reproduced with permission from Eisenbarth & Homann, 2006.)

different models of the development of T1D have been proposed. We will briefly describe all three and focus on one model for further discussion. The first model is the benign: malignant model, which was initially described by Gazda et al. (1997). In this model, the autoimmune process that precedes the development of T1D initiates as a ''benign'' process without the destruction of β-cells. This process is triggered to a ''malignant'' process with resultant β-cell destruction. A second model describes the random destruction of β-cell (Greenbaum et al., 1999) (Fig. 1).

The model we will focus on was initially proposed over 20 years ago by Eisenbarth (1986). This is referred to as the chronic linear model. This model proposes that subjects at increased genetic risk for T1D experience a triggering event in which autoimmunity initiates. Once autoimmunity is triggered, the β-cells are progressively destroyed and metabolic abnormalities develop ultimately resulting in hyperglycemia in the fed state and then fasting hyperglycemia with symptoms and risk for diabetic ketoacidosis. It is useful to note that environmental and genetic factors may play a role at two states: in the initiation of autoimmunity and in the progression of autoimmunity to clinical disease (Rewers et al., 2006) (Table 1).

After a brief introduction to immunology, we will consider the following stages of diabetes development: genetic risk, triggering of autoimmunity, makers of autoimmunity, metabolic abnormalities, and overt diabetes with signs and symptoms of hyperglycemia.

4. Immunology of T1D

It is now widely accepted that T1D is an autoimmune disease resulting in the loss of immunologic tolerance to the β-cell leading to hyperglycemia and clinical symptoms of diabetes. An understanding of the process leading to autoimmunity

Table 1
Stages of diabetes development in the chronic linear model

Stage	Markers	Ability to predict
Genetic prediction	Family history HLA VNTR	5% to >50%
Initiation of autoimmune attack	IAA GAD65 ICA512 T-cells assays	25–50% 5-year diabetes risk
Metabolic abnormalities	Decreased FPIR Impaired fasting glucose Impaired glucose tolerance	>50% 5-year diabetes risk

requires a basic understanding of immunology. Therefore, we will briefly discuss the development and loss of tolerance (Eisenbarth and Homann, 2006).

The immune system is composed of many different cell types including B-cells, which produce antibodies; T-cells, which produce cytokines directing the immune response; and antigen presenting cells (APCs), which interact with T-cells. The interaction between T-cells and APCs influences the direction of the immune response. Within APCs, digested proteins are presented in the groove of the human leukocyte antigen (HLA). The peptide–HLA complex binds to the T-cell receptor (TCR) present on the surface of T-cells. This is the underlying basis of the association of HLA genotypes and autoimmune diseases. The peptides that can be bound to the HLA molecule are determined by which HLA molecule is present. For example, a short peptide for the B-chain of the insulin molecule has been shown to be bound to the HLA DR4-DQ8 molecule (Raju et al., 1997).

Tolerance to self develops in two states: central (within the thymus) and peripheral within lymph nodes and spleen. Central tolerance is dependent upon the production of "peripheral" antigens such as insulin within the thymus (Hanahan, 1998). The gene associated with autoimmune polyendocrine syndrome-I (APS-1), the autoimmune regulatory (AIRE) gene (Nagamine et al., 1997; Bjorses et al., 1998), is a transcription factor that is thought to be important for the transcription of peripheral antigens within the thymus (Anderson et al., 2002). The absence of this transcription factor manifests as multiple autoimmune disorders in the clinical syndrome of APS-1. The thymus plays an important role in the "education" of T-cells and this is where "central tolerance" develops. Cells with a strong reaction to self-peptides presented in the HLA molecules are deleted within the thymus. Cells that do not bind at all to the HLA molecules are also deleted. Cells that bind HLA but do not react to self are released from the thymus. Peripheral tolerance is the second stage of tolerance and occurs in the spleen and local lymph notes. In the periphery, tolerance develops through anergy, in which self-reactive cells are inactivated, and the development of regulatory T-cells. The development of regulatory T-cells is under the influence of another transcription factor, FOXp3 (Fontenot et al., 2003). FOXp3 is expressed on the X-chromosome and deletion of this gene in boys or homozygous deletion in girls results in a syndrome of fulminant autoimmunity with neonatal diabetes, significant gastrointestinal symptoms including malabsorption and resulting in death. Therefore, regulatory T-cells play an influential role in the development of autoimmunity, even with intact central tolerance.

5. Markers of the autoimmune process

Much of our understanding about the natural history of the prediabetic period in T1D is dependent upon the use of serum markers of the autoimmune process. Markers of the autoimmune process in T1D were first identified over 30 years

ago with the observation that people with T1D expressed antibodies against sections of the pancreatic islet, so-called islet cell antibodies (ICA) (Bottazzo et al., 1974, 1980). Additional antibodies reacting to proteins within the β-cell such as insulin (Vardi et al., 1987), GAD65 (Falorni et al., 1995), and IA-2 (ICA512) (Gianani et al., 1995; Lan et al., 1996) have also been identified. The presence of these antibodies indicates that the autoimmune process has against the β-cell has already begun. The presence of these autoantibodies has been used to differentiate type 1A diabetes from type 1B diabetes. However, it is known that these autoantibodies are present in the blood in 80–90% of subjects at onset and their absence does not mean that autoimmunity is not the underlying cause of diabetes.

While it is clear that autoimmunity is generally a process controlled by T-cells, the assays for monitoring the disease are generally autoantibodies that are products of B-cells. In order to improve our ability to predict disease, assays that monitor the T-cell portion of the disease are needed. These assays are currently under development and once validated will likely revolutionalize the field.

The autoimmune process associated with T1D can be detected by the presence in the blood of antibodies to islet-specific antigens including insulin, GAD65, and IA-2 (ICA512). One of the major pitfalls of the use of diabetes-related antibodies is the occurrence of false and transient positively positive autoantibodies. In rigorous studies that have employed stringent safe-guards to detect false positive results, it has been shown that especially at the low levels for positive, a significant proportion (1/3) are false positive, i.e., positive on an initial aliquot of serum but negative on repeat testing of that same serum sample. Additionally, on repeat testing approximately one-third of diabetes-related antibodies become negative, i.e., are transiently positive (Barker et al., 2004). Autoantibodies expressed transiently tend to have a very low level and subjects are usually single autoantibody positive. The risk for diabetes in subjects transiently positive one time for a single diabetes-related antibody is quite low (Yu et al., 2000; Barker et al., 2004).

Certain characteristics of the autoantibody response have been associated with an increased risk for diabetes. For example, in first-degree relatives of subjects with T1D, the number of diabetes-related antibodies expressed differentiates a 5-year risk for diabetes of 20% (one antibody) to 75% (three antibodies) (Verge et al., 1996). Insulin antibody level and affinity for insulin has also been associated with risk for T1D with higher antibody level and increased affinity associated with a higher risk (Achenbach et al., 2004). Achenbach et al. (2006) have proposed models of diabetes risk based upon autoantibody characteristics and have shown that subjects with very high risk ($>75\%$) and relatively low risk (25%) can be identified.

It is known that subjects can express these antibodies for many years prior to the development of disease. Indeed follow-up for >15 years indicates a continual development of diabetes over time that does not appear to decline (Gardner et al., 1999). Therefore, some have stated that in subjects that express diabetes-related antibodies, all will develop diabetes given a long enough follow-up time.

Using diabetes-related autoantibodies, it has become clear that T1D can develop in adults. Indeed, latent autoimmune diabetes in adults (LADA) was first described by the observation that adults with autoantibodies to GAD65 were more likely to require insulin within a relatively short period of time, were leaner and younger compared with GAD65 antibody negative subjects (Tuomi et al., 1993). This distinction of LADA as unique for T1D may be arbitrary. People who develop diabetes as adults compared with children may have a more slowly progressive form of autoimmunity, resulting in the apparent difference in clinical presentation.

6. Stages for diabetes development

6.1. Genetic risk

Twin studies have been used to evaluate the importance of genes to the development of T1D (Fig. 2). Initial studies of twins with one affected sibling with T1D suggested that 50% of identical twins go on to develop diabetes compared with rates in dizygotic twins similar to non-twin siblings (Kyvik et al., 1995; Redondo et al., 1999).

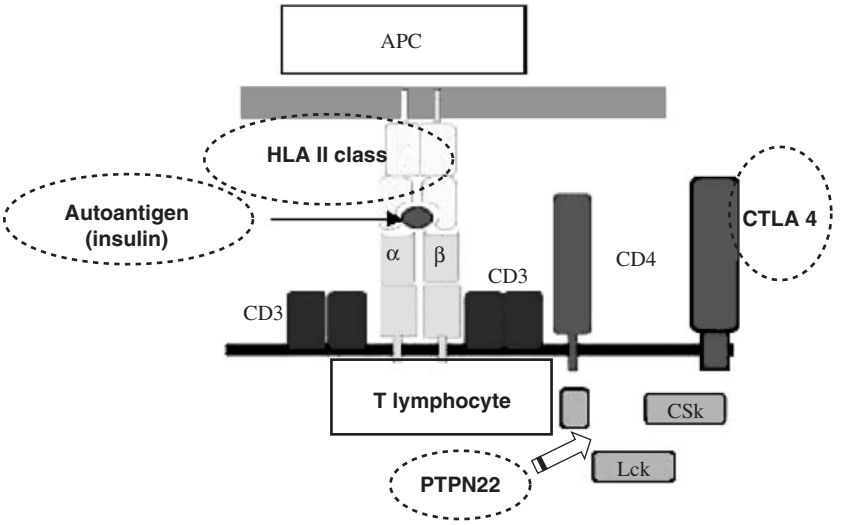

Figure 2. The location of influence of genes that have been associated with T1D. The HLA class II interacts with antigen presenting cells (APCs) to present antigens (such as insulin in the pathophysiologic state) to T-lymphocytes through the T-cell receptor (TCR). Downstream signaling of the TCR is modified by lymphoid tyrosine phosphatase (LYP) protein that is encoded by PTPN22 gene. Cytotoxic T lymphocyte antigen-4 (CTLA-4) is important for interactions within the T-lymphocytes. (Reproduced with permission from Rewers et al., 2006.)

Additionally, the rates of diabetes antibody positivity do not differ between non-twin siblings and dizygotic twins (Redondo et al., 2004). Long-term follow-up of monozygotic twins suggests that the majority of these twins are develop diabetes-related antibodies and diabetes and that the risk for the development of these diseases is dependent upon the age of onset of diabetes in the first affected twin (Redondo et al., 1999).

Many of the genes associated with T1D have products that are active within the immune system, thereby functionally linking observed associations with genes and T1D and pathologic mechanisms. In general, genetic factors can be divided into those genes that influence the development of autoimmunity (regardless of the target organ) and those that identify the target organ (e.g., the β-cell). A long list of genetic loci has been associated with T1D. However, only a handful of the genetic loci identified are consistently associated with T1D across multiple studies. The risk contributed by the HLA is so great that it may over shadow risk from other genetic loci (Jahromi and Eisenbarth, 2006).

Approximately 50% of the genetic risk lies within the HLA (Nerup et al., 1974). Therefore, the association of HLA genotypes and the development of diabetes has a pathophysiologic basis. The highest risk HLA genotype (DR3/4-DQ8) carries a risk for diabetes of approximately 5% by the age of 15 years (Lambert et al., 2004). While this is certainly much higher than the general population risk, it is clear that the majority of subjects with this high-risk genotype do not go on to develop diabetes. However, when evaluating the contribution of the HLA to risk for diabetes, restricting the analysis to subjects with a first degree relative with T1D greatly increases the predictive value of HLA genotyping. Such that offspring of subjects with T1D who are DR3/4 have a 20% risk of developing diabetes-related autoimmunity by 2 years of age (Schenker et al., 1999; Hummel et al., 2004). This risk is greatly increased when looking at children who are identical by descent with their sibling with diabetes for HLA DR3/4. These children have as much as an 80% risk for diabetes-related autoimmunity and 50% risk for diabetes by 10 years of age (Aly et al., 2006). Children with higher risk HLA genotypes and multiple family history of T1D also have a risk of diabetes-related autoimmunity and diabetes that can exceed 50% (Bonifacio et al., 2004).

Factors outside of the HLA have also been associated with diabetes. In particular polymorphisms within the variable number of tandem (VNTR) in the promoter region of the insulin gene have been associated with risk for T1D (Pugliese and Miceli, 2002). VNTR have been divided into three classes: class I (30–60), class II (60–120), and class III (120–170). Class II VNTR is very rarely observed. The level of VNTR is associated with the level of insulin production within the thymus (Pugliese et al., 1997). Class I VNTR is associated with the lowest production of insulin within the thymus. Subjects homozygous for the class I VNTR have the highest risk for T1D and it appears that the protection offered by the class III VNTR is dominant in that subjects heterozygous for the class III VNTR appear to have similar risk as homozygotes. Concordance rates of twins are affected by the VNTR (Metcalfe et al., 2001). There is a complex mechanism of imprinting with the influence of the untransmitted paternal allele suggesting epigenetic phenomena (Bennet et al., 1997).

Polymorphisms of the gene encoding lymphoid tyrosine phosphatase (LYP), PTPN22 have been associated with multiple autoimmune diseases including T1D (Bottini et al., 2004). The mutation results in an amino acid change from arginine to tryptophan at position 620 of the LYP protein, which results in a decrease of the ability of LYP to bind its target molecule CSK and enhances TCR signaling (Cloutier and Veillette, 1999; Bottini et al., 2004).

Combining genetic factors improves the ability to identify subjects at a very high risk for the development of diabetes. For example, in children who are DR3/4-DQ8 and have the highest risk insulin VNTR genotype, the risk for diabetes-related antibodies or diabetes by age 2 years is $>22\%$ compared with $<9\%$ for the lower risk VNTR (Walter et al., 2003). Again, through genetic factors alone, we are able to identify groups of individuals with a very high risk for the development of T1D.

Understanding the genetic factors associated with T1D also allows for the understanding of how autoimmunity develops. The development of autoimmunity may depend on the presence of autoimmune genes and which specific autoimmune disease develops may depend upon the disease-specific risk alleles. For example, subjects with LYP polymorphism associated with increased autoimmunity risk may develop thyroid autoimmunity or diabetes autoimmunity based upon the specific HLA risk or polymorphisms within the insulin gene or thyroid-specific genes. Using combinations of genetic factors and family history, groups with risk for diabetes $>50\%$ can be identified prior to the development of diabetes-related autoimmunity. These groups may be the ideal subjects to target for prevention studies as they can be identified before the autoimmune attack has initiated. It may be easier to prevent the autoimmune attack than it is to reverse the attack once initiated.

6.2. Initiating the autoimmune attack

Genetic factors play an important role in the risk for diabetes. However, they do not tell the whole story. For example, in the general population those subjects with the highest risk HLA genotype DR3/4-DQ8 are at a tenfold risk for the development of diabetes compared with those that do not have the genotype; however, the majority of those subjects will never go on to develop diabetes (Lambert et al., 2004). Therefore, there must be other factors that influence the development of diabetes-related autoimmunity and ultimately diabetes. Factors that may be active in this time period include other genetic loci as describe above, environmental exposures described in detail in the following section, and random events. For example, the TCR is uniquely generated postnatally and is determined by the random rearrangement of adjacent genes.

There is a large body of evidence that supports the notion that insulin is the initial antigen against which the immune attack is directed. Insulin defines a β-cell and as such is a β-cell-specific protein. In the NOD mouse model of diabetes, alteration of expression of insulin within the thymus results in a high rate of insulitis and diabetes, and diabetes can be prevented by the change of a single amino acid within the insulin B-chain (Nakayama et al., 2005). In the NOD

mouse, the immune response to other antigens has been found to occur after that to insulin. Additionally, autoimmunity against insulin characterizes human T1D such that factors such as presence of IAA, IAA level, and affinity of IAA have been associated with an increased risk for diabetes in prospective studies of diabetes development (Achenbach et al., 2004). Therefore, the insulin molecule may play an important role in the initiation of the autoimmune attack.

6.3. Environmental factors

The observation that T1D develops in children with congenital rubella suggested that in at least some children environmental exposures may be important in the development of T1D. Diabetes onset has been shown to have seasonal variation, again suggesting a seasonal environmental exposure. Perhaps the most convincing observation suggesting an environmental exposure is the worldwide increasing incidence of T1D. Over the last several decades, the incidence of T1D has been observed to be increasing at a rate of 3–5%/year. This increase appears to be occurring in populations with both a low and a high underlying risk (Onkamo et al., 1999). Some studies have shown that the increase is greatest in the youngest age group (Karvonen et al., 1999). Researchers have hypothesized that the rate of increase is too great to be accounted for by genetic factors alone and therefore have implicated environmental factors in the etiology of T1D.

Identification of the environmental factors associated with T1D is a focus of several long-term prospective studies. In general, these studies identify children at a very young age, even as neonates, and follow them for the development of diabetes-related antibodies. Generally, children included in these studies are at a higher genetic risk for diabetes including first-degree relatives of patients with T1D and young children with the highest risk HLA genotypes. Follow-up over time includes assessment of dietary intake, viral exposures, vaccinations, medical illnesses, and psychological stress in addition to diabetes-related autoantibodies. The association with the above factors and the development of diabetes-related autoantibodies and diabetes is then determined over time. These studies include the Diabetes Autoimmunity Study in the Young (DAISY) (Rewers et al., 1996), the Prospective Assessment of Newborns for Diabetes Autoimmunity (PANDA) study (Bennett et al., 2004), the Childhood Diabetes in Finland Study (DiME) (Kimpimaki et al., 2000), Finnish type 1 Diabetes Prediction and Prevention Study (DIPP) (Kimpimaki et al., 2001), the German (Hummel et al., 2000) and Australian (Couper, 2001) Baby-Diab Studies, and The Environmental Determinants of Diabetes in the Youth (TEDDY) (Hagopian et al., 2006). These studies have followed thousands of children over time. Factors associated with diabetes-related autoimmunity and diabetes identified through these studies are potential targets for intervention studies.

Dietary factors have been extensively studied in relationship to T1D. Among the earliest exposures that infants experience is infant formula. Cow's milk is known to contain insulin and it was hypothesized that exposure to bovine insulin could be an initiator of the autoimmune destruction of the β-cell. There is conflicting evidence regarding the association of cow's milk formula with some studies showing an increase in diabetes-related autoimmunity and diabetes in children exposed to cow's milk at a young age or exposed to a shorter duration of breast feeding, while other studies fail to show such an association (Gerstein, 1994; Couper et al., 1999; Paronen et al., 2000).

It appears that the timing of introduction of cereals to infants is an important risk factor for the development of diabetes-related autoimmunity. Both the German Baby-Diab (Ziegler et al., 2003) and the DAISY study (Norris et al., 2003) have evaluated timing of cereal introduction in children at high genetic risk for diabetes. The general recommendation from the American Pediatric Association for the initiation of solid food in infants is between 4 and 6 months of age. The German Baby-Diab study shows an increased risk for diabetes with early introduction of gluten and DAISY showed an increased risk with early (<3 months) or late (>6 months) cereal introduction.

The administration of cod-liver oil has been associated with a decreased risk for T1D (Stene et al., 2000; Stene and Joner, 2003). Cod-liver oil contains both docosahexanoic acid (DHA) and vitamin D. DHA has been associated with a decrease in the inflammatory cytokines that mark a pathogenic T-cell response (Endres et al., 1989). Additionally, observational studies have shown that offspring of pregnant mothers and infants who received cod-liver oil had a lower prevalence of diabetes (Stene et al., 2000; Stene and Joner, 2003). Vitamin D consumption has also been associated with a decreased risk for T1D (Hypponen et al., 2001). There is a link between these observations and the underlying genetics of T1D in that polymorphisms in the Vitamin D receptor are associated with T1D (Steck et al., 2005).

Vaccinations have not been shown to increase the risk for diabetes-related autoimmunity or T1D (Blom et al., 1991; Graves et al., 1999). However, there is some evidence that viral infections or lack of viral infections influence the development of diabetes-related autoimmunity and/or T1D. The hygiene hypothesis suggests that our environment is "too clean" and that the lack of viral infections early in life is associated with an increased risk for autoimmunity. Evidence that supports the role of viral infections in the development of T1D includes data from animal and human studies (McKinney et al., 2000). Specific viral pathogens that have been associated with T1D include enteroviruses and rhinoviruses. In mice, viral infections are associated with the development of diabetes-related autoimmunity (Oldstone et al., 1991). However, progression to diabetes requires a second infection. In humans, T1D is associated with the congenital rubella syndrome (Menser et al., 1978) and the development of diabetes in subjects with congenital rubella syndrome is HLA associated (Ginsberg-Fellner et al., 1984). Reports of epidemics of T1D after viral epidemics also suggest an infectious pathogen (Wagenknecht et al., 1991). Infections in utero and during infancy have been associated with T1D (Hyoty et al., 1995; Lonnrot et al., 2000). However, results have not been entirely reproducible across studies (Scherbaum et al., 1991; Graves et al., 2003). Supporting the hygiene hypothesis, there is an inverse correlation between the background rate of enteroviral infections and the incidence of T1D in the same geographic area (Viskari et al., 2004). Viruses can also protect mouse models from diabetes (Oldstone, 1988). These conflicting observations may be linked by the hypothesis that the timing of viral infection is of paramount importance. For example, early exposure to virus in the presence of maternal antibody to virus may be protective compared with a later infection. Although there are tantalizing clues and links between viral infections and T1D, there has been no agent consistently linked with T1D across many studies.

Coincident with the increase in T1D has been the increase in obesity in the general population. The accelerator hypothesis links these increases and suggests that insulin resistance and obesity plays an important role in the development of diabetes-related autoimmunity (Wilkin, 2001). Given that up to 25% of the pediatric population is overweight or at risk for overweight, a significant proportion of our children newly diagnosed with T1D are likely to be overweight. So the coincidence of increased weight and T1D cannot be taken as evidence alone of a relationship. Researchers have noted that children with T1D have a higher birth weight (Stene et al., 2001), are taller and heavier in the years prior to the diagnosis of diabetes (Hypponen et al., 2000), and that markers of insulin resistance such as HOMA-R and insulin levels are independent risk factors for diabetes (Wilkin, 2001). Confounding these relationships is the observation that high-risk diabetes HLA genotypes are associated with an increase in birth weight. Children who are overweight and insulin resistant will need more insulin to maintain normoglycemia. Therefore, any observed association may be secondary to this phenomenon alone and not related to the increased autoimmunity. The real key to the role of insulin resistance and T1D is the timing of the influence. In other words, if insulin resistance is important in the development of autoimmunity, it should play a role prior to the development of diabetes-related autoantibodies. Conversely, if it is important in the progression from diabetes-related autoimmunity to diabetes, it would suggest that it

Table 2
Representative environmental factors involved in the development of type 1 diabetes

	Factors and hypotheses
Infant diet	Cows' milk/breastfeeding
	Timing of cereal introduction
	Cod liver oil (DHA and vitamin D)
Vaccines	No association in multiple studies
Virus	Enterovirus
	Hygiene hypothesis
Obesity	Accelerator hypothesis
	Important in the initiation or progression to diabetes?

is more related to insulin requirements. Further studies evaluating these relationships prior to the development of diabetes-related autoimmunity will help clarify the role of obesity in the development of T1D.

The putative environmental trigger of the autoimmune destruction of the β-cell remains elusive. Long-term prospective studies of young children identified as having a high genetic risk for diabetes have failed to demonstrate a trigger that is solely responsible for diabetes-related autoimmunity. These studies have been following children for over a decade now and no clear environmental exposure has been identified as causative, likely indicating that the relationship between genes and environment in the pathogenesis of T1D is complex and different environmental factors may play a role in subsets of subjects at risk for T1D or multiple staged environmental exposures are required prior to trigger the autoimmunity and promote progression to diabetes (Table 2).

6.4. Metabolic changes prior to diabetes

With continued autoimmunity, the β-mass declines. The exact pace at which this decline occurs varies as evidenced by the fact that T1D can be diagnosed across the life-span. As the β-mass declines, the ability to maintain normoglycemia in the face of a carbohydrate challenge declines and abnormalities of glucose metabolism may be detected. β-cell mass is very difficult to assess. To date, there are no effective methods of imaging the β-cell and histologic evaluation has its obvious limitations. Therefore, researchers have relied upon measures of β-cell function including the intravenous and oral glucose tolerance tests (IVGTT and OGTT, respectively).

The IVGTT is performed with the administration of glucose intravenously and follow-up of insulin levels over the shorter (1 and 3 min) and longer (up to 10 min) time frame (Bingley et al., 1992). The first phase insulin response (FPIR) is defined as the sum of the insulin levels at 1 and 3 min. A diminution of the FPIR is one of the first metabolic abnormalities observed in the prediabetic period. Indeed, the FPIR below the first or tenth percentile for age was used in the Diabetes Prevention Trial-type 1 (DPT-1) to identify a group of autoantibody-positive relatives of subjects with T1D at the highest risk for diabetes (50–75% within 5-years (Diabetes Prevention Trial Type 1 Study Group, 2002)). In studies in which IVGTTs were performed within a 2-week time period, reproducibility ranged form excellent to poor (coefficients of variation from 4 to 36%) (Eisenbarth, 2004). So that subjects with a low FPIR may have repeated testing showing a normal FPIR within a short period of time. Therefore, changes in the FPIR on a single test may not be an accurate assessment of diabetes. Despite the pitfalls of interpretation of individual results of IVGTT, lower FPIR in general is associated with a higher risk for diabetes in diabetes antibody-positive subjects (Chase et al., 2001). Children at high genetic risk for T1D followed in prospective studies for the development of diabetes-related autoimmunity have a decrease in FPIR coincident with the first expression of diabetes-related autoantibodies even within the first several years of life (Keskinen et al., 2002). Thus, the autoimmune process may have preceded detectable autoantibodies in the sera.

Abnormalities of OGTT tend to appear later in the course of disease and are markers of declining β-cell mass. Generally, abnormalities are first detected in the 2 h glucose with impaired glucose tolerance (IGT, 2-h glucose on OGTT >140 mg/dL). Subjects with diabetes-related autoantibodies and IGT have a very high 5-year risk for T1D (Diabetes Prevention Trial Type 1 Study Group, 2002). T1D can be diagnosed on the basis of abnormalities of 2-h OGTT alone (Greenbaum

et al., 2001). At this point in time, subjects are minimally if not asymptomatic. Undetected, the vast majority of subjects with IGT will progress to frank and symptomatic hyperglycemia and/or ketoacidosis.

6.5. Overt diabetes

Overt diabetes develops when the β-cell mass is no longer able to produce sufficient insulin to maintain normoglycemia and prevent the development of ketoacidosis. Primate studies suggest that this occurs when the β-cell mass is approximately 10–20% of the total (McCulloch et al., 1991). There is ample evidence that supports the concept of glucotoxicity in which β-cells exposed to hyperglycemia can no longer produce insulin (Maedler et al., 2002). Therefore, in the weeks prior to the clinical onset of T1D, the rapid onset of symptoms is likely in part due to glucotoxicity. After treatment with insulin, blood glucose levels decline and the remaining β-cells begin to function and subjects enter a period of time known as the honeymoon. During this time, blood glucose control is generally excellent with hemoglobin A1c well within target and approaching the normal range and insulin requirements are generally low (<0.5 units/kg/day) (Chase et al., 2004). Unfortunately, the autoimmune attack continues and over a period of months to a couple of years insulin requirements increase and glycemic control deteriorates. The duration of the honeymoon is highly variable and difficult to predict. In general, younger children may not have a honeymoon, presumably due to the fact that younger children have a more aggressive autoimmune process (Chase et al., 2004; Sherry et al., 2005).

6.6. Prevention trials (Staeva-Vieira et al., 2007)

The effort to understand the pathophysiology of T1D has improved our ability to predict the disease. Prevention strategies can target any stage along the development of T1D including genetic susceptibility, expression of diabetes-related autoimmunity, metabolic abnormalities, and overt diabetes. The paradox of diabetes prevention studies is that our ability to identify subjects at risk for T1D improves as they progress through the prediabetic period. However, the autoimmune process continues to progress during this time period and may be difficult if not impossible to alter. Early in the disease course, prior to the expression of measurable diabetes-related autoimmunity, our ability to predict diabetes on a large scale is poor, but it may be easier to prevent (Atkinson, 2005). Implementing trials at an early stage has practical and ethical issues including the need for large numbers of subjects to be followed over a large period of time to answer the question, the requirement to treat some subjects that would never develop T1D and the need to treat pregnant women and/or children. Therefore, any strategy proposed in the earliest stages must be safe.

Prevention strategies have targeted the different stages in diabetes development. Those targeting high genetic risk neonates and children prior to the expression of diabetes-related autoantibodies are: avoidance of cows' milk protein, administration of DHA, and a planned trial of oral and intranasal insulin. All of these trials target the highest risk children, those with a first degree relative with T1D and high-risk HLA genotypes. They all employ methods that are very safe and follow children for the development of diabetes-related antibodies and/or diabetes.

Several large-scale studies have been designed to prevent diabetes in groups of relatives of subjects with T1D that express diabetes-related autoantibodies, but have not yet develop diabetes. These studies include the DPT-1 that tested oral and parenteral insulin in subjects with a predicted 25–50% (Diabetes Prevention Trial Type 1 Study Group, 2003) and 50–75% (Diabetes Prevention Trial Type 1 Study Group, 2002) risk for T1D respectively and the ENDIT (Gale et al., 2004) study that randomized subjects to nicotinomide or placebo. None of the interventions tested showed a delay in the development of diabetes. Subgroup analysis of subjects in the oral insulin trial of DPT-1 showed a delay of diabetes development of 4 years in those subjects with the highest insulin autoantibodies. Therefore, there is currently a trial

through Diabetes Trial Net of oral insulin in this high-risk group to answer the question: does oral insulin prevent and/or delay diabetes in subjects with diabetes-related autoantibodies. Trials of other agents will likely be performed.

Given the fact that at diagnosis of T1D, 10–20% of the β-cells are still present, one focus of diabetes research has been the prolongation of the honeymoon period. Therefore, trials have been employed to that effect and using as an endpoint c-peptide, which is a marker of endogenous insulin production. The trials completed or underway have identified subjects close to diagnosis of diabetes. Agents employed are generally immunosuppressive with significant toxicity. Among the most promising agents, anti-CD3 has been shown to delay the decrease in c-peptide that occurs in the first year of diagnosis (Herold et al., 2002). However, after 1 year, β-cell function began to decline at a rate similar to that seen in the untreated group (Herold et al., 2005).

As our ability to predict T1D has improved over the past several decades, it has become feasible to implement diabetes prevention trials in groups at high risk for diabetes. Prevention strategies tested in humans have been based on studies in animal models of diabetes and pilot studies of subjects at risk. Diabetes is relatively easy to prevent in mouse models of diabetes, unfortunately this ease in prevention has not translated to human studies (Shoda et al., 2005). Indeed to date, no prevention strategy has been shown to be convincingly effective in humans.

7. Conclusion

T1D is one of the most common chronic diseases of childhood. Our ability to predict T1D has greatly improved such that we are now able to identify subgroups of subjects with risk for diabetes $>50\%$ within 5 years. Genetic and environmental factors likely play a role in its pathophysiology, although the interplay between genes and environment remains an area of active investigation and the relationship between the two is likely complex. Further elucidation of genetic and environmental factors associated with T1D hopefully will identify targets for prevention of diabetes-related autoantibodies and diabetes. Maintenance of near normal glycemia is an important goal for diabetes management with the hope to prevent the development of diabetes-related complications. However, despite the best efforts of patients, their families and the health care team, these goals are very difficult to achieve. Therefore, strategies to prevent the development of T1D are urgently needed and are the focus of much of the research effort in years to come.

Key points

- T1D is the most common cause of diabetes in childhood and is a recognized cause of diabetes in adulthood.
- T1D is an autoimmune disease that is hypothesized to proceed through a series of stages including: genetic risk, triggering of autoimmunity, metabolic abnormalities, and overt diabetes.
- Strategies to prevent or treat T1D have been employed in the research setting at different stages in the natural history of the autoimmunity of T1D.

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CHAPTER 6

The Genetics of Autoimmune Thyroid Diseases

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Abstract

Autoimmune thyroid diseases (AITD), including Graves' disease (GD) and Hashimoto's thyroiditis (HT) are complex diseases that arise due to interplay between environmental and genetic factors. In the past decade, several AITD susceptibility genes have been identified and characterized, including both immune-regulatory genes and thyroid-specific genes. Some of these susceptibility genes are specific to either GD or HT, while others confer susceptibility to thyroid autoimmunity in general. Recent studies began dissecting the mechanisms by which these new genes predispose to thyroid autoimmunity, and the emerging mechanisms focus on abnormalities of the immunological synapse. In this chapter, we will summarize the recent data on the genes predisposing to AITD and the emerging mechanisms by which they confer susceptibility to disease.

1. Introduction

The autoimmune thyroid diseases (AITD) include Graves' disease (GD) and Hashimoto's thyroiditis (HT), both of which are characterized by infiltration of the thyroid by T and B cells, reactive to thyroid antigens, resulting in the production of thyroid autoantibodies, with the resultant clinical manifestations. The hallmark of GD is the production of thyrotropin receptor (TSHR) stimulating antibodies which stimulate the thyroid gland causing overproduction of thyroid hormones resulting in clinical hyperthyroidism (reviewed in Davies, 2000). In contrast, HT is characterized by apoptosis of thyrocyte (caused by the thyroid-infiltrating T cells) leading eventually to thyroid hypofunction and clinical hypothyroidism (reviewed in Weetman, 2000). While the exact etiology of thyroid autoimmunity is not known, there are abundant data supporting a major genetic influence on the development of AITD (reviewed in Tomer and Davies, 2003). Therefore, the current paradigm for the development of AITD is that thyroid autoimmunity develops in genetically predisposed individuals upon exposure to an environmental trigger. In this chapter, we will discuss the recent advances in our understanding of the genetic basis of AITD.

2. Epidemiological data

The AITD have been known to be familial for many years (Hall and Stanbury, 1967). However, familial clustering of a disease is not necessarily due to genetic predisposition to disease, as family members share many environmental factors. One way to estimate the genetic component to familial segregation of a disease is by computing the sibling risk ratio (λ_s). The sibling risk ratio is the ratio of

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the prevalence of the disease in siblings of affected individuals, to the prevalence of the disease in the general population (Risch, 1990). A λ_s of >5 is considered evidence that heritability contributes to the familial aggregation of a disease. We have recently computed a λ_s of 16.9 for AITD, suggesting a strong genetic predilection to develop AITD (Villanueva et al., 2003).

The strongest evidence for a genetic contribution to the etiology of a complex disease comes from twin studies. Indeed, several large twin studies have been performed in AITD, all supporting a strong genetic predisposition. Twin studies in GD showed a higher concordance of GD in monozygotic (MZ) twins when compared to dizygotic (DZ) twins (Brix et al., 1998; Brix et al., 2001; Ringold et al., 2002). Twin studies in HT (Brix et al., 2000) and in patients with thyroid antibodies (TAbs) alone (Phillips et al., 2002) have also shown a higher concordance rate in MZ compared to DZ twins. Thus, the twin data corroborate the presence of a substantial inherited susceptibility to AITD.

3. Identifying complex disease genes

Identifying complex disease genes is a challenging task since complex diseases are caused by several genes with various penetrances, and are strongly influenced by non-genetic factors. However, recent advances in genetic mapping techniques have made complex disease gene identification a reality. Earlier studies focused on candidate genes of known function, but today it is possible to screen the entire human genome for complex disease susceptibility genes without any prior assumptions on their function (a technique called 'reverse genetics').

3.1. Candidate gene analysis

Candidate genes are genes of known sequence and location that are selected, based on their known physiological functions, as possible contributors to disease pathogenesis. For example, one can hypothesize that the *TPO* gene may be a candidate gene for HT because the hallmark of the disease is the presence of TPO antibodies. If a candidate gene causes a disease, then markers inside or flanking this gene will be associated and linked with the disease. The candidate gene approach has been successful in the field of thyroid autoimmunity, as several of the AITD susceptibility genes were identified by the candidate gene approach (e.g., CTLA-4, see below).

3.2. Whole genome screening

Whole genome screening is a powerful tool, as it enables scanning the whole human genome for a disease gene without any prior assumptions on disease pathogenesis (Davies et al., 1994). Whole genome screening by linkage analysis is performed by testing a panel of markers that span the entire human genome for linkage with a disease in a dataset of families in which the disease aggregates. Since linkage of polymorphic markers with a disease spans large genomic distances (10–20 Mb), one can scan the entire human genome by linkage using a relatively small number of markers, approximately 400 (Tomer et al., 2003). If one or more markers in a certain locus show evidence for linkage with the disease this locus may harbor a susceptibility gene for the disease studied. Following the identification of a linked region it can then be fine-mapped and the gene is identified (Glazier et al., 2002).

Recently, genome scanning by associations became possible, too. The main difficulty in genome-wide screening by association analysis is that, unlike the linkage signal which spans 10–20 Mb, the association signal spans short distances (approximately <50 Kb), and therefore up to 500,000 markers are needed to scan the human genome by association. Recently a landmark project, the HapMap project (Altshuler et al., 2005), has made whole genome scanning by association studies a reality. Indeed, this method has already been successfully applied in several complex diseases (Duerr et al., 2006). The HapMap project identified and genotyped more than one million single-nucleotide polymorphisms (SNPs) spanning the entire human genome in four

ethnically distinct human populations (Altshuler et al., 2005). The HapMap analysis demonstrated that the human genome is highly organized into discrete linkage disequilibrium (LD) blocks. This enabled the utilization of 'tagged' SNPs (each SNP representing an LD block) to test the entire human genome for association with disease. Moreover, the HapMap coupled with microarray-based genotyping technology, enabled the typing of up to 500,000 SNPs in a single experiment. Thus, today it is possible to scan the entire human genome using densely spaced SNPs.

3.3. Gene–gene interactions

Recent advances have made it possible to efficiently identify complex disease genes. As a result it became apparent that most complex diseases are influenced by numerous genes which interact with each other, in complex ways. Genes can interact directly at the genomic level (epistasis) or at the protein-functional level. Thus, inheriting certain combinations of susceptibility alleles of genetic variants increases the risk of disease. This genetic risk can then lead to the development of disease upon encounter with environmental risk factors.

Using both the candidate gene approach and whole genome linkage studies, 6 AITD susceptibility genes have been identified so far. These include four immune-regulatory genes, *HLA-DR*, *CD40*, *CTLA-4*, *PTPN22* and two thyroid-specific genes, thyroglobulin (Tg) and TSH receptor. There is also evidence that these genes interact to increase the risk for AITD (Hodge et al., 2006). Additional, AITD genes must contribute to the pathogenesis of AITD, and hopefully they will be identified in the future.

4. Immune-regulatory genes

4.1. Human leukocyte antigen (HLA) class II genes

The major histocompatibility complex (MHC) region is a locus on chromosome 6p21 which encodes the HLA glycoproteins (Todd et al., 1988). This locus has been shown to be associated with many autoimmune diseases including AITD. GD has been consistently shown to be associated with HLA-DR3 (reviewed in Tomer and Davies, 2003). The frequency of DR3 in GD patients was generally 40–50% and in the general population ~15–30%, giving an odds ratio (OR) for people with HLA-DR3 of 3.0–4.0 (Farid, 1981). Among Caucasians, HLA-DQA1*0501 was also shown to be associated with GD (RR = 3.8) (Yanagawa et al., 1993; Barlow et al., 1996), but it appears that the primary susceptibility allele in GD is HLA-DR3 (HLA-DRB1*03) (Zamani et al., 2000).

Since HLA-DR3 is a polymorphic protein it was likely that a specific DR3 sequence predisposes to GD. Indeed, in other autoimmune diseases, such as type-1 diabetes (T1D) (Todd et al., 1987), there is convincing evidence that the disease is associated with specific amino acid sequences of the *DRB1* and *DQ* genes. Therefore, we recently sequenced the HLA-DRB1 locus in a population of GD patients and controls, and identified arginine at position 74 of the HLA-DRβ1 chain (DRβ-Arg-74) as the critical DR amino acid conferring susceptibility to GD (Ban et al., 2004a). These data were later replicated in an independent dataset (Simmonds et al., 2005). Further analysis has shown that the presence of Glutamine at position 74 was protective for GD (Ban et al., 2004a). This suggested that position 74 of the DRB1 chain is critical for GD development. Indeed, structural modeling analysis demonstrated that the change at position 74, from glutamine to arginine, significantly modified the three-dimensional structure of the peptide-binding pocket, and thus could modify the interaction of the DR peptide–binding pocket with antigenic peptides during presentation to T cells. Indeed, sequence variations in the binding cleft of MHC II molecules represent a general paradigm as a susceptibility factor to a number of autoimmune conditions (Todd et al., 1988; Wucherpfennig et al., 1995; Wucherpfennig, 2001; Gebe et al., 2002).

Data on HLA alleles in HT have been less consistent than in GD. Earlier studies showed an association of goitrous HT with HLA-DR5 (RR = 3.1) (Farid et al., 1981) and of atrophic

HT with DR3 (RR = 5.1) in Caucasians (Moens et al., 1978). Later studies in Caucasians reported weak associations of HT with HLA-DR3 (Tandon et al., 1991; Ban et al., 2002) and HLA-DR4 (Petrone et al., 2001). Interestingly, recently we have shown the HLA-DR3 was the primary HLA class II allele responsible for the joint susceptibility for T1D and AITD in families in which both diseases cluster (Golden et al., 2005).

4.2. CD40

CD40 is a co-stimulatory molecule which plays a central role in the regulation of B-cell responses. CD40 is expressed primarily on B cells and other antigen presenting cells (APCs) (Banchereau et al., 1994). CD40 plays a fundamental role in B-cell activation inducing, upon ligation, B-cell proliferation, immunoglobulin class switching, antibody secretion, and affinity maturation (Armitage et al., 1993; Arpin et al., 1995). CD40 signaling cascade has been shown to play a role in a number of autoimmune conditions. For example, blocking CD40 ligand (CD154) suppressed several experimental autoimmune diseases, with a strong humoral component, such as lupus nephritis (Mohan et al., 1995), experimental autoimmune myasthenia gravis (Im et al., 2001), and experimental GD (Chen et al., 2006). Recently, we have identified *CD40* as a novel susceptibility gene for GD. We have identified a C/T polymorphism, at the 5′-untranslated region (5′UTR) of CD40, and have shown that the CC genotype of this SNP was strongly associated with GD (Tomer et al., 2002a). The association between the CC genotype of the CD40 5′UTR SNP and GD has now been replicated in several studies, performed in different populations including Caucasians (Kurylowicz et al., 2005), Koreans (Kim et al., 2003), and Japanese (Mukai et al., 2005; Ban et al., 2006).

The CD40 SNP resides in the Kozak sequence of the 5′UTR of CD40, a region which is essential to the start of translation (Kozak, 1991). Therefore, we hypothesized that the CC genotype predisposed to GD by altering the translational efficiency of CD40. Indeed, further studies have demonstrated that the C-allele of the polymorphism increased the translation of CD40 mRNA transcripts, by 20–30% compared to the T-allele (Jacobson et al., 2005). Therefore, we proposed that there exists a translational pathophysiological facet to GD, due to changes in the levels of CD40 protein, with increased CD40 expression contributing to disease etiology (Jacobson et al., 2005). For example, a thyroid autoreactive B cell expressing higher level of CD40, associated with the CC genotype, may have a lower threshold for activation, thus helping trigger thyroid autoimmunity. Another possibility is that the C-allele of the Kozak SNP enhances the efficiency of CD40 translation in other CD40-expressing tissues including the thyroid gland itself. Indeed, it has been demonstrated that the CD40 is expressed and functional on thyrocytes (Metcalfe et al., 1998), and the thyroidal expression of CD40 is upregulated in GD (Smith et al., 1999).

Since CD40 is a major APC and B-cell co-stimulatory molecule, the question arises whether the CD40 Kozak SNP could play a role in other autoimmune conditions? So far CD40 polymorphisms have been tested in two T-cell–mediated autoimmune diseases, HT (Tomer et al., 2002a) and multiple sclerosis (Buck et al., 2006), and, not unexpectedly, no association was found, as these two conditions have a large Th1 component. Interestingly, we also did not find an association of the CD40 Kozak SNP with Myasthenia Gravis, a classic antibody-mediated autoimmune disease, similar to GD (Jacobson et al., 2007). However, a recent study has shown that the C allele of the CD40 Kozak SNP was strongly associated with high IgE levels in asthma (Park et al., 2007).

4.3. CTLA-4

The cytotoxic T lymphocyte-associated factor 4 (*CTLA-4*) gene encodes for a 188 amino acid glycoprotein which is a major negative regulator of T cell–mediated immune responses (Teft et al., 2006). For example, blocking CTLA-4 with a monoclonal antibody enhances proliferation of T cells and the production of IL-2 (Wu et al., 1997). CTLA-4 is not constitutively expressed on resting, naïve CD4+ CD25– T cells but, in response to T-cell receptor activation, the CTLA-4 expression

is induced, peaking 24–48 h later (Alegre et al., 1996). However, CD4 + CD25 + T regulatory cells constitutively express CTLA-4, although the requirement or lack thereof for CTLA-4 for their function is currently unclear (reviewed in Sansom and Walker, 2006).

Over the past decade the *CTLA-4* gene was shown to be linked and associated with all AITD phenotypes including GD, HT, and TAb (Yanagawa et al., 1995; Nistico et al., 1996; Donner et al., 1997b; Kotsa et al., 1997a; Kouki et al., 2002; Ban et al., 2003a; Ueda et al., 2003). The first CTLA-4 polymorphism identified was a microsatellite marker located at the 3′UTR of the *CTLA-4* gene. The 3′UTR microsatellite showed an association with GD, resulting in an odds ratio of 2–2.5 (Yanagawa et al., 1995; Kotsa et al., 1997a). Later, a SNP at position 49 in the CTLA-4 leader peptide (A/G_{49}) resulting in an alanine/threonine polymorphism was also found to be associated with AITD (Donner et al., 1997b; Yanagawa et al., 1997; Braun et al., 1998; Villanueva et al., 2000; Nithiyananthan et al., 2002). The association between GD and these two polymorphisms has been consistent across populations of different ethnic backgrounds such as Caucasians (Yanagawa et al., 1995; Heward et al., 1999), Japanese (Akamizu et al., 2000), and Koreans (Park et al., 2000). Another SNP (designated CT60), which is located downstream and outside of the 3′UTR of the *CTLA-4* gene was also associated with GD.

CTLA-4 has been tested for association with other AITD phenotypes, except GD. Studies in HT have shown significant associations with CTLA-4 across populations of different ethnicities and geographic locations, including Caucasians (Donner et al., 1997a; Kotsa et al., 1997a; Nithiyananthan et al., 2002), and Japanese (Sale et al., 1997; Akamizu et al., 2000). Similarly, CTLA-4 was shown to confer susceptibility to the production of TAbs (Tomer et al., 2001; Zaletel et al., 2002). The G allele of the A/G_{49} SNP was also found to be associated with higher levels of both thyroglobulin and thyroid peroxidase autoantibodies (Zaletel et al., 2006). Since the development of TAbs often represents the pre-clinical stage of AITD (Vanderpump et al., 1995) it is possible that CTLA-4 predisposes, non-specifically, to the development of thyroid autoimmunity, while additional genetic variants (e.g., CD40) and/or environmental factors (e.g., iodine) trigger the development of specific AITD phenotypes, such as GD (Tomer, 2001).

In view of the function of CTLA-4 as a negative regulator of T cells one would expect it to confer susceptibility to autoimmunity in general and not specifically to one autoimmune phenotype (Tomer, 2001). Indeed, CTLA-4 was reported to be associated and linked with all forms of AITD (GD, HT, and TAbs), as well as with many other autoimmune diseases such as T1D (Nistico et al., 1996; Donner et al., 1997b; Marron et al., 1997; Ueda et al., 2003), Addison's disease (Vaidya et al., 2000), Sjögren's syndrome (Downie-Doyle et al., 2006), systemic lupus erythematosus (SLE, Lee et al., 2005), and myasthenia gravis (Huang et al., 1998).

Since several CTLA-4 variants were found to be associated with autoimmunity it was proposed that one of them might be the causative variant, while the others show association with disease by virtue of their tight LD with the causative variant. Since all CTLA-4 variants associated with autoimmunity are in tight LD, only functional studies can determine which is the causative variant. Mechanistically, a polymorphism that compromises CTLA-4 functionality or reduces its cell surface expression would be expected to cause heightened T-cell activation, and potentially, lead to the development of an autoimmune condition.

Functional studies have been performed for some of the CTLA-4 polymorphisms. The A/G_{49} SNP causing a Thr > Ala substitution in the signal peptide, was reported to cause misprocessing of CTLA-4 in the ER resulting in less efficient glycosylation and diminished surface expression of CTLA-4 protein (Anjos et al., 2002). However, these interesting findings need confirmation. Kouki et al. (2000) have shown an association between the G allele of the A/G_{49} SNP and reduced control of T-cell proliferation, results which were later replicated by us (Ban et al., 2003a). However, this association could be due to a direct effect of the A/G_{49} SNP or due to the effects of another polymorphism in LD with the A/G_{49} SNP. Therefore, Xu et al. (2002) tested

the A/G_{49} SNP directly, by transiently transfecting a T cell line, devoid of endogenous CTLA-4 (Jurkat cells), with a CTLA-4 construct harboring either the G or the A allele of the A/G_{49} SNP. They reported no difference in CTLA-4 expression and/or function when they transfected the cells with a CTLA-4 construct harboring the A or the G allele (Xu et al., 2002). Therefore, it was concluded that the A/G_{49} SNP is not the causative variant, but rather is in LD with the causative variant. A recent comprehensive analysis of the *CTLA-4* gene locus demonstrated that the CT60 SNP of CTLA-4 showed the strongest association with GD, suggesting that it might be the causative SNP (Ueda et al., 2003). Further, functional analysis in a small number of patients has shown that the GG (disease associated) genotype of CT60 was associated with reduced mRNA expression of the soluble form of CTLA-4 (Ueda et al., 2003). However, a recent large study from Sweden could not replicate these results (Mayans et al., 2007), and thus it is unclear whether CT60 is, indeed, the causative variant.

Another CTLA-4 variant that could affect CTLA-4 functionality is the 3′UTR (AT)n microsatellite. Studies analyzing the functional effects of the 3′UTR microsatellite have demonstrated that the longer repeats are associated with reduced CTLA-4 inhibitory function (Takara et al., 2003). Moreover, the 3′UTR AT microsatellite was shown to influence the half life of the CTLA-4 mRNA, with long repeats being associate with significantly shorter half life of CTLA-4 mRNA compared to the short repeats (Wang et al., 2002). Therefore, this could provide an attractive explanation for the association between the short alleles of the (AT)n microsatellite and AITD. Another possibility is that no one CTLA-4 variant is causative and that a haplotype consisting of several variants is responsible for the association with autoimmunity.

4.4. The protein tyrosine phosphatase-22 (PTPN22) gene

The lymphoid tyrosine phosphatase (LYP), encoded by the *PTPN22* gene, is a protein tyrosine phosphatase that, like CTLA-4, is a powerful inhibitor of T-cell activation (Cloutier and Veillette, 1999). Recently, a tryptophan for arginine substitution at codon 620 (R620W) of the LYP protein was found to be associated with rheumatoid arthritis (Begovich et al., 2004), SLE (Kyogoku et al., 2004), and T1D (Bottini et al., 2004; Smyth et al., 2004), as well as GD (Velaga et al., 2004), and HT (Criswell et al., 2005). Unlike CTLA-4 which was associated with AITD across ethnic groups, the *PTPN22* gene showed significant associations only in Caucasians (Ban et al., 2005).

The PTPN22 R620W SNP was found to elicit a functional change in LYP, but somewhat paradoxically, the disease-associated tryptophan variant makes the protein an even stronger inhibitor of T cells, as it is a gain-of-function variant (Vang et al., 2005). One possible explanation for this finding is that a lower T-cell receptor signaling would lead to a tendency for self-reactive T cells to escape thymic deletion and thus remain in the periphery.

5. Thyroid-specific genes

5.1. Thyroglobulin

Thyroglobulin (Tg) is a very large molecule that serves as a precursor and storehouse for thyroid hormones (Charreire, 1989). The thyroglobulin molecule undergoes several important post-translational modifications, including iodination (Kondo and Ui, 1961), which is believed to be a contributor to disease (reviewed in Rose et al., 2002), albeit some studies suggest that iodination is not a prerequisite for the initiation of thyroiditis, but may exert an effect on disease maintenance and/or severity (Kong et al., 1995; Wan et al., 1997). Mouse models have provided additional evidence for the importance of Tg to the development of thyroid autoimmunity. The mouse model for HT, murine experimental autoimmune thyroiditis (EAT), can be induced, in genetically susceptible mice, by immunization with Tg, in conjunction with an adjuvant (reviewed in Stafford and Rose, 2000). EAT, like its human disease counterpart, is

characterized by a cellular infiltrate of the thyroid, anti-Tg T-cell responses, as well as high titers of anti-Tg autoantibodies (Charreire, 1989). Thus, Tg is a critical thyroid-specific protein for the development of AITD in humans and EAT in mice.

Recently, the *Tg* gene was established as a major AITD susceptibility gene (Tomer et al., 2002b, 2003; Collins et al., 2003; Ban et al., 2004b). Sequence analysis of the *Tg* gene has revealed 14 new SNPs. Subsequent case-control association studies demonstrated significant associations between AITD and a SNP cluster in exons 10–12, and another SNP in exon 33 (Ban et al., 2003b). All the associated Tg SNPs (except one on exon 10) were missense SNPs, i.e., they caused an amino acid change in the Tg protein. As a further support for the contribution of thyroglobulin polymorphisms to disease, we identified amino acid variants in the mouse thyroglobulin gene that were associated with murine autoimmune thyroiditis (Ban et al., 2003b). Taken together, these data suggested that amino acid variants in the Tg protein predispose to AITD.

It is likely that Tg amino acid variants predispose to AITD by altering Tg peptide presentation by APCs to T cells. The MHC II molecule exists as a heterodimeric complex, consisting of an alpha chain and a beta chain, which come together to form a cleft that can accommodate peptides of 10–30 residues (Wucherpfennig, 2001). As previously mentioned we have demonstrated that a single amino acid variation in the peptide-binding cleft of HLA-DR, resulting in an arginine at position 74 of the beta chain, was strongly associated with GD, while the presence of glutamine at the same location was protective (Ban et al., 2004a). Further analysis showed that the SNP in exon 33 of Tg, had a statistical interaction with the Arg74 polymorphism of HLA-DR, resulting in a high odds ratio of 15 for GD (Hodge et al., 2006). This statistical interaction may imply a biological interaction between Tg and HLA-DR, most likely by altering Tg peptide presentation. It is possible that the Tg peptide repertoire that is generated due to the associated Tg SNP alleles is pathogenic, while DRβ-Arg74 is able to more optimally present these pathogenic Tg peptides to T cells. Thus, inheriting both the disease-associated Tg SNP alleles and DRβ-Arg74 would result in the production of pathogenic Tg peptides and in their efficient presentation to T cells.

5.2. The TSH receptor (*TSHR*) gene

The hallmark of GD is the presence of stimulating TSHR autoantibodies, and therefore the *TSHR* gene is an excellent candidate for GD. To date, three missense SNPs of the *TSHR* gene have been examined for association with GD (Tonacchera and Pinchera, 2000). Two of these SNPs reside in the extracellular domain of the TSHR: an aspartic acid to histidine substitution at position 36 (D36H), and a proline to threonine substitution at position 52 (P52T). The third SNP lies within the intracellular domain of the receptor, and is a relatively conservative substitution of glutamic acid for aspartic acid (D727E). Bahn and colleagues were the first to report an association between the P52T SNP of the TSHR extracellular domain and GD (Cuddihy et al., 1995). However, this association was not replicated by other groups (Kotsa et al., 1997b; Allahabadia et al., 1998), and therefore it was unclear whether the TSHR is indeed an important susceptibility gene for GD. Further studies gave inconsistent results (Simanainen et al., 1999; Chistyakov et al., 2000; Kaczur et al., 2000). However, a recent study from Japan has shown significant association of the *TSHR* gene with GD (Hiratani et al., 2005). These data were later confirmed in another study from the UK, albeit the associated polymorphisms were different in the Japanese and UK studies (Dechairo et al., 2005). In summary, the *TSHR* gene seems to be associated with GD. However, the magnitude of the contribution of the TSHR to GD susceptibility remains to be determined.

6. Conclusions

The AITD are complex diseases that develop as a result of combined effects of multiple susceptibility genes and environmental triggers. There are now solid epidemiologic data to support an important genetic contribution to the development of AITD,

and in the past decade several loci and genes have shown evidence for linkage and/or association with AITD. The AITD susceptibility genes identified so far can be divided into two broad groups: (1) immune-modulating genes and (2) thyroid-specific genes. The first group includes the *HLA-DR*, *CD40*, *CTLA-4*, and *PTPN22* genes, while the second group includes the *Tg* and *TSHR* genes. It is clear that additional genes contribute to the genetic susceptibility to AITD, and hopefully they will be mapped in the near future.

In order to understand the functional consequences of variants that are associated with AITD, functional studies are needed. Preliminary functional studies have been performed in AITD for HLA (Sawai and DeGroot, 2000), CTLA-4 (Kouki et al., 2000; Xu et al., 2002; Ban et al., 2003a; Ueda et al., 2003;), and CD40 (Jacobson et al., 2005). These functional studies strongly suggest that abnormalities in the immunological synapse might play a significant role in the etiology of AITD. All six AITD susceptibility genes identified so far participate in the immunological synapse and/or the signaling pathways activated by the immunological synapse. This provides a potential molecular explanation for the risk conferred by these genes for disease development. Hopefully, identifying novel mechanisms of disease development through genetic studies will potentially lead to new therapeutic targets.

Key points

- Twin and family studies point to a strong genetic influence on the etiology of autoimmune thyroid diseases, including Graves' and Hashimoto's diseases.
- The genes causing autoimmune thyroid diseases are being identified using the candidate gene approach and whole genome analyses.
- The genes that predispose to autoimmune thyroid diseases include immune-regulatory genes, such as *HLA*, *CTLA-4*, and *CD40*, as well as thyroid-specific genes, thyroglobulin and TSH receptor gene.
- Gene–gene interaction most likely is important in conferring risk for thyroid autoimmunity.
- Using the new methods for gene identification such as micro-array-based SNP analyses many genes will be discovered in the near future.

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CHAPTER 7

Thyroid Dysfunction and the Immune System

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It is now accepted that the neuroendocrine system can influence the development and function of the immune system. Conversely, the information regarding how the immune system participates in the regulation of endocrine activity is rather scant, particularly for immune–endocrine interactions of the hypothalamus–pituitary–thyroid axis. It is known that thyroid-stimulating hormone (TSH) may be produced by many types of extra-pituitary cells such as T and B lymphocytes, splenic dendritic cells, bone marrow hematopoietic cells, and intestinal epithelial cells; however, little is known regarding the physiological role of these TSH pathways. Recent evidence suggests the existence of complex regulatory functions mediated by intra-thyroid bone marrow–derived cells that affect thyroid function in both physiological and pathological conditions.

1. Physiological interactions of thyroid function and the immune system

That TSH is produced by cells of the immune has been known for decades (Smith et al., 1982; Kruger and Blalock, 1986). Leukocytes produce TSH when stimulated by TSH-releasing hormone or with staphylococcus enterotoxin A (Smith et al., 1982; Kruger and Blalock, 1986; Kruger et al., 1989). Additionally, thyroid hormones also may serve as negative feedback regulators of hematopoietic TSH production, as they do in the hypothalamus–pituitary–thyroid axis (Harbour et al., 1989). Splenic dendritic cells (DCs) have also been shown to be an important source of TSH production. When stimulated in vitro, DCs produce threefold to sixfold more TSH than B or T lymphocytes (Bagriacik et al., 2001). Within the bone marrow, TSH is produced by a sub-population of hematopoietic precursor cells bearing the CD45+/CD11b+ phenotype (Zhou et al., 2002; Klein and Wang, 2004). TSH synthesis also takes place in intestinal epithelial cells and intestinal T cells (Wang et al., 1997). TSH production seems to be restricted in sub-villus crypt regions (Scofield et al., 2005)—a site where local T-cell development occurs (Saito et al., 1998)—as well as in certain focal areas of the epithelium (Scofield et al., 2005). In at least two examples, it has been shown that viral infections in the intestine result in an increased local synthesis of TSH (Scofield et al., 2005), which suggests a paracrine action of TSH, which might operate elsewhere in the organism, including the thyroid gland itself.

Although it is known that TSH is produced by cells of the immune system, it is not clear how immune-cell–derived TSH participates in the immunoregulatory circuits in health and disease. There are two possible venues through which immune-derived TSH could modify the immune response: one would be the direct effect of TSH on immune cells, while the other could operate in an indirect fashion through TSH-induced thyroid hormone. Both these possibilities are not mutually exclusive.

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Since TSH is produced by leukocytes, it is possible that TSH acts as a biological response modifier within the immune system, as a cytokine-like molecule. Consonant with this hypothesis is the demonstration of the presence of TSH receptors on lymphoid and myeloid cells (Coutelier et al., 1990; Bagriacik and Klein, 2000) and the proven ability of TSH to induce several immune response-related functions (Fabris et al., 1995, p. 14; Kruger, 1996). TSH alone increases the in vitro production and secretion of antibodies (Blalock et al., 1984; Kruger and Blalock, 1986; Kruger et al., 1989) as well as the in vitro proliferative response of lymphocytes to mitogens (Provinciali et al., 1992). When combined with interleukin-2, TSH increases the potential of this cytokine to induce natural killer cell activity (IL-2) (Provinciali et al., 1992), and stimulation of splenic DCs by TSH increases the activity of interleukin-1β and interleukin-12 in the presence of phagocytic stimuli (Bagriacik and Klein, 2000). In the hematopoietic milieu, TSH increases the synthesis and secretion of TNFα (Whetsell et al., 1999; Klein and Wang, 2004), and triggers phosphorylation of the Jak2 kinase (Whetsell et al., 1999). Inasmuch as these functions are exerted on cells expressing TSH receptors, it is very likely that they are mediated by direct interaction of TSH with its corresponding receptor.

Consonant with the second alternative, that is TSH acting through thyroid hormone release, are several reports of impaired immune function in hypothyroidism. In TSH receptor defective (C.RF-*hyt*/*hyt*) mice, there is impairment of B-cell development (Foster et al., 1999; Dorshkind and Horseman, 2005), which can be corrected by exogenous administration of T_4 (Foster et al., 1999). Additionally, mice that are genetically unable to express T_3 receptor show reduced numbers of T and B lymphocytes, as well as myeloid cells in the bone marrow, thymus, and spleen (Beigneux et al., 2003).

A very attractive possibility is that another role of "immune" TSH would be the micro regulation of thyroid function. This would mainly participate in the communication between the immune system and the thyroid, rather than in the overall regulation of thyroid function which remains the responsibility of the hypothalamus and pituitary. For this to occur, it must be accepted that immune cells that are capable of producing TSH, are also capable of trafficking to the thyroid and exerting paracrine regulation. Two clinical conditions seem to support this hypothesis: the first of them is the Euthyroid Sick Syndrome (ESS); a hypothyroidic condition of humans that occurs in the absence of thyroid disease, where conversion of T_4 to T_3 is impaired in mild forms, but the output of T_4 itself may be found decreased in severe forms. ESS can present as a complication of a variety of infectious and non-infectious inflammatory diseases or after prolonged fasting (De Groot, 1999; Papanicolaou, 2000; Klemperer, 2002; Inan et al., 2003; Brierre et al., 2004). Although the true significance of ESS is not known, it may represent a physiological mechanism aimed at conserving energy during periods of stress (De groot, 1999; Larsen et al., 2002; Fakete et al., 2004). The detailed mechanisms of how the different conditions can trigger ESS have been described elsewhere (Klein, 2006), but it has been forwarded that the immune system, rather than the endocrine axis, is responsible for the recovery of euthyroid function after the underlying pathological condition is resolved. This assumes the existence of an inherent ability of the immune system to continually assess the status of the infectious condition and thus to determine whether it is safe for the host to return to a state of normal metabolic activity.

The second is the ESS-like condition that follows hematopoietic stem cell transplantation, which can be due to total body irradiation (Wehmann et al., 1985; Hershman et al., 1990; Vexiau et al., 1993; Kauppilla et al., 1998; Ishiguro et al., 2004; Carlson et al., 1992; Lio et al., 1988; Matsumoto et al., 2004) or chemotherapy in the absence of irradiation (Toubert et al., 1997; Slatter et al., 2004). In this condition, T_3 and occasionally T_4 levels are diminished in the presence of normal TSH output. Inasmuch as the thyroid is resistant to clinical radiation, the reason for the impairment of thyroid function is unknown but it is not secondary to the thyroid gland damage by the immunosuppressive treatment itself. Moreover, thyroid hormone levels in bone marrow graft recipients remain suppressed in the face of otherwise normal circulating TSH levels. As in ESS, it seems that pituitary TSH has a minimal, if any, influence on thyroid function in these patients. Again, intra-thyroid TSH production by immune system cells is a novel and very interesting explanation.

A simple interpretation of these observations is that inflammatory stress might act through the hypothalamus–pituitary–thyroid axis to initiate and maintain an overall condition of decreased metabolic activity, basically aimed to energy conservation by the host during the period of infection or fasting. Once the infection is eliminated or controlled by the innate and adaptive immune responses, the immune system would provide the initial signal that would trigger an adjustment in thyroid hormone function and the resultant recovery of metabolism.

The relationship between thyroid dysfunction and autoimmunity can be analyzed in pathological conditions, but also from a physiological state that has shed important clues to the understanding of this interaction. In recent years, there has been an increasing interest in disturbances of thyroid function that occur in mothers after delivery, a prevalence that seems to be higher than previously thought. Additionally, it has been proven that patients with previous thyroid dysfunction before pregnancy may have recurrences. In particular, a transient destructive type of postpartum hyperthyroidism has been recognized which may be silent. Preliminary studies on the prevalence of this particular disorder have shown that it is far more common that postpartum Graves' hyperthyroidism (Amino et al., 1976, 1982). For the survival of the foreign fetus to occur, modulation of the maternal immune system to prevent its rejection seems mandatory. Accordingly, the maternal immune response becomes suppressed during pregnancy by a variety of mechanisms, which can also affect the clinical behavior and fate of a variety of autoimmune diseases, resulting in a transient amelioration of their activity during pregnancy, which is followed by a postpartum relapse. Hence, postpartum exacerbation may occur for autoimmune thyroid diseases as Hashimoto's thyroiditis and Graves' disease, as it does for non-thyroid autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus (Ginsburg and Walfish, 1977; Walfish and Chan, 1985). The high frequency of these syndromes likely reflects changes in peripartum immune network regulation; however, the preferential involvement of thyroid in these pregnancy-associated autoimmune disorders remains a conundrum. The most plausible explanation might reside in the bidirectional complex interactions between thyroid function and the immune system.

2. The thyroid gland as a target of autoimmune disease

Cell-mediated as well as antibody-mediated immunity and genetic predisposition all play a role in autoimmune thyroid disease (Weetman, 1992). Multigenic predisposition (Davies, 1998) plus environmental stress is apparently necessary for activation of the autoimmune process. As with other autoimmue diseases, thyroid autoimmunity is more common in women than in men most likely because of both, genetic as well as hormonal factors (Chiovato et al., 1993). Among environmental factors, it is known that excessive dietary iodine intake (Laurberg et al., 1998) and smoking increase the risk of hypothyroidism in Hashimoto's thyroiditis (Fukata et al., 1996) and the risk of ophthalmopathy in Graves' disease.

Several cytokines are involved in the pathophysiology of autoimmune thyroid disease: interferon α, interleukin-2, and macrophage colony-stimulating factor may induce autoimmune thyroiditis (Volpe, 1993). Patients with autoimmune thyroid disease may have a variety of thyroid-specific antibodies, including thyroid-stimulating thyrotropin receptor antibodies, TSH receptor-blocking and inhibitory antibodies, anti-thyroglobulin antibodies, anti-thyroidperoxidase (TPO) antibodies, anti-sodium-iodide symporter, and, possibly, growth-stimulating antibodies (Endo et al., 1996).

Cell-mediated autoimmunity and apoptosis are factors in the destruction of thyroid cells and the development of hypothyroidism in Hashimoto's thyroiditis (McLachlan et al., 1990). Goiter results from lymphocytic infiltration, fibrosis, and, possibly, thyroid stimulation by TSH. In Graves' disease, hyperthyroidism and goiter are caused by autoantibodies against the TSH receptor that mimic the effect of TSH on thyroid follicular cells. The cause of extra-thyroidal manifestations, such as ophthalmopathy and thyroid dermopathy, is less clear. The autoimmune process in the affected tissues may be

due to an antigenic determinant common to both thyroid cells and these tissues (Bahn and Heufelder, 1993; Arscott and Baker, 1998).

Sub-clinical hypothyroidism is defined as mild elevation of serum TSH levels and normal circulating thyroid hormone levels. Anti-thyroid antibodies are positive in 95% of affected patients. Silent thyroiditis is a similar condition that may have autoimmune origin. It is associated with transient excessive thyroid hormone release and low radioiodine uptake. Silent thyroiditis can occur in both men and women, unrelated to pregnancy. In most affected patients, the hypothyroidism is not permanent.

The mechanisms underlying the autoimmune process that leads to thyroid disease must be more complex than those involved in other "organ-specific" autoimmune diseases. Autoimmune thyroid diseases can be found in association with a wide variety of other overt autoimmune diseases or conditions that have been related to autoimmune phenomena. The following is an alphabetical list of such conditions:

Alopecia areata
Autoimmune liver
Autoimmune polyglandular syndromes (e.g., Addison's disease, hypoparathyroidism, type 1 diabetes, ovarian failure)
Celiac disease
Chronic ulcerative colitis
Crohn's disease
Down syndrome
Hepatitis C infection
Idiopathic thrombocythemia
Idiopathic thrombocytopenic purpura
Klinefelter's syndrome
Mixed connective tissue disease
Myasthenia gravis
Polymyalgia rheumatica
Primary biliary cirrhosis
Rheumatoid arthritis
Scleredema
Sjögren's syndrome
Systemic lupus erythematosus
Turner's syndrome
Vitiligo

When analyzing this variety of conditions, it is clear that mechanisms other than antigenic mimicry or cross reactivity of autoantibodies, or self-reacting cells, are operating in patients with such combinations of clinical manifestations. The more is learned about the complex interactions of neuroendocrine regulation and the immune system, specifically about the role of extra-pituitary TSH on several cells and tissues, our understanding of the disease pathophysiology will become clearer. Acceptance that immune TSH might be a ubiquitous regulatory molecule of other physiological processes is, doubtlessly, a most interesting explanation.

Key points

- Cells of the immune system are capable to secrete thyroid-stimulating hormone.
- Immune TSH as well as pituitary may participate in immunoregulatory circuits.
- Physiologic neuro–immune–endrocine interactions are manifold and bidirectional.

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PART II:

Clinical Aspects

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CHAPTER 8

Autoimmunity and the Pituitary Gland

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1. Introduction

About 85 conditions are nowadays classified as autoimmune diseases, and these are among the top 10 causes of morbidity in women (Cooper and Stroehla, 2003). Autoimmune diseases can affect virtually any body site (Cooper and Stroehla, 2003). A common characteristic of organ-specific autoimmune diseases is the mononuclear, mainly lymphocytic infiltration of the target organ, leading to alteration of the normal architecture and loss of function. The appearance of circulating autoantibodies is a common aspect of these diseases. Autoantibodies are, however, more numerous than autoimmune diseases and new antibodies directed against autoantigens are constantly being discovered (Caturegli, 2007).

Autoimmune hypophysitis is still considered rare, but cases with pituitary autoimmunity are being recognized with increasing frequency. Autoimmune hypophysitis represents a significant clinical problem for several reasons. First, it can be associated with important functional defects. In addition, this disorder can mimic, both clinically and radiologically, other pituitary masses such as non-secreting pituitary adenomas, which are relatively common with a population prevalence around 0.1% (Daly et al., 2006) and are appropriately treated with surgical removal. Currently, about 50% of patients with hypophysitis are misdiagnosed as having an adenoma (Leung et al., 2004) and as a result, they undergo unnecessary trans-sphenoidal surgery.

There are two main challenges that must be faced in order to understand autoimmune hypophysitis and associated disorders. First, it is necessary to identify the pathogenic pituitary autoantigens that induce autoimmune hypophysitis. Second, it would be worthwhile to establish registries for patients with autoimmune hypophysitis to allow researchers to assemble study populations of sufficient size to conduct statistically meaningful research and develop novel diagnostic strategies (Caturegli, 2007).

The goal of this review is to examine different aspects of autoimmunity related to the pituitary gland.

2. Pathophysiology

There are anatomic and functional correlations among the nervous, endocrine, and immune systems. Chesnokova and Melmed (2002) reported

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that these systems both express and respond to several common regulatory molecules including steroids, neuropeptides, cytokines, and neurotransmitters. This system provides the molecular basis for integrated, bidirectionally coordinated neuroendocrine-immune responses to disturbances of homeostasis induced by stress, inflammation, or infection (Wilder, 1995; Besedovsky and del Rey, 1996; Turnbull and Rivier, 1999; Chesnokova and Melmed, 2002). Hypothalamic-pituitary-adrenal (HPA) axis activation, under primary hypothalamic control by CRH (Chrousos, 1998), is critical for maintaining physiological homeostasis under these circumstances. Anterior pituitary hormone responses to inflammatory, psychological, and environmental stressors are complex (Chrousos, 1998; Reichlin, 1999; McEwen, 2000). Specific trophic hormoneal responses depend on hypothalamic humoral control and duration and degree of stress, as well as dynamic changes in the secretion and action of peripheral and central cytokines (Chesnokova and Melmed, 2002).

It is thought that alterations in the neuroendocrine system are caused by an accumulation of genetic, environmental, and behavioral factors, as well as other influences, before disease is clinically apparent. These varied influences may cause a chronic imbalance in homeostatic mechanisms maintained by neuroendocrine, microvascular, and immune processes. Chronic inflammatory stress mediated by humoral and neural signs during active disease, and autoantibodies directed against structures in the neuroendocrine system, may also have a role in the neuroendocrine dysfunction (Imrich, 2002).

Immune cytokines represent a large group of pleiotropic and redundant polypeptides that are quickly induced in response to tissue injury, infection, or inflammation. Cytokines may function as classic endocrine secretions released from proximal tissues, passing into the circulation and reaching a distal target (Reichlin, 1999). In addition, cytokines can function as paracrine or autocrine cell regulators mediating adjacent cell functions. Cytokines act in the brain through one or more of the following mechanisms: (1) binding to cytokine receptors in the blood–brain barrier cerebral endothelium with subsequent triggering of PGE2 and activation of the HPA axis (IL-1β, TNF-α, but not gp130 cytokines); (2) activating specific transport mechanisms for IL-1, IL-6, leukemia inhibitory factor (LIF), INF, and TNF-α (Kastin et al., 1999); (3) penetrating into the brain through circumventricular organs (central sites with capillaries that have open junctions and abundant fenestrations) (Reichlin, 1999); (4) de novo synthesis in the central nervous system (Wilder, 1995; Besedovsky and del Rey, 1996; Licinio and Wang, 1999; Turnbull and Rivier, 1999); (5) acting on peripheral nerves that signal to the brain (Dantzer et al., 1998). It is unclear which of these non-mutually exclusive mechanisms are involved in specific pathophysiology of the neuroimmune interface (Licinio and Wang, 1999).

Cytokines have complex interactions that are described as overlapping, synergistic, and antagonistic actions, and cytokines are classified as either ''pro-inflammatory'' or ''anti-inflammatory'' based on their peripheral actions. Chesnokova and Melmed (2002) reported that cytokines do not necessarily translate directly into central nervous system actions and/or central regulation of the HPA axis (Allan and Rothwell, 2001). Cytokines and their receptors are expressed in the hypothalamus, as well as within anterior pituitary cells. Specifically, the gp130 cytokine family [LIF, IL-6, IL-11, ciliary neurotrophic factor (CNTF), and Oncostatin M (OSM)] participates in ACTH regulation and mediates the immunoneuroendocrine interface (Auernhammer and Melmed, 2000; Arzt, 2001). Two POMC inducers, CRH and gp130 cytokines, act in synergy and signal through cAMP and the JAK/STAT/SOCS pathways, respectively (Melmed, 2001). Ligands for the gp130 receptor cytokine family signal via common intracellular molecules, often exerting redundant functions (Arzt, 2001).

Cytokines, including IL-1, IL-6, and LIF, mediate pituitary development and cell proliferation and mature ACTH hormone secretion and have a negative feedback regulation on the HPA axis, especially as mediators of the complex response to stress or inflammation (Chesnokova et al., 1998). Mice with LIF deficiency (LIFKO) mount an attenuated ACTH response to restraint and immobilization. Conversely, LIF replacement restores ACTH levels (Chesnokova et al., 1998).

During inflammatory stress, cytokines that stimulate corticotroph POMC expression and ACTH secretion are produced peripherally and in the hypothalamus and pituitary (Chrousos, 1998; Turnbull and Rivier, 1999), and by stimulating the HPA axis they antagonize their own peripheral pro-inflammatory action.

Inflammatory cytokines also trigger central ACTH secretagogues such as noradrenaline (Giovambattista et al., 2000), pituitary adenylate cyclase-activating polypeptide (Hannibal et al., 1999), vasopressin (Chikanza et al., 2000), and other cytokines (Givalois et al., 1994). Pituitary cytokine expression and action (Arzt and Stalla, 1996; Ray and Melmed, 1997; Arzt, 2001) and HPA regulation by cytokines (IL-1, IL-6, TNF) (Besedovsky and del Rey, 1996; Turnbull and Rivier, 1999) have been extensively reviewed.

Synergistic cross-talk of different signaling cascades allows the HPA axis to quickly respond to inflammatory and stress stimuli (Auernhammer et al., 1999). gp130 receptor cytokines activate the HPA axis even in the absence of CRH (Bethin et al., 2000). Both IL-6 and LIF mediate HPA responses to stress and inflammation by inducing the ACTH axis in the course of the inflammatory process and can directly stimulate POMC expression and ACTH secretion in mouse corticotrophs (Auernhammer and Melmed, 2000). Experiments with CRH-deficient mice that demonstrated activation of the HPA axis by IL-6 lend support to this hypothesis (Bethin et al., 2000). IL-6 also directly induces release of adrenal corticosteroids in humans (Mastorakos et al., 1993) and from rat adrenal cells (Franchimont et al., 2000), and is important for prolonged activation of neurons of the paraventricular nucleus and continued CRH expression during the late phases of inflammation (Vallieres and Rivest, 1999).

Similarly, hypothalamic LIF that is induced during the course of the chronic inflammatory process is important for sustaining response to inflammation of the HPA axis (Chesnokova and Melmed, 2000). The diffusible form of murine LIF is induced in the hypothalamus and pituitary after injections of lipopolysaccharide (Wang et al., 1996), IL-1 (Auernhammer et al., 1998), turpentine or complete Freund's adjuvant (Chesnokova and Melmed, 2000). Brain IL-6 and gp130 are similarly induced after lipopolysaccharide injection (Melmed, 2001). LIF, administered before lethal septic shock, has a protective effect in preventing sepsis-induced tissue damage and lowering mortality (Waring et al., 1995). LIF is also a protective factor for neurons and peripheral tissues during injury (Sugiura et al., 2000).

3. Anti-pituitary antibodies

Anti-pituitary antibodies (APAs) are not considered reliable markers of lymphocytic hypophysitis (LYH) because measurement of the antibodies is hampered by discrepancies in methodology and clinical interpretation. The clinical relevance of these antibodies, detected using different methods, has been reduced because the test results are not consistent. A longitudinal study demonstrated that APAs can disappear over time (Kajita et al., 1991), and for this reason the time of testing could influence their identification (Barbaro and Loni, 2000). The complement consumption test, using plasma samples against human pituitary homogenate, was the first method that was used to detect APAs. Testing with complement consumption was soon replaced by immunofluorescence, immunoblotting, and radioligand assay (De Bellis et al., 2005).

Crock (1998) reported the use of immunoblots to detect antibodies to a 49-kDa cytosolic pituitary protein in the serum of patients with LYH. These antibodies were also detected in patients with other autoimmune or pituitary disorders, such as Addison's disease (42%), pituitary tumors (20%), thyroid autoimmunity (15%), and rheumatoid arthritis (13%). The antigen appeared to be the ubiquitous glycolytic enzyme, alpha enolase (O'Dwyer et al., 2002a). Autoantibodies detected in the serum of patients with LYH also react against another isoform of this enzyme, gamma enolase, which is better known as neuron-specific enolase (NSE) and restricted to neuronal tissue and neuroendocrine cells (O'Dwyer et al., 2002b). NSE is expressed in placental tissue as well as the pituitary, and the presence of the antigen in both sites may explain the association of LYH with

pregnancy. Although its expression in normal pituitary tissue appears to be very heterogeneous, NSE is likely expressed by all pituitary cell subtypes (Van Noorden et al., 1984). Many studies that have analyzed the expression of NSE in pituitary cells have usually used antibodies that could cross-react with other enolase isoforms. For this reason, additional experiments are needed to determine if specific enolase isoforms are expressed differentially in subtypes of pituitary cells. The presence of such isoforms would explain the particularly high susceptibility of the corticotroph, and to a lesser extent the thyrotroph, in LYH.

Other anti-pituitary autoantibodies have been detected in patients with LYH and seemingly unrelated conditions, such as type 2 diabetes mellitus. Because they lack specificity, these autoantibodies are considered by some researchers to be an epiphenomenon rather than a cause of the disease (Kristof et al., 1999), a supposition that contradicts a putative autoimmune etiology for LYH.

The discrepancies between results of immunoblotting and radioligand methods of detecting APAs inspired De Bellis et al. (2005) to reevaluate testing. Indirect immunofluorescence was performed, using cryostat sections of young baboon pituitary glands. Human tissue was not used because of legal difficulties in obtaining human fetal pituitary tissue (Pinol et al., 2000). It was concluded that a simple immunofluorescence method, using pituitary of young baboon as substrate, was a good approach for the detection of APAs (De Bellis et al., 2005).

APAs are a reliable marker only when present in high titers. This finding is true in patients with apparently isolated idiopathic GHD as well as adults with autoimmune endocrine diseases with selective GHD. Rivera (2006) expressed the opinion that APAs, despite their lack of absolute specificity for disease, are most likely at the core of the mechanism of diseases such as LYH.

4. Lymphocytic hypophysitis

Autoimmune hypophysitis, often referred to as LYH, is the most common chronic inflammation that primarily affects the pituitary gland (Caturegli et al., 2005). Yet, it is still considered a rare condition. LYH was first described by Simmonds (1917) in pituitaries examined at autopsy. The first antemortem case of LYH is generally credited to Goudie and Pinkerton in 1962 (Asa et al., 1981), and was thought to be have an autoimmune etiology.

4.1. Epidemiology

The number of reported cases of LYH has increased over time. Non-invasive pituitary imaging techniques and the trans-sphenoidal surgery have made definitive diagnosis possible, and awareness of LYH has increased in the medical community. The 1 per 9 million per year incidence estimate derived from the data of Buxton and Robertson (2001) may well be an underestimate. Some cases may go undiagnosed because of their indolent, subclinical course. In a review, Caturegli et al. (2005) stated that LYH is more common in women, who tend to present at a younger age than men. LHY manifests during pregnancy or postpartum in a significant percentage of women. Lymphocytic infundibulo-neurohypophysitis (LINH) appears to affect males and females equally. Lymphocytic infundibulo-panhypophysitis (LIPH) is slightly more common in women. Both LINH and LIPH are not associated with pregnancy and have a mean age at presentation of 42 ± 17 years that is significantly higher than that of LHY in women.

4.2. Etiopathology

Some facts support an autoimmune etiology for LYH: LYH is often associated with other autoimmune conditions, occurrence is increased in women, it is associated with pregnancy; and the affected pituitary tissue obtained by biopsy or at necropsy has pathologic findings compatible with autoimmune disease.

4.3. Clinical findings

4.3.1. Lymphocytic adenohypophysitis (LAH)

This form of LYH is usually the only one described in textbooks and scientific articles, and it is characterized as a disease typical of women during the peripartum period. Actually, LAH does occur more often in women compared to men, and 60% of cases are diagnosed in relation to pregnancy and parturition, typically during the third trimester or the postpartum period. A few cases have been described that presented during the second trimester (Hashimoto et al., 1997). The classical clinical picture includes headache and mass-effect symptoms (50–70% of cases) (Thodou et al., 1995; Hashimoto et al., 1997; Heinze and Bercu, 1997; Beressi et al., 1999; Bellastella et al., 2003), symptoms of adenohypophysial hypofunction (66–97%) (Thodou et al., 1995; Heinze and Bercu, 1997), hyperprolactinemia (20–38%). Symptoms of neurohypophysis involvement (Thodou et al., 1995) are found in 14–20% of cases of LAH (Thodou et al., 1995; Hashimoto et al., 1997, 2002).

4.3.2. Lymphocytic infundibulo-neurohypophysitis (LINH)

In cases of suspected LYH where diabetes insipidus is the presenting or most prominent symptom, LINH is the most likely diagnosis. Patients usually have mass-effect symptoms and may show evidence of other pituitary hormone deficiencies. In LINH, mass-effect symptoms have been described as limited to frontal or generalized headache and lethargy (Ahmed et al., 1993; Lee et al., 1994; Tubridy et al., 2001).

4.3.3. Lymphocytic infundibulo-panhypophysitis (LIPH)

LIPH is especially common in children and adolescents. These are cases of otherwise typical LINH that present with clinical evidence of extensive, severe adenohypophysial involvement, and typical histopathological findings (Maghnie et al., 1998; Maraver et al., 1998; Tubridy et al., 2001; Vega et al., 2001; Hashimoto et al., 2002; Ouma and Farrell, 2002).

4.3.4. Association with autoimmune diseases

LYH is frequently associated with organ-specific autoantibodies and other autoimmune diseases, and this finding argues in favor of an autoimmune mechanism in LYH (Bellastella et al., 2003). Cycles of remission and relapse also support the autoimmunity hypothesis (Matta et al., 2002; Lecube et al., 2003; Yamagami et al., 2003). LYH is most commonly associated with Hashimoto's thyroiditis and Graves' disease (Barbaro and Loni, 2000). Other clinical associations are diabetes insipidus, type 1 diabetes mellitus, Addison's disease, hypoparathyroidism, chronic atrophic gastritis, and pernicious anemia (Ezzat and Josse, 1997; Presotto et al., 1997; Betterle et al., 1998). Systemic lupus erythematosus, autoimmune hepatitis, and primary biliary cirrhosis are less frequent associations (Nishiki et al., 1998; Pinol et al., 2000; Ji et al., 2000).

Autoimmune polyendocrine syndrome (APS) can be diagnosed in patients with LYH when two or more autoimmune diseases are present. As a consequence, all patients with LYH and other autoimmune diseases can be considered as having an APS (De Bellis et al., 2003). Most cases of LYH show characteristics of complete APS type 3 (autoimmune thyroid disease with or without other autoimmune diseases, but not Addison's disease and hypoparathyroidism) (Pinol et al., 2000; De Bellis et al., 2003). APS type 1 and type 2 can also occur to a lesser extent (Betterle et al., 1998, 2002). Moreover, LYH may be included in incomplete APS type 2, as some cases had chronic autoimmune thyroiditis and ACA, 21-OHAb, and ICA. When LYH does not fall within the above-mentioned combination, it can be included in type 4 APS. In this setting, LYH may be associated with autoimmune central diabetes insipidus (Kamel et al., 1999; De Bellis et al., 2003).

4.4. Natural history

The natural history of LYH is in accord with other autoimmune diseases. The initial inflammation with enlargement of the gland corresponds to the

period of mass-effect symptoms and, usually, subclinical hormone deficits that can be demonstrated by non-specific dynamic testing. Progression to tissue destruction and atrophy is associated with permanent hypopituitarism (Bellastella et al., 2003). However, in some cases the disease course can be rather insidious and cases of relapsing/remitting LYH have been reported (Matta et al., 2002).

Similarly, in LINH the inflammatory process can be self-limited and radiological follow-up can show regression in about 2 years time (Imura et al., 1993). Complete or partial CDI may, however, be permanent and the reason probably is neuronal destruction (Takahashi et al., 1999). Pathologic changes suggest that the process progresses from inflammation to fibrosis and subsequent atrophy, resulting in an empty sella in imaging studies (Honegger et al., 1997; Leggett et al., 1999). Spontaneous resolution has been reported by several authors (Cameroglu et al., 1997).

4.5. Diagnosis

A presumptive diagnosis of LYH can be made on clinical and laboratory findings and imaging studies, but confirmation requires histopathology, i.e., pituitary biopsy.

Rivera (2006) suggested that the clinician should suspect LYH in a patient with evidence of pituitary dysfunction in the presence of three or more scenarios (Table 1).

MRI diagnostic criteria are summarized in Table 2 and shown in Fig. 1. Typical histopathologic changes provide the gold standard in establishing a definite diagnosis. In many cases, a conservative approach is convenient and biopsy may not be necessary (Kristof et al., 1999).

Characteristic changes include a diffuse polyclonal lymphocytic infiltration with predominance of T cells, particularly $CD4^+$ cells (McCutcheon and Oldfield, 1991; Imura et al., 1993; Abe et al., 1995; Nishioka et al., 1996). Scattered plasma cells, a few eosinophils, edema, and fibrosis replacing pituitary *acini* are also commonly present (Thodou et al., 1995; Beressi et al., 1999). Electron microscopy has shown interdigitation of inflammatory cells with pituicytes (Cosman et al., 1989) and lysosomal bodies and oncocytic changes in some pituitary cells (Thodou et al., 1995). The absence of multinucleated giant cells, epitheliod histiocytes, and true granulomas distinguishes LYH from granulomatous hypophysitis, which tends to occur

Table 1
Clinical context to suspect lymphocytic hypophysitis according to Rivera (2006)

Scenario	Notes
(a) Women in the peripartum period	
(b) Young patients	Especially when <30 years old
(c) Early, isolated, or combined deficit of ACTH and TSH secretion	When changes on MR imaging do not support severe anterior pituitary deficiency
(d) Presence of other autoimmune conditions and/or positive autoantibodies (Fig. 1)	Thyroid peroxidase antibodies, antinuclear antibodies, anti-gastric parietal cells, adrenal antibodies, anti-smooth muscle antibodies (Crock, 1998), and anti-pituitary antibodies
(e) Acute onset of headache with mass-effect symptoms such as ophthalmoplegia, visual field defects, nausea, or vomiting	Pituitary apoplexy may have a similar onset but it usually has a more severe presentation associated with distinct MR findings (pituitary hemorrhage); these conditions, however, are not mutually exclusive and pituitary apoplexy can even occur in a patient with LYH
(f) Acute onset of DI with headache and mass-effect symptoms	Granulomatous and infiltrative diseases like sarcoidosis and histiocytosis, which generally are of more insidious presentation should be excluded
(g) Lymphomonocytic pleocytosis in the CSF	In the absence of clinical meningitis and antiviral antibodies

Table 2
Characteristic MRI findings in the different forms of lymphocytic hypophysitis

Diseases	MRI findings
Lymphocytic adenohypophysitis and lymphocytic infundibulo-panhypophysitis	Intrasellar mass triangular shaped and/or affecting the diaphragma sellae with marked contrast enhancement or diffuse, ill-defined, symmetrical pituitary enlargement or suprasellar extension, especially ''tongue-like'' extension or all the above scenarios with delay of complete enhancement time in dynamic MRI (>90 sec)
Lymphocytic infundibulo-neurohypophysitis and lymphocytic infundibulo-panhypophysitis	Diffuse thickening of the pituitary stalk with or without enhancement after gadolinium and loss of the normal posterior ''bright spot'' on T1-weighted images

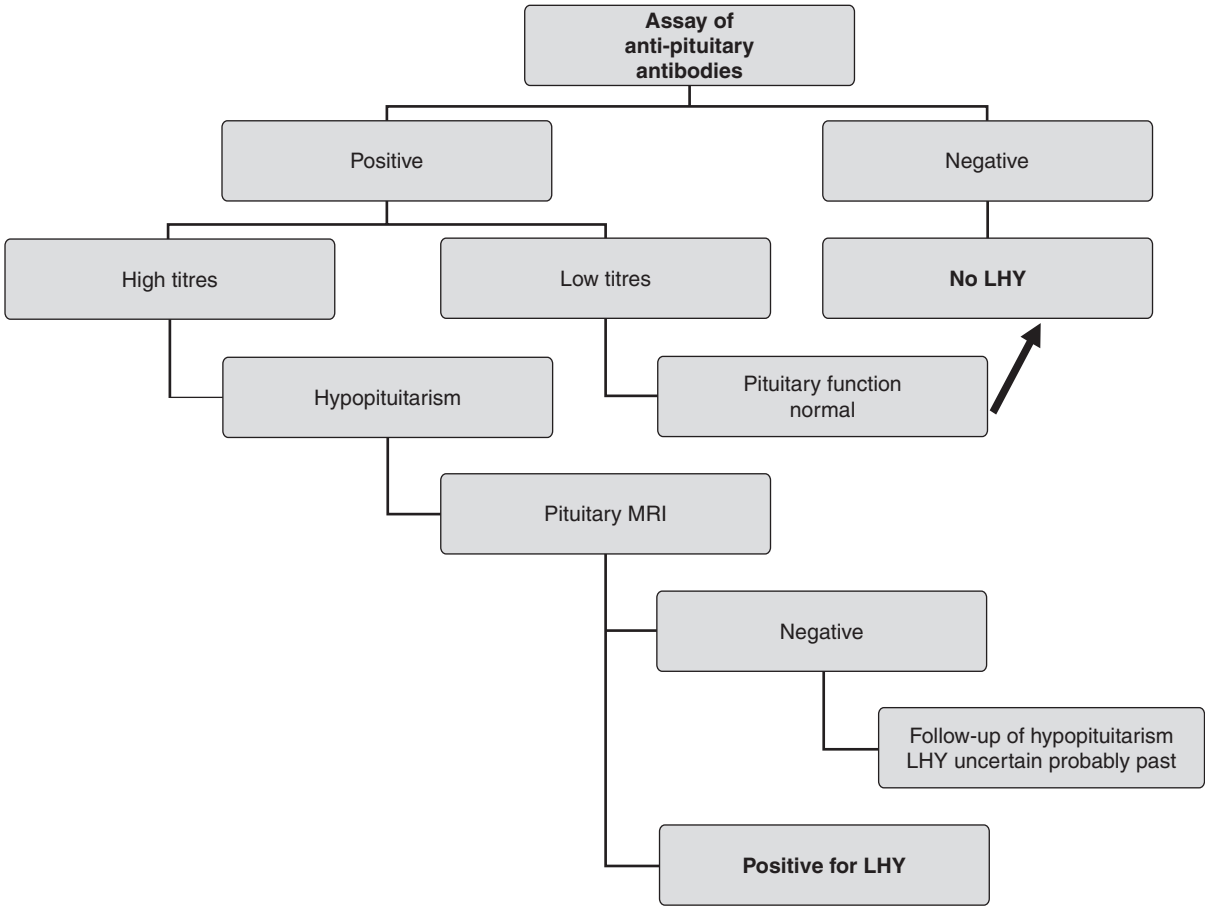

Figure 1. Algorithm of diagnosis of lymphocytic hypophysitis.

in older patients and is associated with systemic granulomatous diseases.

4.6. Treatment

Currently, treatment of LYH is essentially symptomatic and includes reducing the size of the pituitary mass and/or replacing defective endocrine functions. Mass reduction can be achieved by pituitary surgery, lympholytic drugs (glucocorticoids, azathioprine, or methotrexate), or radiotherapy (Caturegli et al., 2005).

Surgery has been the most common form of treatment in LYH and was performed on 243 (64%) of the total of 379 patients reported thus far. Surgery provides tissue to make a histological diagnosis and is very effective in achieving rapid decompression of the sellar mass and prompt resolution of headaches and visual deficits (Caturegli et al., 2005). The role of surgery in the treatment of LYH is under debate, but surgical treatment should be chosen when preoperative diagnosis of a pituitary mass is undefined or there are serious and progressive deficits in visual fields, visual acuity, or ocular movements without response to medical treatment (Caturegli et al., 2005).

Glucocorticoids can be effective for treating LYH, both as anti-inflammatory agents to reduce the size of the pituitary mass or the thickened stalk and as replacement for defective adrenal function. The most commonly used glucocorticoids have been prednisone (from 20 to 60 mg/day) (Pestell et al., 1990; Beressi et al., 1994; Nishioka et al., 1997), hydrocortisone (Hayes and McKenna, 1996; Gagneja et al., 1999), and methylprednisolone (120 mg/d for 2 weeks) (Kristof et al., 1999; Li et al., 1999; Waki et al., 1999; Lecube et al., 2003; Yamagami et al., 2003). Improvement in adenopituitary function and/or appearance on MRI have been shown in some patients (Kristof et al., 1999). More recently, immunosuppressive drugs such as azathioprine (Lecube et al., 2003) and methotrexate (Tubridy et al., 2001; Leung et al., 2004) were used to treat patients who responded poorly to glucocorticoids.

Pituitary radiation, using either conventional fractionated external-beam radiotherapy or, recently, γ-knife radiosurgery, has long been a therapeutic option for managing tumors in the sellar region, but radiation is still controversial in cases of LYH. Currently, the first option is the use of supraphysiological doses of glucocorticoids (Beressi et al., 1994; Virally-Monod et al., 1996).

5. Conclusions

Autoimmunity is an entity that is increasingly recognized and studied, and many diseases that were first categorized as idiopathic are now known to have an autoimmune origin. In recent years, detecting circulating autoantibodies and the increased sensitivity of imaging methods have made it possible to make a timely diagnosis. Identification of the antigens that make pituitary cells a target for autoantibodies requires further investigation. LYH is still considered a rare disease, but its resemblance to Hashimoto's thyroiditis is remarkable. Hashimoto's thyroiditis was considered rare when it was first reported, and it is now known that this is not the case. LYH may be more frequent that is realized, and currently is likely to be largely unrecognized. Insights into the diagnosis and treatment of LYN are expected in the near future.

Key points

- Pituitary autoimmunity is increasingly diagnosed.
- LYH can be recognized by a characteristic MRI pattern.
- APAs can be detected in the serum, but their assay is performed only in highly specialized centers.
- In patients with non-functioning pituitary lesions, hypopituitarism with or without diabetes insipidus of rapid onset, the diagnosis of LYH should be verified.

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CHAPTER 9

Adrenal Involvement in Systemic Autoimmune Diseases

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1. Introduction

Research exploring interactions between the hypothalamic-pituitary-adrenal (HPA) axis and the immune system has significantly advanced the understanding of the etiopathogenesis of some systemic autoimmune diseases (SAD) (Sternberg, 1997). The HPA axis is the main component of the stress system. Stress induces increased serum concentrations of glucocorticoids, which are essential for the prevention of autoreactive or unrestrained amplification of the immune response. Neuroendocrine regulation of immune responses occurs through the antiinflammatory action of glucocorticoids released after stimulation of the HPA axis regionally, through local production of glucocorticoids in immune organs such as the thymus, and locally at sites of inflammation, through release of proinflammatory neuropeptides and neurohormones. A dysfunction of the HPA axis may confer a high susceptibility of developing autoimmune disorders. Female Lewis (LEW/N) rats, characterized by a defective hypothalamic corticotrophin-releasing hormone (CRH)-response, are highly susceptible to a wide variety of experimental autoimmune disorders (Sternberg et al., 1989).

The etiopathogenic role of a blunted HPA axis has been analyzed in patients with rheumatoid arthritis (Neeck et al., 1990; Cash et al., 1992; Chikanza et al., 1992; Cutolo et al., 1999; Gutierrez et al., 1999), fibromyalgia (Crofford et al., 1994), chronic fatigue syndrome (Demitrack et al., 1991), systemic lupus erythematosus (SLE) (Gutierrez et al., 1999), and Sjögren's syndrome (SS) (Johnson et al., 1998). This review focuses on current knowledge of adrenal involvement in patients with SAD, including SLE, antiphospholipid syndrome (APS), SS, systemic sclerosis (SSc), and giant cell arteritis (GCA).

2. Adrenal involvement in SLE

There is preliminary evidence that a defective HPA axis is present in both murine and human lupus. A significantly lower increase in plasma corticosteroid concentrations after stimulation with recombinant IL-1 has been demonstrated in the MRL/lpr murine model of lupus (Lechner et al., 2000). In addition, aging of mice, which is accompanied by increased autoantibody production, is associated with a decrease in hypothalamic expression of the CRH-mRNA (Shanks et al., 1999). Studies of the HPA axis in patients with SLE are limited and often influenced by concomitant glucocorticoid treatment.

2.1. Studies of the HPA axis in SLE

Some reports have suggested that SLE is associated with dysfunction of the HPA axis (Koller et al., 2004). Zietz et al. (2000) found altered HPA

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function in patients with moderately active disease, who presented lower serum levels of androstenedione, cortisol, and dehydroepiandrosterone (DHEA), but with normal levels of ACTH, both at baseline and after stimulation. Gutierrez et al. (1998) also reported a significantly lower cortisol response to induced hypoglycemia in females with active SLE in comparison with controls. In contrast, Koller et al. (2004) have recently found no significant alterations in the HPA axis in treatment-naïve SLE patients. These contrasting results may be related to several factors, such as the small number of SLE patients, the variability in the evaluation of SLE activity, and the different hormonal tests used.

2.2. Therapeutic implications

Dehydroepiandrosterone is produced primarily by the adrenal glands and is a precursor of estradiol and testosterone. The potential role of DHEA in the treatment of SLE is suggested by studies showing low circulating levels of DHEA and DHEA-S in patients with active disease (Lahita et al., 1987), the immunomodulatory effects of DHEA (Straub et al., 2000b), and results from NZF1 lupus murine models (Lucas et al., 1985). Two randomized, double-blinded, placebo-controlled clinical trials have tested the use of DHEA in women with SLE (Chang et al., 2002; Petri et al., 2002). The first study found that DHEA treatment (200 mg/d) significantly reduced the number of flares and improved patients global assessment of disease activity (Chang et al., 2002). The second study found that disease activity remains stable even though the corticosteroid dose was reduced (Petri et al., 2002). Both studies suggested a beneficial role for DHEA in patients with mild-to-moderate SLE, with the improvement of some minor features such as oral ulcers and myalgia, patient and physician overall assessments and SLEDAI score, and reduction in the total dose of glucocorticoids. However, DHEA was associated with a high frequency of side effects, especially acne and hirsutism that were reported in 33 and 16%, respectively, of 381 SLE women included in the study by Petri et al. (2002), 11 (6%) indicated these events as reasons for treatment discontinuation. DHEA may represent a therapeutic option in SLE patients with mild/moderate disease, with a similar therapeutic role to that of antimalarial drugs, although the high frequency of side effects should be taken into account (Van Vollenhoven et al., 1995).

3. Adrenal involvement in APS

Some recent studies have focused on the identification and characterization of adrenal involvement in patients with APS. Espinosa et al. (2003) have reviewed the main characteristics of 86 patients with APS and adrenal involvement, of whom 70% had primary APS. Adrenal failure was the first clinical manifestation of APS in 35% of patients. The main symptoms at clinical presentation were abdominal pain, fever, nausea and vomiting, and hypotension. Patients often complained of weakness and fatigue (Table 1). Hyponatremia and/or hyperkalemia were the most common laboratory abnormalities, observed in more than 80% of patients. Reduced baseline cortisol levels, together with raised adrenocorticotrophic hormone levels, were detected in 96% of patients, and the cosyntropin stimulation test was positive in all cases. Thrombocytopenia was reported in 60% and hemolytic anemia in 48% of patients. Lupus anticoagulant (LA) was detected in more than 95% of patients and anticardiolipin antibodies (aCL) in more than 90%. Imaging techniques (computed tomography and/or magnetic resonance of the adrenal glands) demonstrated mainly adrenal hemorrhage in 60% of patients, while signs of adrenal infarction appeared in 15%. Adrenal involvement was bilateral in 75% of patients. Histopathological findings included hemorrhagic infarction with vessel thrombosis (55%), adrenal hemorrhage (27%), adrenal infarction (5%), and normal findings (9%). Steroid replacement therapy was the most frequent treatment (84% of patients), followed by anticoagulation (52%) and aspirin (6%).

Table 1
Epidemiologic and clinical characteristics of 86 patients with antiphospholipid syndrome and adrenal involvement (Espinosa et al., 2003)

Gender[a]	
Male	47 (55%)
Female	38 (45%)
Mean age	43 years
Autoimmune diseases	
Primary antiphospholipid syndrome	61 (71%)
Systemic lupus erythematosus	14 (16%)
Lupus-like	7 (8%)
Discoid lupus	1 (1%)
Drug-induced lupus	1 (1%)
Paraneoplasic antiphospholipid syndrome[b]	2 (2%)
Clinical manifestations	
Abdominal pain	46 (55%)
Hypotension	45 (54%)
Fever	34 (40%)
Nausea or vomiting	26 (31%)
Weakness, fatigue, malaise, or asthenia	26 (31%)
Lethargy, altered mental status, or confusion	16 (19%)
Silent	12 (14%)
Weight loss	11 (13%)
Skin hyperpigmentation[c]	8 (10%)
Ileus	5 (6%)
Diarrhea	3 (4%)

[a] In one case gender was not reported.
[b] Undifferentiated carcinoma and metastatic bronchopulmonary cancer.
[c] Indicating longstanding adrenal insufficiency.

A high degree of clinical suspicion is necessary for the diagnosis of primary adrenal failure in patients with APS. It should be suspected in patients presenting with abdominal pain, weakness, asthenia, and/or hypotension. These patients should be tested for hypoadrenalism, including serum sodium/potassium values and, if necessary, abdominal imaging studies. The diagnosis may be confirmed by basal plasma adrenocorticotrophic hormone and cortisol measurements and the cosyntropin stimulation test. Although primary adrenal failure should be considered an infrequent complication of APS, it may be the first clinical manifestation of APS in one-third of cases. This suggests the need for systematic screening for LA and aCL in all patients with adrenal infarction or hemorrhage and for screening procedures in APS patients with weakness, asthenia, and altered electrolyte values.

4. Adrenal involvement in Sjögren's syndrome

The epidemiological pattern of primary SS, with an overwhelming predominance of female patients (Brennan and Fox, 1999), supports a role for hormonal factors in the etiopathogenesis of the autoimmune exocrinopathy (Ishimaru et al., 2003; Jonsson et al., 2003; Kassi et al., 2003, Shim et al., 2004). Some recent studies have analyzed the role of the HPA axis in the etiopathogenesis of primary SS.

4.1. HPA dysfunction

Johnson et al. (2006) found normal basal and CRH-stimulated cortisol levels in female patients with primary SS, in spite of a tendency to low ACTH levels at baseline and after stimulation, while a study of eight women with primary SS (Johnson et al., 1998) showed slightly reduced basal levels of ACTH and cortisol. Johnson et al. (2006) have also reported that patients with SS had significantly lower levels of ACTH and cortisol and a blunted pituitary and adrenal response to ovine CRH compared to controls, with a lower peak of plasma ACTH and cortisol levels in SS patients.

4.2. DHEA levels

Valtysdottir et al. (2001) found that women with primary SS had normal serum levels of testosterone, androstenedione, LH, and ACTH (at baseline and after stimulation). However, these patients had decreased circulating levels of DHEA-S. These findings (normal testosterone/androstenedione and decreased DHEA-S serum levels) suggest adrenal dysfunction. A recent study (Sullivan et al., 2000) has also shown that women with SS are androgen deficient. Adrenal-mediated immune mechanisms may be involved in the abnormal

levels of adrenal androgens and cortisol found in patients with SS.

4.3. Therapeutic options

Low levels of serum DHEA have been described in patients with SLE, RA, and SS. This may suggest a possible therapeutic role for this steroid hormone in these autoimmune diseases (Van Vollenhoven, 2002). Pillemer et al. (2004) recently carried out a 24-week, randomized, double-blinded, placebo-controlled trial to evaluate the safety and potential use of DHEA in patients with primary SS. In contrast with previous promising results found in SLE patients, this study showed no evidence to support the potential efficacy of DHEA in the treatment of primary SS.

5. Adrenal involvement in systemic sclerosis

Altered neuroendocrine function may contribute to the complex etiopathogenesis of SSc (Brezinschek et al., 1993). Some studies have found lower levels of adrenal androgens in patients with SSc, suggesting a possible dysfunction of the HPA axis. Imrich et al. (2006) evaluated the HPA function in premenopausal women with SSc using the insulin-induced hypoglycemia test and found reduced basal levels of DHEA and a lower response to the insulin-induced hypoglycemia test, suggesting down-regulation of the production of adrenal androgens in SSc. Although responses of DHEA and ASD (an intermediate adrenal androgen) to hypoglycemia were lower in SSc compared to controls, ACTH responses were similar in both groups, suggesting that the hypothalamic-pituitary axis was not affected but that adrenal function seems to be blunted in patients with SSc.

6. Adrenal involvement in giant cell arteritis and polymyalgia rheumatica

The possible etiopathogenic role of the HPA axis in patients with systemic vasculitides has not been studied, except in the cases of GCA and polymyalgia rheumatica (PMR). The abrupt onset of PMR/GCA, with clinical features similar to the steroid withdrawal syndrome, the rapid response to exogenous corticosteroids, and relative adrenal dysfunction supports the hypothesis that PMR/GCA may be HPA axis-driven diseases. Genetically determined alterations of the HPA axis may enhance the possible susceptibility of developing PMR/GCA. However, Gonzalez-Gay et al. (2002) found no association between polymorphisms of the promotor region of the corticoliberin gene and increased susceptibility to PMR/GCA.

Several studies have reported a relative adrenal failure in untreated patients with PMR (Nilsson et al., 1994; Straub et al., 2000a; Cutolo et al., 2002a–c). Since DHEA-S has a well documented immunomodulatory function (Daynes et al., 1993; Spencer et al., 1996; Straub et al., 1998; Imrich, 2002), this suggests a possible link between the endocrine and immune systems in the pathogenesis of PMR. Furthermore, Cutolo and Straub (2000) found a correlation between low DHEA-S levels and raised acute phase reactants (ESR and CRP) in female patients with PMR. Two additional studies (Straub et al., 2000a; Cutolo et al., 2002b) have also found an inverse correlation between DHEA-S levels and IL-6 concentrations in patients with PMR.

In contrast, recent studies have found no significant differences between PMR patients and controls in various tests evaluating the HPA axis. Some studies have found that cortisol levels at PMR diagnosis did not significantly differ between patients and controls (Straub et al., 2000a; Pacheco et al., 2003). In another study, no significant differences in the response to ACTH or cortisol were found between PMR patients and healthy controls, while Pacheco et al. (2003) did not confirm adrenal hypofunction in PMR/GCA, as no differences were found in serum DHEA-S levels between patients with PMR/GCA and healthy controls.

Although some studies have found changes in steroidogenesis in terms of DHEA-S reduction or relative cortisol deficiency, more extensive studies are needed to clarify the etiopathogenic role of DHEA-S dysregulation in patients with PMR/GCA.

Table 2
Organ-specific and systemic autoimmune diseases associated with Addison's disease: reported cases

Organ-specific autoimmune diseases	Systemic autoimmune diseases	References
Addison	SLE	Koren and Hanly (1997
Addison, thyroiditis	Sarcoidosis, SS	Seinfeld and Sharma (1983)
Addison, lupus vulgaris	–	Drago et al. (1988)
Addison	Sarcoidosis, inflammatory myopathy	Selva-O'Callaghan et al. (2006)
Addison, thyroiditis	Sarcoidosis	Watson and Lewis (1996)
Addison	Sarcoidosis	Papadopoulos et al. (1996)
Addison	Sarcoidosis	Papadopoulos et al. (1996)
Addison, thyroiditis	Sarcoidosis	Walz and From (1990)
Addison	Sarcoidosis	Jacobs et al. (1988)
Addison	Sarcoidosis	Umeki et al. (1987)
Addison	Sarcoidosis	Guillevin et al. (1978)

Abbreviations: SLE, systemic lupus erythematosus; SS, Sjögren syndrome.

7. Adrenal involvement in other autoimmune diseases

In contrast to organ-specific autoimmune diseases that are frequently associated with SAD, such as thyroiditis, Addison's disease (AD) has rarely been described in association with SAD. Isolated cases have been reported in SLE (Koren and Hanly, 1997), SS (Seinfeld and Sharma, 1983), lupus vulgaris (Drago et al., 1988), and inflammatory myopathy (Selva-O'Callaghan et al., 2006) (Table 2). The association of AD with sarcoidosis is relatively more frequent, with a total of nine reported cases (Guillevin et al., 1978; Umeki et al., 1987; Jacobs et al., 1988; Walz and From, 1990; Papadopoulos et al., 1996; Watson and Lewis, 1996), some of which presented multiple associated autoimmune diseases, both systemic and organ-specific (three patients had a triple association between AD, sarcoidosis, and thyroiditis, the so-called Schmidt's syndrome).

Key points

- Studies of the HPA axis in patients with SLE are limited and often influenced by concomitant glucocorticoid treatment.
- DHEA may represent a therapeutic option in SLE patients with mild/moderate disease, with a similar therapeutic role to that of antimalarial drugs.
- Although primary adrenal failure should be considered an infrequent complication of APS, it may be the first clinical manifestation of APS in one-third of cases.
- Adrenal-mediated immune mechanisms may be involved in the abnormal levels of adrenal androgens and cortisol found in patients with SS.
- The abrupt onset of PMR/GCA, with clinical features similar to the steroid withdrawal syndrome, the rapid response to exogenous corticosteroids, and relative adrenal dysfunction supports the hypothesis that PMR/GCA may be HPA axis-driven diseases.

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CHAPTER 10

Endometriosis and Autoimmunity

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1. Introduction

Endometriosis (EM) is a disorder of the female reproductive system characterized by the presence of endometrial tissue outside the uterine cavity, most commonly on fallopian tubes, ovaries, pelvic peritoneum, rectovaginal septum, bladder, and rectum. Occasionally, endometrial cells may spread beyond the pelvic and abdominal regions, and endometrial implants have been found in pleura, pericardium, and brain (Giudice and Kao, 2004).

EM is a major cause of pelvic pain and infertility in women during reproductive age. The prevalence of pelvic EM is 6–10% in the general female population and 35–50% in patients with pelvic pain and/or infertility (Barbieri, 1990; Balasch et al., 1996). The severity of pain in endometriosis is variable. The diagnosis should be confirmed by a surgical procedure, generally laparoscopy, to identify, excise, and histologically evaluate the pelvic lesions (Ueki et al., 1995; Balasch et al., 1996).

2. Etiology and pathogenesis

The etiology and pathogenesis of EM are unknown, but two major theories have been suggested in the literature.

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2.1. Coelomic metaplasia

In 1919, Meyer proposed that the ectopic endometrium originates from the metaplasia of peritoneal cells (coelomic metaplasia), since the peritoneal and eutopic endometrial tissues have embryologically the same origin. In addition, excessive hormonal stimulation may induce the differentiation of coelomic epithelium to endometrial tissue (Giudice et al., 1998).

2.2. Retrograde menstruation

The most accepted pathogenic mechanism for pelvic endometriosis is the retrograde menstruation/implantation theory, referred to as Sampson's theory (Giudice and Kao, 2004). In support of this hypothesis, laparoscopic studies have confirmed the occurrence of retrograde menstruation in more than 90% of EM patients. Retrograde flow is also observed in normal women, but in smaller volumes (Halme et al., 1984; Liu and Hitchcock, 1986).

These two theories do not, however, completely explain endometriosis, especially with regard to involvement outside the pelvic and abdominal cavities. Therefore, the roles of immunological, hormonal, genetic, and environmental factors have gained new attention (Fig. 1). These influences do not seem to cause endometriosis, but may contribute to attachment of endometrial cells derived from retrograde menstruation to the peritoneum,

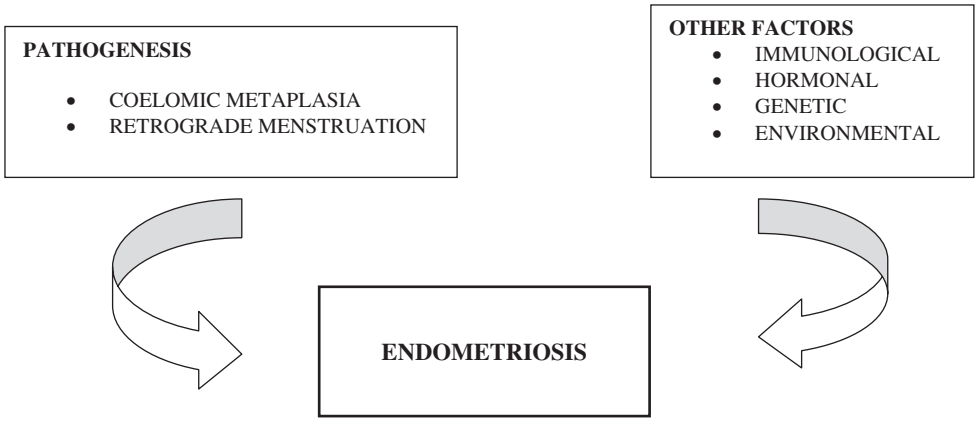

Figure 1. Etiology and pathogenesis of endometriosis.

invasion of peritoneal epithelium, development of a blood supply, establishment of an inappropriate response that does not adequately clear the endometrial implants, resulting in their continued growth (Giudice et al., 1998), and development of endometriosis (coelomic metaplasia), particularly of the ovarian cysts lined by endometrial tissue (endometriomas) or rectovaginal endometriosis (Nisolle and Donnez, 1997).

Among these factors, alterations of the immunological system are by far the most widely and extensively documented. In considering hormonal factors, exposure to estrogenic stimulation is relevant, as endometriosis is associated with an increased number of menstrual cycles, early age of menarche, short menstrual cycles, and inverse relationship to parity (Hummelshoj et al., 2006). The heritable characteristic is suggested by the sixfold higher risk for EM in first-degree relatives of patients with severe EM, compared to relatives of unaffected women (Simpson et al., 1980). In addition, an increased risk of EM has been found in monozygotic twins (Moen, 1994). Environmental factors might be involved in EM pathogenesis. In this regard, Belgium, with the highest dioxin pollution in the world, has the highest incidence of EM. Nevertheless, prospective studies from Italy and Belgium found no significantly elevated risk of this disorder in women who have been exposed to dioxin (Giudice and Kao, 2004).

3. Immunological abnormalities

Immunological abnormalities may be subdivided into three lines of evidence: association with autoimmune diseases, humoral immune responses, and cellular immune responses.

3.1. Association of EM with autoimmune diseases

There are several studies in the literature suggesting an intriguing association of EM with autoimmune diseases, particularly SLE (summarized in Table 1). A retrospective evaluation of 22 EM patients treated with total abdominal hysterectomy found that a previous history of SLE was significantly more frequent in EM patients (9%) than in women with uterine fibroids (0/185) (Smith et al., 1993). In contrast, another case-control study of 109 SLE patients revealed that EM was associated with a twofold increased risk of SLE, but the association was not statistically significant (Grimes et al., 1985). Nevertheless, a precise criterion in the diagnosis of EM was not applied.

A large cross-sectional survey of EM patients based on mailed self-reported information questionnaires found higher frequencies of SLE and other diseases (fibromyalgia, chronic fatigue syndrome, rheumatoid arthritis, Sjögren's syndrome,

Table 1
Endometriosis and autoimmune diseases

Number of patients/diagnosis	Study design	SLE association	Other associations	Weakness
22/EM (Smith et al., 1993)	Retrospective	Yes	ND	Study design, number of patients, patient selection[a], control group[b]
109/SLE (Grimes et al., 1985)	Case-control	No	ND	EM diagnosis
3680/EM (Sinaii et al., 2002)	Mailed self-reported	Yes	Fibromyalgia, chronic fatigue syndrome, rheumatoid arthritis, Sjögren's syndrome, hypothyroidism, multiple sclerosis, asthma, and atopic diseases	Study design, SLE diagnosis, control group[c]
45/EM (Pasoto et al., 2005)	Prospective	No	Fibromyalgia, diffuse myalgias, arthralgia	Number of patients

ND, not described.
[a] All EM patients were severe cases.
[b] Ethical distribution was significantly different in patients and controls.
[c] EM patients were significantly more likely to be of reproductive age, compared with control group.

hypothyroidism, multiple sclerosis, asthma, and atopic diseases) with EM, compared with published rates in the female population of the United States (Sinaii et al., 2002). However, women completing the questionnaire were significantly more likely to be of reproductive age, 15–44 years old (89%), compared with the general control population (43%) (Sinaii et al., 2002). This is a relevant issue, because SLE characteristically affects women of reproductive age (Masi and Kaslow, 1978). Furthermore, it is important to take into account the fact that diagnosis of most rheumatic diseases is based on extensive clinical and laboratory evaluations and supported by well-established classification criteria (Tan et al., 1982; Wolfe et al., 1990). Failure to use established clinical and laboratory criteria for SLE might have misdiagnosed this disease.

In contrast, we found no evidence of an association between SLE and EM in a prospective study that included extensive clinical and humoral evaluations of EM patients (Pasoto et al., 2005). Forty-five women with histologically confirmed pelvic endometriosis were studied, and none fulfilled SLE criteria. However, EM patients did have significantly higher frequencies of arthralgia, general myalgia, and fibromyalgia compared to controls. We used rigorous criteria for diagnosing EM and required histological confirmation and exclusion of any hormonal therapy for at least 3 months prior to the study. These precautions assured that EM was diagnosed accurately (Pasoto et al., 2005). Oral contraceptives have been associated with drug-induced lupus (Yung and Richardson, 1994), and gonadotrophin-releasing hormone analog has been implicated in the development of musculoskeletal symptoms, particularly fibromyalgia (Toussirot and Wendling, 2001). Interpretation of these studies is complicated, in view of the fact that the prevalence of SLE is low (15–50:100,000 in USA) (Hochberg, 1990) and larger prospective evaluations are necessary to determine accurately the possible association of EM and SLE.

Associations between EM and other diseases have been described (Table 1). Recently, a case-control study of 58 patients with primary Sjögren's syndrome and 157 controls based on self-administered questionnaires showed that a previous history of EM was four times more common in primary

Sjögren's syndrome patients than in controls (Haga et al., 2005). However, the majority of these patients and controls did not report previous gynecological surgery (Haga et al., 2005), a fundamental procedure required to make a precise diagnosis of pelvic EM (Ueki et al., 1995; Balasch et al., 1996).

Previous histories of eczema, atopic diseases, and asthma were also significantly more frequent in women with EM than normal controls (Lamb and Nichols, 1986; Nichols et al., 1987; Sinaii et al., 2002). However, a controlled study of 879 women of reproductive age with benign gynecological conditions has shown that patients with histologically confirmed pelvic EM do not have an increased risk of asthma (Ferrero et al., 2005).

With regard to thyroid disorders, Poppe and Velkeniers (2003) observed a high prevalence of positive TPO antibody in women with infertility in association with EM. Reforcing this finding, Alviggi et al. (2006) reported one case with pelvic EM, alopecia universalis, autoimmune thyroiditis, and multiple sclerosis. In addition, we and others have found a high prevalence of generalized musculoskeletal complaints and fibromyalgia in EM (Sinaii et al., 2002; Pasoto et al., 2005).

3.2. Alterations of humoral immune response

3.2.1. Complement

Reduced serum levels of C3 and C4 complement components (Meek et al., 1988), as well as deposition of C3 and immunoglobulin G (IgG) in eutopic endometrial tissue were observed in women with EM (Weed and Arquembourg, 1980; Kreiner et al., 1986). In support of these findings, elevated C3 levels were detected in peritoneal fluid from EM patients (Badawy et al., 1984; Bartosik, 1985). In this aspect, it is interesting that ectopic endometrial tissue produces and secretes C3 in vitro, mainly from glandular epithelium (Isaacson et al., 1989). It is therefore speculated that C3 in peritoneal fluid might contribute to the pathogenesis of some phenomena observed in EM—infertility, pelvic inflammation, increased number of activated macrophages, and elevated concentrations of several cytokines (as interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor (TNF-α)) in peritoneal fluid (Nothnick, 2001). In fact, peritoneal fluid from these patients, with high concentrations of cytokines, growth factors, and activated macrophages, has been shown to be toxic to sperm function and embryo survival (Giudice and Kao, 2004).

3.2.2. Antiendometrial antibodies

Antiendometrial antibodies have been detected in serum (Mathur et al., 1982), peritoneal fluid (Mathur et al., 1988), and ectopic or eutopic endometrial tissue (Mathur et al., 1982) from EM patients. However, comparison among different studies with regard to the frequency of these antibodies is impaired because different methods were used to test for the antibodies, including passive hemaglutination (Mathur et al., 1982), immunodifusion (Badawy et al., 1984), indirect immunofluorescence (Wild and Shivers, 1985), immunoblotting (Mathur et al., 1988), immunohistochemistry (Kennedy et al., 1990), and ELISA (Moncayo et al., 1991). These antibodies were detected in 87% of sera and 100% of peritoneal fluid samples from EM patients by immunoblotting analysis (Mathur et al., 1988). Interestingly, severity of EM correlated with high titers of antiendometrial antibodies (Iborra et al., 2000).

3.2.3. Antinuclear antibodies (ANA) and antiphospholipid antibodies

An impressively high prevalence of several autoantibodies has been reported in serum from EM patients (Table 2). It is possible that the antibodies are an epiphenomenon (Gleicher, 1990) or a product of non-specific polyclonal B-cell activation. However, a cluster of circulating autoantibodies associated with autoimmune diseases such as ANA (Gleicher et al., 1987; Taylor et al., 1991; Malinowski et al., 1995; Kim et al., 1997; Iborra et al., 2000; Dias et al., 2006), antihistone (Crha and Ventruba, 1996), anti-Ro (SS-A)/anti-La (SS-B) (Taylor et al., 1991; D'Cruz et al., 1996), anticardiolipin (Kennedy et al., 1989; Taylor et al., 1991; Abrao et al., 1997; Kim et al., 1997), lupus

Table 2
Autoantibodies in endometriosis

EM	Blood	Frequency (%)	Peritoneal fluid[a]
ANA	Yes	18–47	ND
Anti-histone	Yes	0–26	Yes
Anti-polinucleotides	Yes	3–16	ND
Anti-Ro/SS-A	Yes	2	ND
Anti-cardiolipin	Yes	9–65	Yes
Lupus anticoagulant	Yes	46	ND
Anticarbonic anhydrase	Yes	35	ND

ND, not described.
[a] Confino et al. (1990).

anticoagulant (Gleicher et al., 1987; Taylor et al., 1991; Kim et al., 1997), antiendothelial cells (Fernández-Shaw et al., 1993), and rheumatoid factor (Kim et al., 1997) raises the hypothesis that a common immunological disturbance underlies EM and rheumatological autoimmune diseases. In this regard, pelvic EM is characterized by defective clearance of apoptotic endometrial cells in a pro-oxidant inflammatory environment. This disturbance has been described in lupus patients (Seery, 2006). It is proposed that this combination of alterations triggers autoantibody production in EM patients. It is important to emphasize, however, that lupus-specific autoantibodies such as anti-dsDNA and anti-Sm were not found in EM patients in our prospective study (Pasoto et al., 2005).

In addition, anti-smooth muscle (Taylor et al., 1991), a known antibody specificity associated with liver disease, and anticarbonic anhydrase (Kiechle et al., 1994; Brinton et al., 1996; D'Cruz et al., 1996), an antibody without a clear clinical association, have also been described in EM patients.

3.3. Altered cellular immune responses

3.3.1. Leukocyte subpopulations

Peritoneal fluid from EM patients has elevated number of macrophages, T helper, and natural killer cells (Halme et al., 1987; Hill et al., 1988). In contrast, natural killer cell activity is decreased in blood (Oosterlynck et al., 1991) as well as peritoneal fluid (Oosterlynck et al., 1992) in EM. The low NK activity might impair clearance of pelvic endometrial implants, resulting in their continued growth (Witz and Schenken, 1997). In fact, EM patients have reduced cytotoxicity to autologous endometrium (Steele et al., 1984).

Table 3
Alterations of cellular immune response in endometriosis

EM	Blood	Peritoneal fluid
Leukocyte subpopulations		
Number of macrophages	ND	↑
Number of T helper	ND	↑
Number of NK	ND	↑
NK activity	↓	↓
Monocyte production		
IL-4	↑	↑
IL-2	=	=
IL-10	=	=
IFN-γ	↓	↓
Concentration of soluble factors		
IL-1	ND	↑
TNF-α	ND	↑
IL-4	↑	↑
IL-5	ND	↑
IL-6	ND	↑
IL-8	ND	↑
IL-10	ND	↑
TGF-β	ND	↑

ND, not described.

3.3.2. Soluble factors

Increased concentrations of soluble factors derived from immunologically active cells have been reported in EM, including IL-1 (Fakih et al., 1987; Taketani et al., 1992), TNF-α (Eisermann et al., 1988; Taketani et al., 1992; Harada et al., 1997), IL-4 (Hsu et al., 1997), IL-5 (Koyama et al., 1993), IL-6 (Koyama et al., 1993; Rier et al., 1994; Punnonen et al., 1996; Harada et al., 1997), IL-8 (Ryan et al., 1995; Rana et al., 1996), IL-10 (Punnonen et al., 1996), and transforming growth factor-β (TGF-β) (Oosterlynck et al., 1994) (Table 3).

Since these cytokines and growth factors have inflammatory, mitogenic and/or angiogenic activities, they could contribute to development of EM

(Witz and Schenken, 1997). In this regard, the peritoneal fluid from EM patients stimulates endometrial stromal cell proliferation in vitro (Surrey and Halme, 1990), and a similar result was obtained with TGF-β (Hammond et al., 1993). Elevated levels of angiogenic growth factors (Oosterlynck et al., 1993), including the vascular endothelial growth factor (VEGF) (Shifren et al., 1996), were detected in peritoneal fluid from EM cases.

3.3.3. Mediators of cellular immune response

Increased production of IL-4 was observed in circulating monocytes from EM patients, whereas levels of TNF-γ were suppressed and no change was observed for IL-2 or IL-10 (Hsu et al., 1997). These cells also stimulated endometrial stromal cell proliferation in vitro (Braun et al., 1994).

3.3.4. Haptoglobin and macrophage phagocytic activity

Recently, haptoglobin was found to be expressed in endometriotial epithelial cells but not in eutopic endometrial epithelium. It was also demonstrated that this protein binds to peritoneal macrophages, increases macrophage production of IL-6, and reduces macrophage phagocytic activity by blocking adherence (Sharpe-Timms et al., 2002).

4. Conclusions

- Various mechanisms may be involved in the pathogenesis of EM. Hormonal, genetic, environmental, and immunologic factors together may generate the favorable conditions for endometrial implants growth.
- Endometriosis does not appear to be associated with autoimmune rheumatological diseases. These patients do, however, have a high frequency of musculoskeletal symptoms and fibromyalgia.
- Clustering of autoantibodies associated with autoimmune diseases occurs in EM patient sera, suggesting that a common mechanism may underlie both EM and rheumatological autoimmune diseases. However, it is important to note that lupus-specific autoantibodies such as anti-dsDNA and anti-Sm are not observed in EM.
- Altered cellular immune responses with increased number of macrophages and lymphocytes; high concentrations of interleukins, growth factors, and angiogenic growth factors; and decreased natural killer activity have been observed in EM and may be relevant to its pathogenesis.

Key points

- Premature ovarian failure, antisperm antibodies, and endometriosis may have an autoimmune origin.
- Autoantibodies such as, antiphospholipid antibodies, antithyroid antibodies, and antinuclear antibodies may be associated with infertility, in addition to pregnancy loss.
- Systemic lupus erythematosus and diabetes mellitus have been associated with infertility.

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CHAPTER 11

Autoimmunity in Turner's, Down's, and Klinefelter's Syndromes

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Abstract

This review opens with descriptions of the range of clinical features observed in Turner's syndrome, Down's syndrome, and Klinefelter's syndrome. This is followed by an account of the disturbances of chromosomal number and structure, and the underlying mechanisms leading to the various karyotypes are outlined. Next follows an overview of the mechanisms of autoimmune disease and risk factors including genes and environmental influences, such as stress and infection. The evidence for the nature, prevalence, and clinical consequences of autoimmune disorders in Turner's syndrome, Klinefelter's syndrome, and Down's syndrome is then reviewed, emphasising the similarities and differences between them. Finally potential mechanisms underlying autoimmunity in chromosomal disorders are examined including sex steroids, parental autoimmunity, X-monosomy, X chromosome inactivation, and the possible importance of skewing of X-inactivation. The special issues addressed relating to Down's syndrome include evidence for premature ageing of the immune system and over-expression of genes on chromosome 21.

1. Introduction

Major disturbances of chromosomal number, structure, or function are frequently lethal. Those permitting prolonged survival, albeit often shorter than normal, frequently involve relatively small quantities of genetic material, as exemplified by the three common chromosomal disorders causing Down's syndrome, Turner's syndrome, and Klinefelter's syndrome. Down's syndrome is caused by trisomy of chromosome 21—one of the smallest chromosomes. Turner's syndrome and Klinefelter's syndrome involve abnormalities in the X sex chromosome. This chromosome is large compared with the male conferring Y sex chromosome. Probably because of the need to balance the amount of genetic material in the two sexes, in men with the normal 46,XY karyotype both of the sex chromosomes are fully activated; in females with the normal 46,XX, karyotype only one chromosome is fully active, where as the other is largely inactive—a process eponymously termed Lyonization (Lyon, 1961). Were the whole of the second X chromosome inactivated there would theoretically be no difference from normal in women with the typical Turner karyotype 45,X, save that there would be only one X chromosome to be expressed in all cells, in contrast to the

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randomized expression of paternal and maternal-derived X chromosomes in 46,XX women. Similarly, if Lyonization led to complete inactivation of all second X chromosomes in classical Klinefelter's syndrome with 47,XXY karyotype or the further chromosomes on the rarer poly-X variants, this too should not greatly affect phenotypic expression. The differences which are seen presumably relate to the fact that the p-terminal region of the X chromosome is highly homologous with the Y chromosome and is not inactivated by the processes underlying Lyonization. Thus expression of this small segment, present in normal women would be absent in the cells with the typical Turner karyotype and present in Klinefelter's syndrome for each supernumerary X chromosome in addition to the expression of the Y chromosome present. Hence the differences apparent in both Turner's syndrome and Klinefelter's syndrome are accounted for by lack or extra amounts of genetic material approximating to that on the very small Y chromosome.

The detailed analysis of genetic mechanisms is dealt with below. The relationship to autoimmune diseases, particularly those affecting endocrine glands will also be addressed in full. The evidence for such relationships is abundant and conclusive for Turner's syndrome and Down's syndrome. The received wisdom among clinical endocrinologists is that much the same applies for Klinefelter's syndrome and seems supported in most text books of Endocrinology by brief sentences such as: "Association with the following disorders is greater than chance: chronic pulmonary disease, emphysema or chronic bronchitis, varicose veins, glucose intolerance or mild diabetes mellitus and primary hypothyroidism" (Forest, 2006) and "There is a slightly increased incidence of certain systemic diseases associated with Klinefelter's syndrome including diabetes mellitus, thyroid dysfunction, restrictive lung disease, autoimmune disorders such as systemic lupus erythematosus (SLE), and lower limb extremity varicose veins and venous stasis" (Matsumoto, 2001). The validity of any such relationships will be examined in detail.

2. Clinical manifestations of Turner's, Down's, and Klinefelter's syndrome

2.1. Turner's syndrome

Turner's syndrome occurs in about 1 in 2000 live female births. The typical features of Turner's syndrome include short stature, infertility, and hypogonadism. Other features often found include small jaw with carp-like mouth, epicanthic folds or ptosis, and multiple lentigenes. These can lead to delayed diagnosis even into late adult life. Early diagnosis is, however, increasingly frequent including prenatally when ultra-sonography discloses nuchal translucency or other features which can be confirmed karyotypically by chorionic villus sampling or amniocentesis, or indeed serendipitously when these procedures are performed for other reasons. Shortly after birth, Turner's syndrome may be suspected because of congenital lymphedema causing puffiness of the hands and feet, a short wide neck, widely spaced nipples or occasionally severe neck webbing, and/or congenital cardiac defects. During childhood short stature may become progressively noticeable as may be the increased carrying angle of the elbows. Otitis media is particularly troublesome and common and sometimes behavioral problems or specific problems especially with spatial awareness lead to the diagnosis. Teenage diagnosis is commonly on the basis of failure of breast development and either primary amenorrhea or rapidly developing secondary amenorrhea as well as the increasingly conspicuous growth failure. There is growing recognition of the need for lifelong medical surveillance of women with Turner's syndrome (often in multidisciplinary special clinics) in order to diagnose and treat only when necessary complications occur over the life span. These include increased risk of thyroid dysfunction, diabetes mellitus, and autoimmune conditions such as celiac disease and liver disease which are the focus of this review. Other important issues include hypertension, renal disease, risks of thoracic aortic dissection, osteoporosis, progressive sensori-neural deafness, and assisted conception with associated increased risks of congenital malformations in the offspring (Belchetz, 2003).

2.2. Down's syndrome

Most cases arise from meiotic non-disjunction in the maternal gamete (Fig. 1) and the occurrence is strikingly influenced by maternal age which at 20–24 years carries a risk of 1 in 1490 rising to 1 in 60 at maternal age 40 and 1 in 11 at maternal age 49. Because most births occur with younger women, 80% of children with Down's syndrome are born to women younger than 35. These facts mean that prenatal screening with non-invasive tests which at best detect 90–95% and give a false positive result of 2–5% give about ten times as many false positive as true results hence needing further invasive testing by amniocentesis.

The physical features commonly found in Down's syndrome include tendency to short stature, oblique eye fissures with epicanthic folds, flat occiput, small nose with flattened bridge, white spots on the iris (Brushfield spots). A single palmar crease is typical but not diagnostic as it is seen with other chromosomal abnormalities and in some normal individuals. The tongue protrudes from the mouth and there is muscle hypotonia. Mental retardation is the rule though a wide range is observed.

Complications of Down's syndrome include a markedly increased incidence of congenital heart defects affecting 25–50% of patients and particularly involving arterio-ventricular canal defects but also ventricular septal defects, tetralogy of Fallot, and persistent ductus arteriosus. Other problems include hearing defects, Alzheimer's disease of early onset, leukemia, epilepsy, and reduced fertility in both sexes. Average life expectancy is markedly reduced. Immune defects are common as are autoimmune conditions especially involving the thyroid and diabetes mellitus but also liver disease, celiac disease, and the antiphospholipid syndrome.

2.3. Klinefelter's syndrome

The incidence of Klinefelter's syndrome has been ascertained in surveys of newborn boys and is

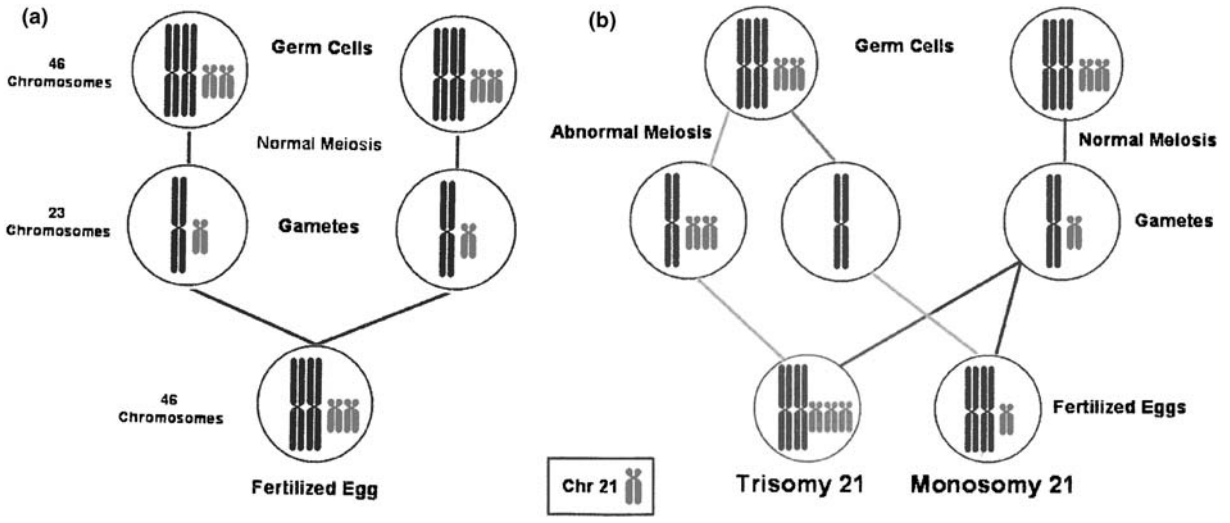

Figure 1. Patterns of meiosis involving chromosome 21 (shown in gray as small chromosomes) and any other normal autosome (shown as black large chromosomes). The left panel shows normal meiosis leading to haploid chromosomes in gametes. The right panel shows non-disjunction of chromosome 21 from germ cells from one parent, usually maternal, at meiosis, leading to half the resulting gametes with either two or no chromosomes 21 and following fertilization by normal gametes, leading to fertilized eggs either with trisomy 21 (Down's syndrome) or monosome 21 (non-viable).

considerably higher than the majority of prevalence studies based on diagnosed cases. This in part can be accounted for by the wide phenotypic variation with some men showing very few features apart from small testicles and infertility, contributing to clinical under-ascertainment. The commonly recognized features fall into two groups: men who are tall and overweight with features of feminization such as pronounced gynecomastia and female fat distribution, while others are tall and thin and have poor muscular development. Testicular failure is a key feature, with most affected men having infertility due to azoospermia or very severe oligospermia associated with atrophic, shrunken, and hyalinized seminiferous tubules, responsible for the remarkably reduced volume of the testicles. Features of hypogonadism due to failure of testosterone production from Leydig cells are common but variable in degree and tend to appear later in adult life even if normal until a decade or so after puberty. In other cases, puberty scarcely occurs or else arrests after only incomplete development. In such individuals, in contrast to the normal marked adolescent growth spurt and its final arrest on epiphyseal fusion associated with the rise from the low childhood to the adult male levels of testosterone, the limbs continue on a prolonged and more even growth trajectory and eventually become excessively long, with leg length exceeding trunk height and span exceeding total height, i.e., eunuchoidal proportions. Such early pubertal failure also causes failure of secondary sexual hair growth and muscle bulk and strength. Long-term untreated hypogonadism leads to osteoporosis (Belchetz, 2003).

3. Genetics of chromosomal disorders

3.1. Genetic basis of Down's syndrome

3.1.1. Chromosomal basis

Down's syndrome (trisomy 21) was the first medical condition shown to result from a chromosome abnormality and is the most common single known cause of learning problems with the highest birth incidence of any chromosomal abnormality. The overall birth incidence of trisomy 21 is 1 per 700 live births. The incidence of conception is much greater, but more than 60% are spontaneously aborted and at least 20% are stillborn.

In most cases (95%) the chromosomal basis is non-disjunction, usually at the first (75%) but sometimes at the second meiotic division. The extra chromosome 21 is maternal in 90% of cases and paternal in 10% (Fig. 2). In the small percentage of paternal errors the non-disjunction occurs equally meiosis in I or meiosis II. Chromosome fluorescence in-situ hybridization (FISH) studies of sperm of fathers who had trisomy 21 conceptions of paternal origin show no increase in the incidence of disomy 21 sperm (Hixon et al., 1998). There is an excess of male Down syndrome individuals when the extra chromosome 21 is paternal in origin for reasons which are not understood. Klinefelter's syndrome co-exists in 0.25% as a result of double non-disjunction. Approximately 2–3% of patients with Down's syndrome are mosaic with a normal cell line. Mosaicism results from non-disjunction with the trisomic 21 zygote losing a chromosome 21 post-zygotically at anaphase lag. Mitotic non-disjunction can also cause mosaic Down's syndrome.

In about 4% of cases one parent carries a balanced translocation involving chromosome 21, i.e., the extra chromosome 21 is fused with another large or small acrocentric chromosome (13, 14, and 15 are D-group acrocentrics; 21 and 22 are G-group acrocentrics). The fusion is, in fact a whole arm exchange with loss of the satellite material (which contains no active genetic material). This is termed a Robertsonian translocation in recognition of Robertson's contribution from his studies on insect cytogenetics early last century. There are no clinical differences between individuals with trisomy 21 and those with Robertsonian translocation Down's syndrome but there are obviously differences in recurrence risks for offspring of the parents. In cases with translocation Down's syndrome about one-third are inherited from one parent who has the translocation. In about 90% of cases the mother is the carrier of a translocation involving one of the D-group acrocentrics. In 7% of cases of affected individuals with translocations involving one of the G-group acrocentrics have a carrier parent and the parent is most frequently the mother. Recurrence risks for further children depends on which parent

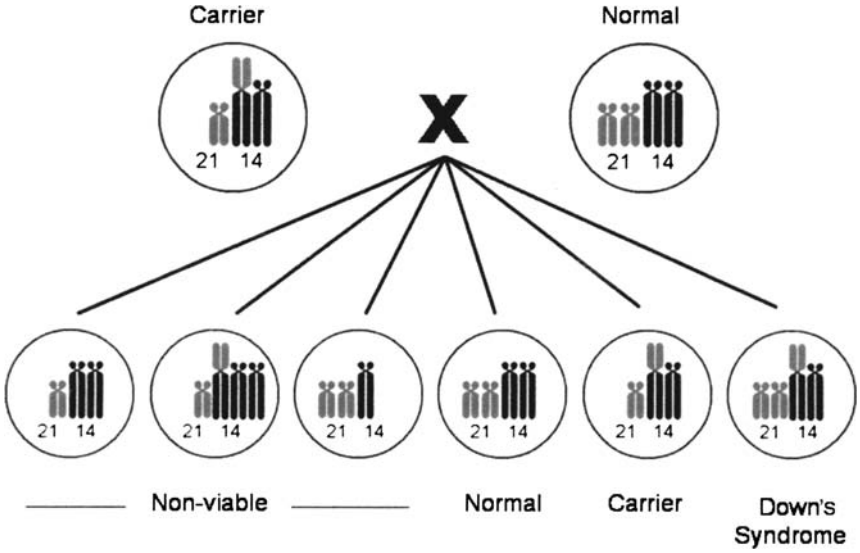

Figure 2. Robertsonian translocation of the long arm of chromosome 21 to another chromosome (14) leading to an asymptomatic carrier. Material from chromosome 21 is shown in gray, and material from chromosome 14 is black. Gametes from a carrier parent mated with gametes from parents with normal karyotype lead to six potential karyotypes, of which three are non-viable, one normal, and one with the Robertsonian translocation type of Down's syndrome.

carries the translocation, e.g., if the father carries the (14q21q) translocation the risk for further children is approximately 2.5% whereas if the mother carries the translocation the risk is approximately 10% (Harper, 2006). If either parent carries a (21q21q) translocation, the recurrence risk is 100%.

The risk of having a child with trisomy 21 rises with maternal age and other risk factors have been postulated but not confirmed. There has been some suggestion that earlier age of menopause with high FSH may be a risk factor for aneuploidy (Warburton, 2005) but this has not been proven.

3.2. Genotype/phenotype correlation in Down's syndrome

The recent sequencing of 283 protein-encoding genes on chromosome 21 (Hattori et al., 2000) has allowed some genotype–phenotype correlation to take place and has pointed towards candidate genes and functional pathways potentially associated with the cognitive defects observed in individuals with Down's syndrome. Classifying the function of genes on chromosome 21; 45% have as yet unknown function, but 13% are involved in cell to cell communication processes which are critical in brain development and function (Kahlem, 2006). Another group of genes (16 or 6%) on chromosome 21 are involved in cellular metabolism and mitochondrial energy generation. These could be the cause of the increased neuronal deaths seen in the brains of patients with Down's syndrome. Several studies have linked mitochondrial dysfunction with Down's syndrome and Alzheimer's disease (Busciglio et al., 2002; Capone et al., 2002). The neuropathological changes of Alzheimers's have also been attributed to triplication of the *APP* gene. However, other factors such as apoliprotein Eε4 alleles, estrogen deficiency, karyotypes, and gender affect age of onset of dementia (Schupf, 2002).

3.3. Genetic basis of Klinefelter's syndrome

3.3.1. Chromosomal basis

Klinefelter's syndrome was first described in 1942 on the basis of a patient with gynecomastia and

testicular failure (Klinefelter et al., 1942). The overall birth incidence of Klinefelter's syndrome is 1 per 500–1000 males (Bojesen et al., 2003). The chromosomal basis of the condition is an extra X chromosome, 47,XXY. Approximately 80% are 47,XXY the other 20% are mosaic for 47,XXY/46,XY or higher grade sex chromosomal aneuploidy or structurally abnormal X chromosomes (Bojesen et al., 2003).

The extra X chromosome is of maternal origin in 40–50% and paternal in 50–60% of cases (Harvey et al., 1991; Lorda-Sanchez et al., 1992). It may arise as non-disjunction at either the first or second maternal meiotic division, but in the male it can only arise when the first meiotic division produces an XY sperm, since errors at MII or during an early cleavage division result in 47,XXX or 47,XYY conceptuses, not in 47,XXY (Thomas and Hassold, 2003). The relationship between parental age and the non-disjunction is complex. One group found that fathers who had recently had a child with the condition had an increase in XY sperm per year of age (Eskenazi et al., 2002). In cases with the extra X maternally derived, there is a reported increase in maternal age but this effect is limited to the subset of cases originating in MI.

3.4. Genotype/phenotype correlation in Klinefelter's syndrome

The clinical phenotype of Klinefelter's syndrome is variable. There are several genetic explanations for this. In contrast to Down's syndrome, the phenotype is not simply due to a gene dosage effect due to X-inactivation of the majority of the extra X-chromosome. Firstly the phenotype may be explained because of a dosage effect of X-linked genes that escape inactivation and there may be difference in expression of these genes. This has been shown in normal females (Carrel and Willard, 2005). Second, mosaicism for 46,XY or other cell lines in other tissues could influence phenotype as has been shown in Turner's syndrome. Thirdly, the pattern of X-inactivation could account for variation in the phenotype (Iitsuka et al., 2001). Non-random X-inactivation could unmask an X-linked mutation. Fourth, the parental origin of the extra X chromosome could affect phenotype due to imprinted genes. Lastly there could be an effect of androgen-related genes such as the androgen receptor on phenotype (Zinn et al., 2005).

3.5. Genetic basis of Turner's syndrome

3.5.1. Chromosomal basis of Turner's syndrome

The chromosomal basis for Turner's syndrome was established in 1959 when the first patient who was studied cytogenetically was found to have a 45,X karyotype (Ford et al., 1959). Later it was shown that some patients had an X chromosome missing from only some cells of the body with two normal X chromosomes in other cells or an abnormal chromosome in some cells combined with monosomy X in other cells. There are a number of different karyotypes that can result in the Turner's syndrome phenotype. It is difficult to know the exact percentage of each of these as most studies are relatively small. Magenis et al. (1980) looked at 651 patients and found that 45,X was found in approximately 50%, structural abnormalities of the second X 45,X/46, Xi(Xq) in 28%, 45,X/46,XX mosaicism in 9.5%, 45,X/47,XXX or 45,X/46,XX/47,XXX in 3.5%, 45,X/46,XY in 5.5%, 45,X/46,X+ marker in 3%, and more complex karytopes in the remainder.

The 45,X genotype is associated with high intrauterine lethality and approximately 10% of all embryonic and fetal deaths after 5 weeks gestation are associated with this karyotype (Warburton et al., 1981). Less than 1% of 45,X conceptuses survive pregnancy (Hook and Warburton, 1983) and the mortality is particularly high at 10–15 weeks gestation. This has led to the hypothesis that in fact all liveborn Turner children are mosaic for a normal cell line in some organ or tissue necessary for fetal survival (Hook and Warburton, 1983). There has been extensive work to try and prove or disprove this hypothesis but although it can be shown that most patients have low level mosaicism in some tissues, no study has

yet proven that all liveborn patients are mosaic, however, it is obviously impossible to test ALL tissue (Fernandez-Garcia et al., 2000; Wiktor and Van Dyke, 2005).

The 45,X genotype can arise from loss of either sex chromosome. There are four possible mechanisms for the origin of the 45,X zygote, firstly fertilization of an ovum lacking an X chromosome, secondly fertilization of an ovum by a sperm lacking a sex chromosome, thirdly development of a 45,X cell line following a post-zygotic error, or lastly loss of a sex chromosome between syngamy and the fusion of the pronucleus. The first mechanism is unlikely because approximately two-thirds of females retain the maternal X chromosome (Lorda-Sanchez et al., 1991; Chu et al., 1994) and it is not associated with increased maternal age. The second mechanism is also unlikely because there is no corresponding increase in 47,XXX or 47,XXY fetuses and sperm cytogenetic studies have shown no unusually high incidence of paternal non-disjunction. The relative importance of the other two mechanisms is presently unclear. A final rare cause of Turner's syndrome may be the result of a parental translocation involving the X chromosome. The parent would have a balanced translocation but during cell division the offspring would inherit an unbalanced karyotype with loss of part of the X chromosome.

3.6. Genotype/phenotype correlation in Turner's syndrome

The phenotype of Turner's syndrome is extremely variable. The main features of the condition are thought to be due to either haplo insufficiency for genes which remain active on the X chromosome, uncovering of a recessive mutation on the single remaining X, or a parental origin effect.

One of the main findings in recent years has been the discovery of the *SHOX* gene. This gene was found by researchers who were hunting for the gene which caused short stature in Turner's syndrome and were looking in the Xp-Yp pseudoautosomal region at genes that escaped X-inactivation (Rao et al., 1997). It became clear later that *SHOX* was also responsible for a condition called Leri-Weill dyschondrosteosis when in heterozygous form and Langer mesomelic dysplasia in homozygous state (Zinn et al., 2002). Leri-Weill dyschondrosteosis shares similarities with Turner's syndrome in that affected individuals have short stature and Madelung deformity of the wrists and the condition is worse in females. It is probable that estrogens affect the phenotype. *SHOX* point mutations have also been found in some short children (Jorge et al., 2007). It is postulated that the tall stature in Klinefelter's syndrome is due to three copies of *SHOX*.

Parental origin of the retained X may have a bearing on phenotype. Chu et al. (1994) found an increased incidence of structural heart anomalies and neck webbing in patients who retained the maternal X chromosome, Skuse et al. (1997) found that individuals retaining the paternal X were socially better adjusted with superior verbal skills and Sagi et al. (2007) found that only individuals retaining the maternal X chromosome had renal malformations. They also found that ocular abnormalities were more common in those patients retaining the paternal X chromosome. All these studies seem to indicate that there may be a parent-of-origin effect on the phenotype in Turner's syndrome but all these effects are probably also modified by tissue specific mosaicism and the different karyotypes.

4. Mechanisms of autoimmunity

The increased susceptibility to autoimmunity in Turner's syndrome, Down's syndrome, Klinefelter's syndrome is by mechanisms that are not entirely clear but likely to involve an interaction between genetic predisposition, impairment in peripheral or central tolerance, and exposure to environmental factors. Autoimmune diseases can be broadly divided into those affecting mainly one organ, such as thyroid disease and type 1 diabetes, and those affecting multiple organs, such as SLE and rheumatoid arthritis. Several mechanisms

are implicated in offering protection from self-reactivity and autoimmune disease which are briefly outlined below.

4.1. Protective mechanisms against autoimmune disease

4.1.1. T cell tolerance

Central tolerance of self-reactive T cells occurs in the thymus during ontogeny through a process of negative selection that involves both elimination (clonal deletion) and deactivation (clonal anergy). Although very effective, central tolerance can never be complete and other mechanisms operate in the periphery to ensure suppression of auto-reactive T cells escaping the thymus. These mechanisms include active suppression by $CD8^+$ cells and peripheral tolerance mediated by the induction of anergy in T cells, by a mechanism similar to that seen in the thymus (Jones and Diamond, 1995). Testosterone receptors are expressed in the thymus, and the male hormone has been shown to have a role in central tolerance (Olsen and Kovacs, 1996). Therefore, a defect in central tolerance may be one mechanism for the increased susceptibility to autoimmunity in Klinefelter's syndrome. Furthermore, testosterone has been shown to increase activity and number of suppressor $CD8^+$ cells and testosterone deficiency may therefore release the immune system from this inhibitory effect leading to initiation of autoimmunity (Oktenli et al., 2002).

4.1.2. B cell tolerance

Both clonal deletion and anergy of autoreactive B cells have been described similar to T cells (Goodnow, 1992). It is worth noting that the presence of activated T cells is of importance for B cell induction and proliferation, as T cells produce cytokines that mediate B cell activation and result in immunoglobulin class switching (Liu et al., 1992; Garside and Mowat, 1995). Androgens have been shown to reduce peripheral B cell numbers resulting in reduction in antibody production. Therefore the lack of testosterone may result in enhancement of humoral immunity predisposing in the process to autoimmune disease (Olsen and Kovacs, 1996).

4.1.3. Cryptic and cross-reactive antigens

Self-tolerance of non-exposed (cryptic) antigens means that potentially autoreactive T cells are not subjected to the normal deletion response. Self-tolerance to these antigens continues as far as they remain immunologically hidden. However, exposure of these antigens (through tissue damage) could trigger an autoimmune response. A classical example of this includes the immune response against lens protein after eye trauma.

An attractive concept explaining the initiation of autoimmunity is the existence of cross-reactive antigens (molecular mimicry). If a pathogenic antigen resembles a host self-antigen, then tolerance of self may be broken. An example is rheumatic fever, where antibodies to a streptococcal protein cross-react with cardiac muscle myosin leading to carditis. Both Down's syndrome and Turner's syndrome are associated with increased susceptibility to infection, which may be one factor contributing to the increased prevalence of autoimmunity seen in these conditions (discussed below).

4.2. Risk factors for autoimmune disease

4.2.1. Genetic predisposition

4.2.1.1. MHC genes. Major histocompatibility complex (MHC) genes, also known as human leukocyte antigens (HLA), are located on chromosome 6 and have been extensively studied in a variety of autoimmune conditions (Ballotti et al, 2006a, b). Association of DR3 and DQA have been documented for organ-specific autoimmunity, whereas DR2 and DR3 associations have been documented for systemic lupus erythematosus (Ballotti et al, 2006a, b). However, these associations have been rather weak indicating only a limited role for these genes in the pathogenesis of autoimmunity. Although there is no clear association between MHC polymorphisms and Turner's,

Down's, Klinefelter's syndromes, a number of polymorphisms in MHC may render these individuals susceptible to autoimmunity, which has no effect in normal individuals. For example, DQA 0301allele is associated with thyroid autoimmunity in Down's syndrome, whereas it has a little effect on normal subjects (Nicholson et al., 1994). Similarly, a recent study suggested that Down's syndrome subjects with organ-specific autoimmunity are more likely to carry low risk MHC genotypes compared with the normal population, but the number of patients studied was too small to draw definitive conclusions (Gillespie et al., 2006).

4.2.1.2. Immune genes. Polymorphisms in the cytotoxic T lymphocyte associated 4 (CTLA-4), a gene mapped to chromosome 2, have been shown to be associated with autoimmunity by mechanisms that include alteration of the T cell responses (Simmonds and Gough, 2004). The lymphoid tyrosine phosphatase (LYP), encoded by protein tyrosine phosphatase-22 (PTP-22) on chromosome 1, is a powerful modulator of T cell receptor activation. A C/T single nucleotide polymorphism at codon 620, results in arginine to tryptophan residue change and is associated with both thyroid autoimmunity as well as type 1 diabetes mellitus (Bottini et al., 2004; Velaga et al., 2004). Polymorphisms of molecules involved in B cells activation such as CD40 have also been associated with autoimmunity in some but not all studies (Ajjan and Weetman, 2001). The role of CTLA-4, PTP-22, and CD40 polymorphisms in predisposition to autoimmunity in subjects with Turner's, Down's, or Klinefelter's syndromes has not been investigated and this remains an area for future research.

4.2.1.3. Disease-specific genes. Thyroid disease and type 1 diabetes mellitus are among the commonest of organ-specific autoimmune disease. Genetic variants of thyroid stimulating hormone receptor (TSHR) (chromosome 14) and thyroglobulin (TG) (chromosome 8) are associated with thyroid autoimmunity but the risk conferred is relatively small (Ajjan and Weetman, 2006). Chromosome 1 insulin gene variable number tandem repeats (INS VNTR) is associated with type 1 diabetes mellitus, which was proposed to be due to a defect in central tolerance. Disease-specific genes have not been studied in Turner's, Down's, or Klinefelter's syndromes and their role for increased autoimmunity in these conditions remains unclear. As none of the above genetic polymorphisms is located on the sex chromosomes or chromosome 21, it is not unreasonable to conclude that they have no role in increased susceptibility to autoimmunity in endocrine chromosomal disease above what is found in the normal population.

4.2.2. Environmental factors

Increased stress interferes with the hypothalamic pituitary axis and induces catecholamine production resulting in neuroendocrine disequilibrium, subsequently affecting the immune system (Calcagni and Elenkov, 2006). It can be argued that stress levels are increased in individuals with Turner's, Down's, and Klinefelter's syndromes due to psychological factors and this may cause immune dysfunction, leading in the process to predisposition to autoimmunity. However, no studies have been conducted in this area and therefore the role of stress in predisposition to autoimmunity in these individuals is unknown.

The implication of bacterial or viral infections in the predisposition to autoimmune disease has always been an attractive concept and mechanisms proposed include incorporation of the virus into the human genome, host cell destruction with subsequent release of autoantigens, and molecular mimicry between the infectious agents and host antigens. Down's syndrome and Turner's syndrome subjects seem to have an increased susceptibility to infections, which may be due to defective immunoregulation, predisposing in the process to autoimmunity (Cuadrado and Barrena, 1996; Stenberg et al., 2004). However, this remains a hypothesis which could be tested by a longitudinal study investigating the prevalence of autoimmunity in Down's syndrome and Turner's syndrome subjects with repeated infections.

5. Autoimmune disease in Turner's syndrome

5.1. Turner's syndrome and thyroid autoantibodies

Autoimmune thyroid disease is the commonest autoimmune condition recognized in Turner's syndrome. The relationship between thyroid disease and Turner's syndrome was first described by Aria et al. (1948) who reported the postmortem findings of a small thyroid gland in a patient with Turner's syndrome. Engel and Forbes (1961) first described the occurrence of thyroiditis in a patient with gonadal dysgenesis. Later, Williams et al. (1964) found thyroid autoantibodies in 13 of 25 patients with Turner's syndrome. The titers of autoantibodies were generally low except in those with mosaic karyotypes. The incidence of thyroid autoantibodies ranges from 12 to 87% depending on the method of measurement and ages of patients studied, with incidence increasing with age. Fleming et al. (1988) found that 48% of 52 patients had levels of thyroid antibodies diagnostic of Hashimoto's thyroiditis.

The youngest age at which the thyroid peroxidase antibodies and thyroglobulin antibodies have been reported in various series is 4 years (Medeiros et al., 2000), increasing throughout childhood and teenage years (without any obvious relationship to stage of pubertal development, Livadas et al., 2005), and with further increasing incidence throughout adult life. TSH receptor antibodies are much less commonly detected but when present correlate strongly with clinical disease (Chiovato et al., 1996).

5.2. Thyroid autoantibodies and karyotype in Turner's syndrome

The relationship between karyotype and thyroid autoantibodies in Turner's syndrome was discovered early. Doniach et al. (1968) looked at thyroid autoantibodies in patients with sex chromosome abnormalities and found that the highest incidence occurred in patients with mosaic karyotypes, especially those with an X isochromosome. Conflicting evidence from different studies, hampered by their frequent small size, leaves unresolved the question of any relationship between karyotype and propensity to autoimmunity, especially whether ring X chromosome leads to a higher incidence of Turner's syndrome (Sparkes and Motulsky, 1963; De Kerdanet et al., 1994; Radetti et al., 1995; Elsheikh et al., 2001; Livadas et al., 2005; El Mansoury et al., 2005, Bettendorf et al., 2006).

Clinical hypothyroidism occurs commonly as well but with much less frequency than the high incidence of thyroid autoantibodies. The most frequent pattern is mild hypothyroidism. When this is associated with serum thyroxine levels within the reference range and mildly elevated TSH concentration, the condition used to be designated subclinical hypothyroidism. It is now recognized that healthy euthyroid individuals maintain serum thyroxine within a much tighter band than the broad population-derived normal range (Andersen et al., 2002). Furthermore, with the succession of ever more sensitive TSH assays, there has been a tendency for the upper limit of TSH to be set at progressively lower values. Thus, while for many years a value of 6 mU/L was the upper normal value used by many laboratories, this is now commonly reduced to around 4 mU/L and there is a current unresolved debate (particularly in the United States) whether in fact 2.5 mU/L should be the upper cut-off point (Brabant et al., 2006). Finally, subtle changes in well being are often ascribed to these small-scale deviations from the unequivocally normal. This reflects apparently wide differences in sensitivity between individuals to perturbations in thyroid function. Thyrotoxicosis occurs with about a tenth the frequency of hypothyroidism. It has been suggested that relative to classical Graves' disease, cases with toxic phase of Hashimoto's thyroiditis are more frequent in Turner's syndrome (Idris and O'Malley, 2000).

The availability of a range of autoantibodies indicating a high likelihood of celiac disease—including antigliadin, antiendomysial, and antitissue transglutaminase antibodies has revealed their relatively high frequency in the Turner population, greater

than in women with normal karyotypes but again often presenting mild or atypical features (Ivarsson et al., 1999; Bonamico et al., 2002).

The use of growth hormone therapy in girls with Turner's syndrome is well documented to improve final adult height especially when initiated early in life. A large, longitudinal, multicenter study from Germany revealed that girls with thyroid and/or tissue transglutaminase antibodies achieved relatively poorer growth responses to growth hormone therapy (Bettendorf et al., 2006).

Other autoimmune gastrointestinal abnormalities in Turner's syndrome include Crohn's disease and ulcerative colitis. The prevalence of irritable bowel syndrome in Turner's syndrome is between 2 and 3%, and the prevalence of Crohn's disease is twice as common as ulcerative colitis, in contrast to the general population (Price, 1979). Autoimmune skin conditions have also been described in Turner's syndrome, including psoriasis and alopecia areata (Lee and Yoo, 1996; Rosina et al., 2003), but these have been sporadic cases and it is unclear whether the incidence of autoimmune skin conditions is increased in Turner's syndrome.

Although the Danish registry study has shown a 10-fold increase in insulin treated diabetes in subjects with Turner's syndrome (Gravholt et al., 1998), there is no clear evidence for an increased prevalence of type 1 diabetes mellitus in these patients (Elsheikh et al., 1999). The reason for the increased incidence of diabetes in Turner's syndrome women is probably due to deranged insulin secretion by mechanisms that are not entirely clear, and is not related to autoimmunity against pancreatic β-cells (Bakalov et al., 2004). Obesity is associated with raised leptin levels and also use of exogenous estrogen, though insulin dose and glycemic control show no such relationship (Danne et al., 1997).

The other autoimmune conditions of rheumatoid arthritis and chronic autoimmune liver disease are again common in Turner's syndrome and warrant being tested for repeatedly at intervals along with the other autoimmune conditions mentioned above and appropriate investigations, monitoring, and treatment (Zulian et al., 1998; Wihlborg et al., 1999; Invernizzi et al., 2004; Selmi et al., 2006).

6. Autoimmune disease in Klinefelter's syndrome

The original concept that autoimmune conditions could be separated into two categories, organ-specific and non-organ specific, has been replaced by the concept of a spectrum of disorders running between these two extremes. In considering the incidence of autoimmune conditions in Klinefelter's syndrome, it is apparent that there is a much stronger association with conditions of a non-organ specific nature, especially SLE, than the classical single organ conditions exemplified by Hashimoto's thyroiditis. This distinction from the situation seen in Turner's syndrome was indicated in early studies (Burch et al., 1966; Ferguson-Smith et al., 1966; Vallotton and Forbes, 1967). The apparently high incidence of SLE in Klinefelter's syndrome implicates hormonal factors, including raised estrogen and reduced androgen levels (Schattner and Berrebi, 1989).

6.1. Sex hormones

The female predisposition to autoimmune disease implicates estrogen as a trigger, particularly as a factor that exacerbates the disease (Cutolo et al., 2004). However, the onset of autoimmunity after the menopause, the presence of severe disease in men, and the absence of female predominance in some autoimmune conditions are arguments against hormones as the sole factors determining autoimmune disease initiation or maintenance (Gleicher and Barad, 2007). An immunological predisposition in Klinefelter's syndrome is evident by elevated immunoglobulin levels, increased $CD4^+/CD8^+$ ratio, and high levels of Th1 and Th2 cytokines (Aoki, 1999; Kocar et al., 2000; Oktenli et al., 2002).

Interestingly, these abnormalities normalize with testosterone replacement, directly implicating low levels of this hormone in the immunological disturbances found in Klinefelter's syndrome. Furthermore, animal studies suggest that testosterone has a role in maintaining central tolerance (Aoki, 1999), which may be another mechanism for the increased risk of autoimmunity in Klinefelter's syndrome subjects.

Other autoimmune disorders are increased in Klinefelter's syndrome, including progressive systemic sclerosis (Kobayashi et al., 1991) and rheumatoid arthritis (Kobayashi et al., 1994). The role of androgens is complex, since men with rheumatoid arthritis have low testosterone levels (Gordon et al., 1988; Spector et al., 1989) and an association between Klinefelter's syndrome and juvenile chronic arthritis has been reported (Mirkinson et al., 2005). Benefit has been claimed for clinical and immunological features of autoimmune diseases in patients with Klinefelter's syndrome treated with testosterone (Bizzarro et al., 1987). Evidence that there is enhanced susceptibility to rheumatoid/autoimmune disease in males with untreated hypogonadism, irrespective of aetiology, has come from a study including hypergonadotrophic hypogonadism other than Klinefelter's disease and hypogonadotrophic hypogonadism (Jimenez-Balderas et al., 2001) and a large comparative study of patients with Klinefelter's syndrome and hypogonadotrophic hypogonadism (Oktenli et al., 2002). Both groups of patients displayed enhanced cellular and humoral immunity. However, Klinefelter's syndrome patients had striking, specifically elevated incidence of anticardiolipin and antiextractable nuclear antibodies. Whether these findings, especially in Klinefelter's syndrome and SLE, predispose to a higher risk of the catastrophic antiphospholipid syndrome (Asherson, 2006) is conjectural. This rare but frequently fatal syndrome generally requires a trigger such as trauma, infection, or neoplasia. Of interest from an endocrine perspective is the high incidence of adrenal insufficiency associated with the antiphospholipid syndrome (Espinosa et al., 2003).

Patients with Klinefelter's syndrome may display a phenotypic variation on the basis of androgen production as well as response to androgen which is mediated by the intracellular androgen receptor. The androgen receptor gene is situated on the long arm of the X chromosome and contains a variable length CAG trinucleotide repeat sequence coding for a polyglutamine tract, greater length of which reduces the androgenic response. One investigation into the impact of this on variation in phenotypic features only found inverse correlation with penile length (Zinn et al., 2005). Further studies related increased CAGn length to taller height and gynecomastia (Lanfranco et al., 2004) and later onset of puberty (Wikström et al., 2006). The latter study also related a delay in onset and progress of puberty in 3 boys with a paternal origin of the supernumerary X chromosome compared with 11 boys with maternal origin for the extra X chromosome. The causes of the wide variation in phenotypes in Klinefelter's syndrome remain largely unresolved. In obese patients there is unsurprisingly an increased incidence of type 2 diabetes and the metabolic syndrome (Bojesen et al., 2006) while overall there is debatably also an increased incidence in type 1 diabetes (Aoki, 1999). Management of both types of diabetes may be more difficult in patients with learning difficulties (Rovet et al., 1996; Somango-Sprouse, 2001) or behavioral and psychological problems which are found in a significant subset of patients with Klinefelter's syndrome (DeLisi et al., 2005).

7. Autoimmune disease in Down's syndrome

Down's syndrome, among the common conditions caused by chromosomal aneuploidy, exhibits the highest frequency of significant autoimmune conditions. The situation in many ways resembles that in Turner's syndrome. The dominant associations are with thyroid disease, diabetes, and celiac disease but the spectrum is wide (Soderbergh et al., 2006).

The long recognized high prevalence of thyroid autoimmune disease in Down's syndrome often starts in early life, including infancy (Shalitin and Phillip, 2002). A longitudinal study of 85 children with Down's syndrome in Uppsala County Sweden, with annual checks up to age 25, revealed 28 children developed hypothyroidism, half before the age of 8. Only one had detectable thyroid antibodies (Karlsson et al., 1998). By contrast, after this age nearly all those developing hypothyroidism had thyroid antibodies. There was no sex difference in this series. Importantly, children with Down's syndrome who became hypothyroid before the age of 11 years had greatly reduced

growth velocity in the year before diagnosis compared to sex and age-matched euthyroid controls with Down's syndrome. However, seven out of eight showed increased growth velocity, often very striking, in the year that treatment with thyroxine was started. Two children developed hyperthyroidism. A study of 138 community-based patients with Down's syndrome revealed 28 previously undiagnosed hypothyroid and 2 hyperthyroid cases (Friedman et al., 1989). In this series 78.5% of hypothyroid patients were female, most between 30 and 50 years. A prospective study of the natural history of hypothyroidism in a group of 344 patients with Down's syndrome followed for 2–7 years disclosed subclinical hypothyroidism in 32.5% compared with 1.1% of age and sex-matched controls (Rubello et al., 1995). Though prevalence of antithyroid antibodies in Down's syndrome patients (18%) was three times higher than controls, it was similar in subclinical hypothyroid (18.7%) and euthyroid (15.8%) Down's patients. On follow-up, 35.7% of thyroid antibody patients with subclinical hypothyroidism developed clinical thyroid disease which did not occur in any of the antibody negative patients with subclinical hypothyroidism. With the high frequency of cognitive problems and early onset Alzheimer's disease in Down's syndrome, it has been suggested that even mild "subclinical" hypothyroidism may contribute to cognitive decline (Percy et al., 1990). Hyperthyroidism occurs in about 2% of patients with Down's syndrome but may give rise to major behavioral disturbances which respond to treatment rendering the patient euthyroid including thionamide drugs and radio iodine. Hepatitis B infection may be associated with autoimmune thyroid disease and is common in Down's syndrome. A study of 57 adults with Down's disease showed threefold higher frequency of thyroiditis in carriers of hepatitis B surface antigen, than non-carriers, but no such relationship in 450 age-, sex-, and environmentally matched mentally retarded patients without Down's syndrome (May and Kawanishi, 1996).

Use of antibody measurements has facilitated the diagnosis of celiac disease in Down's syndrome, and antitissue transglutaminase and antiendomysial antibodies are especially helpful. Several series recorded prevalence in the range of 10–16% (Carlson et al., 1998; Zachor et al., 2000; Bonamico et al., 2001; Book et al., 2001; Agardh et al., 2002; Cogulu et al., 2003). Many patients diagnosed with celiac disease were relatively asymptomatic other than bloating, but growth retardation was frequent.

Type 1 diabetes has long been recognized as more common than expected in Down's patients. A 4.2-fold increased prevalence in a nationwide population study of Down's syndrome was reported from Denmark (Bergholdt et al., 2006). In agreement with other studies (Gillespie et al., 2006), there is an association with HLA and low risk genotypes. Furthermore, the median age of onset (6 years) is about 2 years younger than the general population. Finally, in contrast to Klinefelter's syndrome, where diabetes confers a major risk of increased mortality (Swerdlow et al., 2005), a small study from Scotland suggests that there were fewer problems in controlling diabetes in Down's syndrome, which the authors ascribed to a settled mode of existence (Anwar et al., 1998). However, as in Klinefelter's syndrome, there is a suggestion of an increased prevalence of antiphospholipid antibodies and stroke in Down's syndrome (Gatenby et al., 2003).

The association between chromosomal endocrine conditions and autoimmune disease is summarized in Table 1.

Table 1
Summarization of the association between Turner's syndrome, Down's syndrome, Klinefelter's syndrome and autoimmune conditions

Autoimmune conditions	Turner's syndrome	Down's syndrome	Klinefelter's syndrome
Thyroid disease	↑	↑	Dubiously ↑
Celiac disease	↑	↑	Probably ↔
Type 1 diabetes mellitus	↔	↑	↔
Systemic lupus erythematosus	Unclear	Probably ↑	↑
Systemic sclerosis	Probably ↔	Probably ↔	↑
Juvenile rheumatoid arthritis	↑	Probably ↔	↑
Inflammatory bowel disease	↑	Probably ↔	Probably ↔

Symbols: ↑, increased; ↔, no effect.

8. Possible mechanisms for autoimmunity in chromosomal abnormalities

8.1. Parental autoimmunity and chromosomal abnormality

Clinical observation of a frequent occurrence of goiter and thyroid disease in mothers of babies with Down's syndrome led to investigations of the link between parental autoimmunity and chromosomal abnormalities. Since there was a known link between gonadal dysgenesis and thyroid autoimmunity, it was suspected that there was a common cause for the chromosomal abnormalities. Fialkow (1964) suggested that the parental autoantibodies themselves predisposed to aneuploidy, either in mitosis or meiosis. Several studies (Fialkow, 1964, 1967; Burgio et al., 1965) did show that women who had a baby with Down's syndrome had higher thyroid antibody titers than controls. However, many of the studies did not use age-matched controls and most were carried out some time after the child was born. Cuckle et al. (1988) looked at stored serum from pregnant women who had subsequently had a baby with Down's syndrome and compared them with controls. Although not statistically significant, the differences were consistent with increased levels of antibodies in mothers of children with Down's syndrome.

Torfs et al. (1990) came to a different conclusion. They studied serum taken during early pregnancy from a large group of women who had given birth to a baby with a trisomy and controls and some of the husbands. They found no differences in thyroid autoantibodies between mothers of Down's syndrome babies and control mothers. This finding was supported by Gustafsson et al. (1995), who looked at 29 mothers of babies with Down's syndrome and controls, but the blood was taken at delivery because pregnancy may decrease thyroid antibodies. They found no difference in frequency of thyroid antibodies between cases and controls.

Several studies have looked at thyroid autoantibodies in patients with Klinefelter's syndrome and their parents. Ferguson-Smith et al. (1966) looked at 86 patients, 27 fathers and 43 mothers. They found no increased frequency of thyroid, gastric, or nuclear autoantibodies in patients with Klinefelter's syndrome or their parents. This finding was confirmed in a similar study by Vallotton and Forbes (1967). Both groups concluded that predisposition to autoimmunity was not an important factor in non-disjunction in this group of patients.

In contrast, several studies of thyroid disease frequency or thyroid antibodies in parents and offspring with Turner's syndrome produced conflicting results. This may be because some studies looked at thyroid autoantibodies while others looked at actual thyroid disease. Fleming et al. (1988) found that 48% of 53 women had raised autoantibodies and 8 patients had a first degree relative with thyroid disease. They did not, however, ask about thyroid disease in a control population of infertile women. Vallotton and Forbes (1967) analyzed thyroglobin antibodies in a series of 45 patients with Turner's syndrome, with both parents for 17 of the cases. They found a significantly higher incidence of thyroid autoantibodies in patients and parents compared with controls. Wilson et al. (1996) studied 60 patients with 50 mothers compared with 127 controls. They found that 30% of patients were positive for either thyroid peroxidase and/or thyroglobin antibodies and 22% of mothers were also positive. This was significantly different from controls. Other studies have shown conflicting results but these have used different methods of measuring antibodies and different age groups of patients. In contrast to these studies Larizza et al. (1999) studied 95 patients with Turner's syndrome, 72 fathers and 78 mothers ranging in age from 1 to 32.3 years (mean 12 years). They looked at organ-specific and non-organ specific autoantibodies and found that the frequency was higher in patients than controls. The percentage of autoantibodies in parents was not significantly different from controls. They did find that in 17% of cases only the father had evidence of autoimmunity. They therefore concluded this showed preferential paternal transmission. However, the percentage of patients positive for thyroid microsomal antibodies (15.8%) is lower than in most other studies possibly due to the young age of some of their patients.

In most of the above studies, absolute numbers of positive cases were low, the age range of patients was very wide, control groups varied and were not always representative of the general population, controls were not age-matched to patients, and parents and laboratory methods varied. Firm conclusions cannot therefore be drawn from available evidence, however, it would seem more likely that antibodies would be missed due to sampling at the wrong age or using insensitive methods, rather than finding false positive samples in parents. There would seem to be, therefore, some connection between Turner's syndrome and family history of autoimmune thyroid antibodies. What is unclear is whether this is because of a familial tendency to autoimmunity or whether the autoimmunity is implicated in the aneuploidy.

8.2. Autoimmunity and X monosomy

Autoimmune thyroid disease is more common in women. Epidemiological studies have consistently shown female to male predominance of 5:1 to 10:1 with a peak incidence in the two decades that precede the menopause (Mogensen and Green, 1980). This finding could be explained by an effect of estrogens or testosterone on the immune system or by an effect of genes on the sex chromosomes. Such a gene on the Y chromosome seems unlikely as there is very rarely male-to-male transmission of such traits.

The fact that patients with Turner's syndrome have an increased risk of autoimmunity strengthens an argument in favor of X-linked autoimmunity genes. It also weakens the argument that autoimmunity is influenced by estrogens, as women with Turner's syndrome will not produce endogenous estrogens. Immune genes have been localized to the X-chromosome, and loss of some of these genes is associated with severe disturbance in immune cells (Ochs et al., 2005).

Invernizzi et al. (2004) tested the hypothesis that there are X-linked genes for autoimmunity by looking at patients with primary biliary cirrhosis in which there is a 9:1 female predominance. They assessed the frequency of X monosomy cells in peripheral blood in 100 women with primary biliary cirrhosis and compared this with 50 controls and 50 women with chronic hepatitis C. They found a highly significant greater frequency of monosomy X in the patients with primary biliary cirrhosis than in controls. In 2005, the same group studied 44 women with systemic sclerosis and 44 women with autoimmune thyroid disease and found similarly that they had a significantly higher frequency of monosomy X in peripheral blood cells compared to age-matched controls (Invernizzi et al., 2005).

There are two genetic models to explain the connection between autoimmune disorders and candidate genes. First, a polygenic model could be proposed in which genes on the X chromosome escape X inactivation. Alternatively, there could be protective genes on the Y chromosome. A combined hypothesis would be that genes which escape X-inactivation have equivalent genes on the Y chromosome. There is some experimental evidence for an X linked locus for Graves' disease on the X chromosome at Xq21.33-22 (Barbesino et al., 1998).

8.3. X-inactivation and autoimmunity

Loss of immunologic tolerance to self-antigens is an important feature of autoimmune disorders. A potential mechanism through which lack of exposure to X-linked self-antigens could occur in women is a skewing of X-chromosome inactivation. In women, one of the two X chromosomes is inactivated in early embryonic life which means that females are mosaic for two cell lines; cells would contain either the maternal or the paternal X chromosome as the active X. If there is a gene for autoimmunity carrying a mutation on one of the X chromosomes, this may be expressed if there is skewing of X inactivation.

The hypothesis that there are genes for autoimmunity on the X chromosome which escape inactivation is strengthened by studies of X inactivation patterns in patients with autoimmune diseases. Ozbalkan et al. (2005) looked at X inactivation

patterns in peripheral blood samples of 70 women with scleroderma, 12 women with rheumatoid arthritis, and 9 patients with SLE and compared them with controls. In scleroderma, 64% of patients exhibited skewed patterns vs. 8% of controls and 8% of rheumatoid arthritis patients. This was a significant difference between the patients with scleroderma and controls, and interestingly, different tissues exhibited a difference in patterns.

It should be noted that skewed X inactivation may occur late in life due to somatic cell selection or clonal loss of myeloid stem cells, and this may explain late occurrence of autoimmune disease (Hatakeyama et al., 2004). Skewed X inactivation was found in peripheral blood cells from patients with autoimmune thyroid disease and scleroderma (Selmi et al., 2006). Ozcelik et al. (2006) showed a similar and significant skewed X inactivation pattern in 110 women with autoimmune thyroid disease (34%) compared with 160 female controls (8%). Analysis of two familial cases showed skewing only in affected individuals. Brix et al. (2005) looked at 32 female twins with autoimmune thyroid disease and a control group of 96 healthy female twins. The frequency of skewed X inactivation in the patients was higher than in the control population. The frequency of skewed X inactivation was much higher in female twins with autoimmune thyroid disease than healthy co-twins.

8.4. Mechanisms of autoimmunity in Turner's syndrome

These hypotheses could help explain why patients with Turner's syndrome have an increased risk of autoimmunity. If there are genes for autoimmunity on the X chromosome which escape X inactivation, patients with Turner's syndrome would be haploinsufficient for the gene/genes and more at risk. This hypothesis, however, does not explain the increased risk in women with the particular karyotype 45,X/46,X,i(Xq). It is possible that an imbalance between genes on the short arm and long arms of the X chromosome are the cause of this increased risk, since the risk is not seen in Klinefelter's syndrome where there are also three copies of the X chromosome long arm.

8.5. Mechanisms of autoimmunity in Down's syndrome

8.5.1. Premature aging of the immune system

In Down's syndrome, the mechanism of increased risk for autoimmunity is less clear. Some authors have suggested a "premature aging" effect with a prematurely increased percentage of $3G5^+$ (age-related) T cells. Rabinowe et al. (1989) found four children under the age of 10 with a significantly higher number of these cells than age-matched controls, and the increased numbers would have been expected at 50–70 years of age. Cuadrado and Barrena (1996) showed a similar pattern of abnormal proportions of peripheral blood lymphoid subsets indicating "early senescence."

8.5.2. Disomic homozygosity at the APECED locus

Another theory about the increased frequency of autoimmunity in Down's syndrome is that in contrast to Turner's syndrome, autoimmunity is related to overexpression of genes on chromosome 21. A rare autosomal recessive condition, autoimmune polyglandular syndrome type 1 (APECED), has been mapped recently to chromosome 21q22.3 and is due to mutations in the gene *AIRE*. Individuals with this condition have increased susceptibility to mucocutaneous candidiasis and autoimmune endocrinopathies including hypoparathyroidism and adrenal insufficiency. Patients can also have abnormalities of the pancreas, nail dystrophy, and alopecia. Shield et al. (1999) looked at 16 children with Down's syndrome with diabetes and their parents compared with 99 children with Down's syndrome without diabetes. There was no evidence of increased disomic homozygosity in the region of the APECED locus in the Down's syndrome patients with diabetes compared with the patients without diabetes. This study, however,

looked at one candidate gene and the results do not exclude the possibility that other genes on chromosome 21 could cause of the increased frequency of autoimmunity.

Key points

- The three common chromosomal disorders: Turner's, Down's, and Klinefelter's syndromes, are associated with autoimmune disease.
- Disturbances of chromosomal number and structure arise by several mechanisms.
- Genetic and environmental risk factors are involved in proposed mechanisms leading to autoimmune disease.
- Organ-specific autoimmune disorders such as thyroid, liver, and celiac diseases are associated with Turner's and Down's syndromes.
- Klinefelter's syndrome is more strongly related to non-organ-specific autoimmune disease such as SLE.
- Mechanisms proposed for the association between chromosomal disorders and autoimmunity include sex steroids, parental autoimmunity, X-monosomy, and skewing of X-inactivation.
- Features of Down's syndrome potentially enhancing the prevalence of autoimmunity include premature ageing of the immune system and overexpression of genes on chromosome 21.

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CHAPTER 12

Autoimmune Polyendocrine Syndromes (APS) or Multiple Autoimmune Syndromes (MAS)

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1. Historical considerations in autoimmune disorders of the endocrine glands

The origin of endocrine autoimmunity goes back to 1956, when it was found that patients with chronic thyroiditis had autoantibodies against thyroglobulin (Roitt et al., 1956), those with Graves' disease had a factor stimulating the thyroid gland (Adams and Purves, 1956) and chronic thyroiditis could be produced in animals by immunization with homogenates of autologous tissue (Rose and Witebsky, 1956). It was first shown in 1957 that patients with "idiopathic" Addison's disease (AD) had circulating autoantibodies against adrenal cortex extracts (Anderson, 1957). As early as 1855, Addison (1937) described lymphocytic infiltration of the adrenals in idiopathic adrenal insufficiency, and this finding was confirmed by McIntyre Gass (1962). Hashimoto (1912) described mononuclear leukocyte infiltration in patients with an enlarged thyroid gland. In 1940, similar infiltrates ("insulitis") were described around and inside the pancreatic islets of patients with type 1 diabetes mellitus (DM) (Von Mayenburg, 1940).

Based on these findings, Witebsky et al. (1957) codified the criteria that defined a disease as autoimmune (Table 1). Following these criteria, several patients previously labeled as having idiopathic disease were reclassified as having autoimmune diseases. To date, "more than 80 diseases are attributable to autoimmunity and one or another affect some 7% of the population," to quote the book that celebrated the 50th anniversary of the discovery of autoimmunity (Rose and MacKay, 2006).

2. Autoimmune polyendocrine syndromes or multiple autoimmune syndromes

In 1980, Neufeld proposed a classification of autoimmune polyendocrine syndrome (APS) based on clinical criteria and identifying four types of APS (Table 2) (Neufeld and Blizzard, 1980). Following the publication of Neufeld's criteria, a large body of new data has been acquired in the field of autoimmune diseases, and for this reason we believe that the proposed classification needs some modifications.

The term APS is used historically but is not fully appropriate because it indicates not only multiple autoimmune endocrine diseases but also autoimmune endocrine diseases associated with non-endocrine autoimmune diseases (such as type 1 DM and celiac

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Table 1
Criteria for defining a disease as autoimmune (Witebsky's postulates)

Demonstration of serum autoantibodies and/or cell-mediated events
Demonstration of a lympho-monocyte infiltration in the target organ
Possibility of identifying and isolating autoantigens
Possibility of inducing experimentally the disease in animals by immunization with autoantigens and to transfer the disease passively by serum or lymphocytes

Table 2
Classification of autoimmune polyglandular syndromes (APS) according to Neufeld and Blizzard (1980) (modified)

Type 1 APS or APECED	Chronic mucocutaneous candidiasis Chronic hypoparathyroidism Addison's disease (at least two present)
Type 2 APS or Schmidt's syndrome	Addison's disease (always present) + Thyroid autoimmune diseases and/or Type 1 diabetes mellitus
Type 3 APS or thyro-gastric syndrome	Thyroid autoimmune diseases + Other autoimmune diseases (excluding Addison's disease)
Type 4 APS	Combinations not included into the previous groups

disease) and associations between non-endocrine autoimmune diseases (for instance, vitiligo and alopecia). For these reasons, we believe it would be more appropriate to use the term, multiple autoimmune syndromes (MAS), to denote these overlapping autoimmune conditions. Therefore, we will use the abbreviation, MAS, instead of the traditional designation, APS, in this chapter.

During the last three decades, the number of recognized autoimmune diseases has greatly increased and the various autoimmune associations have become more complex. It is now recognized that the autoimmune disorders are chronic and their natural history occurs in three separate phases: (a) potential, (b) subclinical, and (c) clinical. The potential phase is characterized by a genetic predisposition and the presence of circulating autoantibodies and/or lymphocytic infiltration of the target organs, without any impairment of the target organs. The subclinical phase is characterized by circulating autoantibodies and/or lymphocytic infiltration of the target organs, associated with evidence of subclinical impairment of the target organs, and the clinical phase is revealed by circulating autoantibodies and/or lymphocytic infiltration of the target organs associated to typical signs and symptoms of the disease. For this reason, the concept of autoimmune disorder not only may refer to the clinical disease but can also apply to the presence of the relevant autoantibodies, along with either subclinical failure or normal function of the target organ(s) (Fig. 1). Therefore, the term MAS should be applied not only to subjects with two or more overt autoimmune diseases (the "tip of the iceberg"), but also to those with the combinations summarized in Table 3.

Another important aspect of this group of disorders is that each autoimmune disease has a privileged association with other autoimmune disorders, and in the majority of cases the future disease can be heralded by detection of the relevant circulating autoantibodies (Betterle et al., 2002; Schatz and Winter, 2002). Table 4 lists autoimmune diseases with their preferred autoimmune associations. Hence, clinicians who follow patients with one of these diseases should always look for the potential, subclinical, or clinical coexistence of other autoimmune disorders that have the potential to "complicate" the main autoimmune disease by determining the specific autoantibody markers (Betterle et al., 2002; Schatz and Winter, 2002). Moreover, because autoantibodies may be acquired during the life span, a test for autoantibodies that is negative at the first screening should be repeated every 2 or 3 years.

2.1. Pathogenesis of MAS

A hypothesis that has been proposed to explain multiple organ involvement in MAS stated that tissues derived from the same germ layer could

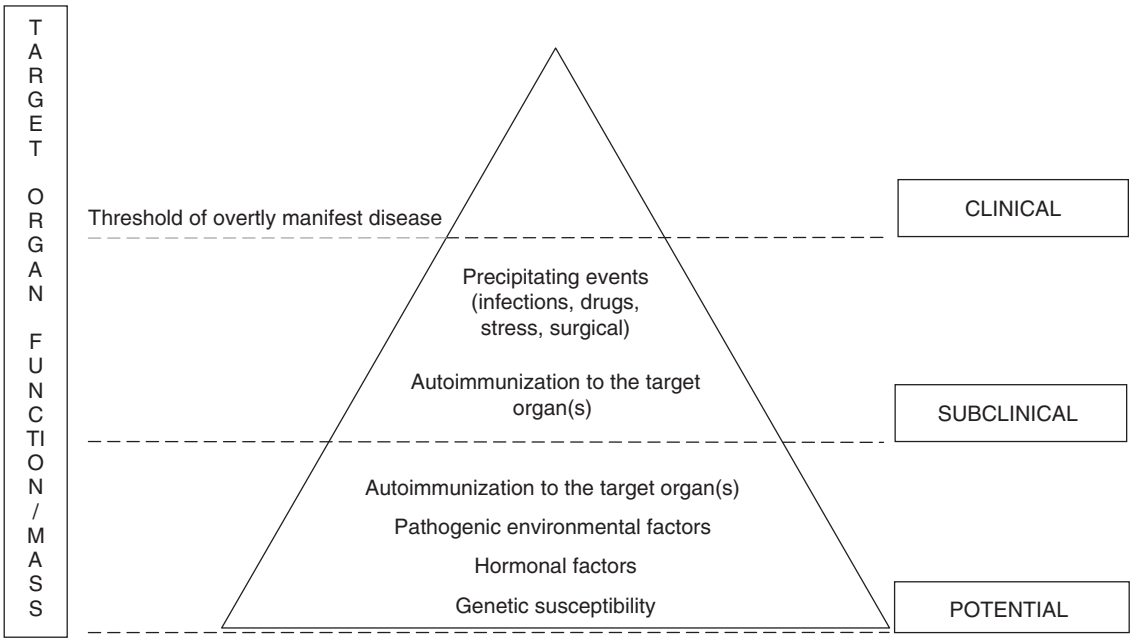

Figure 1. The autoimmune iceberg.

Table 3
Examples of combinations defining a multiple autoimmune syndrome

Combinations	Examples
Two or more clinical autoimmune diseases	Chronic thyroiditis or Graves' disease with clinical dysfunction + Chronic adrenalitis with clinical adrenocortical failure
One clinical + another subclinical autoimmune disease	Chronic thyroiditis or Graves' disease with clinical dysfunction + Adrenal cortex autoantibodies with subclinical adrenocortical failure
One clinical + another potential autoimmune disease	Chronic thyroiditis or Graves' disease with clinical dysfunction + Adrenal cortex autoantibodies with normal adrenocortical function
Two or more subclinical autoimmune diseases	Thyroid autoantibodies with subclinical thyroid dysfunction + Adrenal cortex autoantibodies with subclinical adrenocortical failure
One subclinical + one potential autoimmune disease	Thyroid autoantibodies with subclinical thyroid dysfunction + Adrenal cortex autoantibodies with normal adrenocortical function
Two or more potential autoimmune diseases	Thyroid autoantibodies with normal thyroid function + Adrenal cortex autoantibodies with normal adrenocortical function

express common germ-layer–specific antigens which serve as targets for autoimmune responses (Tadmor et al., 1992). This theory can explain type 3 MAS that includes thyroid and stomach, organs originating from the same endodermal germ layer, but does not explain type 2 MAS, in which the targets are the adrenal cortex (derived from mesoderm), the thyroid gland, and the

Table 4
Heralding autoimmune disease (first row) and preferential multiple association with other autoimmune diseases (columns), in order of prevalence

Thyroid autoimmune disease	Type 1 diabetes mellitus	Celiac disease	Addison's disease	Vitiligo	Hypoparathyroidism
Autoimmune gastritis	Thyroid autoimmune disease	Thyroid autoimmune disease	Thyroid autoimmune disease	Thyroid autoimmune disease	Addison's disease
Type 1 diabetes mellitus	Autoimmune gastritis	Type 1 diabetes mellitus	Autoimmune gastritis	Autoimmune gastritis	Thyroid autoimmune disease
Celiac disease	Celiac disease		Type 1 diabetes mellitus		
Addison's disease	Addison's disease		Hypergonadotropic hypogonadism		

pancreas (derived from endoderm). Moreover, if this theory is correct, the MAS should appear simultaneously, but this occurs rarely.

2.2. Animal models of MAS

The difficulty in understanding the pathogenesis of MAS is related to the fact that animal models that develop spontaneous autoimmunity generally express potential MAS, in which autoimmunity is limited to detection of circulating autoantibodies. For example, the White Leghorn Chicken, a strain that spontaneously develops lymphocytic thyroiditis, develops autoantibodies to adrenal cortex, gastric mucosa, and other tissues. However, no impairment of the target organs usually occurs (Koury et al., 1982; Ikegami, 2002). Thyroid and gastric autoantibodies are also detected in type 1 diabetic BioBreeding (BB) rats, but this animal model of type 3 MAS never achieves clinical expression of thyroid or gastric disease (Asamoto et al., 1986; Ikegami, 2002). In the non-obese diabetic mouse, which spontaneously develops type 1 DM, autoimmunity is directed against submandibular and lachrymal glands (Makino et al., 1980), or parathyroid infiltration can be observed (Krug et al., 1991), but also this model of MAS remains latent. Spontaneous type 2 MAS has been described only in a boxer dog affected by primary hypothyroidism and partial adrenocortical deficiency, and pathological studies revealed thyroid atrophy and lymphocytic adrenalitis (Kooistra et al., 1995).

Apart from these spontaneous models, there are animal models of experimental MAS induced by environmental triggers (i.e., viruses, toxic substances), thymectomy, or genetic manipulation (Onodera et al., 1981; Bartolomaeus et al., 1988). Type 2 MAS affecting thyroid, adrenals, ovary, pancreatic islets, and stomach in various combinations has been found in mice treated with cyclosporin A at birth, followed by removal of the thymus (Sakaguchi, 1989). This experience suggests that MAS is the result of a deeper T-cell unbalance than that required for the induction of single-organ disease.

Following the discovery that AutoImmune REgulator (AIRE) gene mutations are interrelated with MAS type 1, AIRE gene knockout animal models were developed. However, these models of type 1 MAS differ from the spontaneous human disease (Pontynen et al., 2006). In conclusion, despite the stimulating information provided by animal models, data in animals do not necessarily reflect human disease in vivo.

2.2.1. Type 1 autoimmune polyglandular syndrome (APS), or autoimmune polyendocrinopathy–candidiasis–ectodermal–dystrophy (APECED), or type 1 multiple autoimmune syndrome (MAS)

Type 1 APS or APECED, the preferred term for type 1 MAS, is a rare autosomal recessive disorder caused by mutations in the AIRE gene located on

chromosome 21. The prevalence of type 1 MAS varies greatly, ranging from 1:9,000 inhabitants among the Iranian Jewish community (Zlotogora and Shapiro, 1992) and 1:10,000,000 in Japan (Sato et al., 2002). In Italy, three ''hot areas'' have been identified in Sardinia (1:14,400) (Clemente et al., 1998), Apulia (1:35,000) (Meloni et al., 2002), and Veneto regions (1:4,400) (Betterle et al., 2002). The three main components of APECED are chronic mucocutaneous candidiasis (CMC), chronic hypoparathyroidism (CHP), and Addison's disease (AD). The frequency in different populations is summarized in Table 5. CMC occurs at a very young age (5.4–6.7 years) (Ahonen et al., 1990; Betterle et al., 1998), and usually involves the finger nails, although in some patients it leads to chronic esophagitis with esophageal stricture and dysphagia. In adults, CMC may result in carcinoma of the oral mucosa, tongue, or esophagus (Betterle et al., 1998; Perheentupa, 2006). CHP is the second disease, appearing at 6–11 years of age (Ahonen et al., 1990; Betterle et al., 1998; Perheentupa, 2006). The parathyroid glands are atrophic and/or infiltrated by mononuclear cells (Whitaker et al., 1956; McIntyre Gass, 1962; Perheentupa and Miettinen, 1999). In patients with type 1 MAS and CHP, autoantibodies to calcium-sensing receptors have been identified (Li et al., 1996), but the reported frequency of these antibodies varies, depending on the method employed for their detection (Betterle, 2006; Galavas et al., 2007). Hypocalcemia manifests with tremor, tetany, and eventual convulsions. Treatment of CHP is based on chronic administration of calcium and vitamin D. AD is the third main disease (the second endocrinopathy), presenting at 10–15 years of age (Ahonen et al., 1990; Betterle et al., 1998; Perheentupa, 2006). Autopsy studies showed atrophy and lymphocyte infiltrates in the adrenals (McIntyre Gass, 1962; McNicol and Laidler, 1996). At the onset of AD, antibodies to the adrenal cortex and/or 21-hydroxylase (ACA and/or 21-OHAbs) are detectable in more than 90% of cases (Betterle et al., 2006). Computerized tomography scanning in AD patients shows normal or atrophic adrenals. The symptoms of AD are hypotension, asthenia, hyperpigmentation, and hypoglycemia. Treatment of AD is based on oral administration of 20–30 mg of hydrocortisone or 25–37.5 mg of cortisone acetate (in two or three daily doses) and fludrocortisone in one dose of 50–200 μg (Oelkers, 1996; Arlt and Allolio, 2003).

In patients with CMC and/or CHP but without AD, ACA, and/or 21-OHAbs can be detectable in about 50% of cases and all antibody-positive individuals develop AD within 3 years of follow-up, indicating that these antibodies in these subjects are markers of absolute risk of AD (Coco et al., 2006).

Regarding minor diseases summarized in Table 5, the most frequent is hypergonadotropic hypogonadism that is more prevalent in females (68%) than in males (28%). In most cases, hypogonadism precedes AD (Betterle et al., 1998; Perheentupa, 2006). Steroid-producing cell antibodies (StCA) and/or antibodies to 17α-hydroxylase (17α-OHAbs) and/or P450 side-chain cleavage enzyme (P450sccAbs) are present in the majority of patients with gonadal failure, and examination of ovarian tissue discloses lymphocytic oophoritis (Hoek et al., 1997). StCA are positive in about 20% of the patients with type 1 MAS without hypergonadotropic hypogonadism, and these patients are at high risk of developing gonadal failure (Betterle, 2006). Alopecia areata is another disease marked by the presence of tyrosine hydroxylase autoantibodies (Hedstrand, 2000). Autoimmune thyroid diseases (AITD) usually occur as chronic thyroiditis, with thyroid autoantibodies as a serological marker (Betterle et al., 1998; Perheentupa, 2006). Autoimmune hepatitis can also occur, but the clinical course is variable and extends from subclinical to fatal fulminating forms. Therefore, it needs to be recognized promptly and treated with immunosuppressive drugs (Betterle et al., 1998; Perheentupa, 2006). Autoimmune hepatitis is associated with liver–kidney microsomes (whose autoantigens are CYP-IA2 and CYP-2A6) (Clemente et al., 1997, 1998) and to the enzyme aromatic-L-amino-acid decarboxylase (Rorsman et al., 1995). When vitiligo develops (Betterle et al., 1998; Perheentupa, 2006), it is marked by antibodies to melanocytes (Hertz et al., 1977; Betterle et al., 1984) or to transcription factors SOX9 and SOX10 (Hedstrand, 2001). Autoimmune gastritis, with or without pernicious

Table 5
Frequency (percent) of the major and minor clinical features in type 1 MAS

Nation	USA	Finland	Iran	USA	Sardinia	Southern Italy	Norway	Japan	Northern Italy	Slovenia	Ireland	Poland	Total cases
Cases	$n=71$	$n=91$	$n=23$	$n=16$	$n=18$	$n=11$	$n=36$	$n=7$	$n=55$	$n=12$	$n=31$	$n=14$	$n=385$
Authors	Neufeld et al. (1981)	Ahonen et al. (1990) and Perheentupa (2006)	Zlotogora and Shapiro (1992)	Wang et al. (1998)	Rosatelli et al. (1998) and Meloni et al. (2002)	Perniola et al. (2000)	Myhre et al. (2001) and Wolff et al. (2007)	Sato et al. (2002)	Betterle et al. (1998) and personal data	Trebusak Podkrajsek et al. (2005)	Dominguez et al. (2006)	Stolarski et al. (2006)	Range
CMC	73	100	17	75	83	100	70	86	83	100	90	92	17–100
CHP	76	88	96	100	88	100	79	71	89	83	84	100	71–100
AD	100	84	22	95	83	82	67	43	82	58	68	50	22–100
Hypogonadism	15	47	38	24	28	18	20	n.d.	22	8	68[a]	28	8–47
Alopecia	32	39	13	40	33	n.d.	35	14	36	33	19	21	13–40
Type 1 diabetes	4	33	4	n.d.	5.5	0	9	43	4	8	13		0–43
AITD	25	31	4	31	0	36	11	n.d.	11	25	6.4	n.d.	0–36
AG/PA	13	31	9	n.d.	33	27	n.d.	n.d.	24	n.d.	6.4	n.d.	0–33
AH	25	18	n.d.	31	22	27	3	n.d.	19	8	n.d.	34	5–31
Malabsorption	22	48	n.d.	6	28	18	9	14	11	25		14	6–28
Vitiligo	10	31	n.d.	12	17	n.d.	20	n.d.	22	8	6.4	n.d.	8–25
Keratitis	n.d.	22	0	n.d.	17	n.d.	9	n.d.	9	17	6.4	14	0–22
Cancer	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7	n.d.	n.d.	n.d.	1–7
F/M	1.1	1	1.1	n.d.	n.d.	0.8	0.8	0.7	1.8	0.3	1.4	2.5	0.3–1.8

Data on different series.
Abbreviations: CMC, chronic mucocutaneous candidiasis; CHP, chronic hypoparathyroidism; AD, Addison's disease; AITD, autoimmune thyroid disease; AG/PG, autoimmune gastritis/pernicious anemia; AH, autoimmune hepatitis; n.d., not defined.
[a] Of the females.

anemia, can also be found (Betterle et al., 1998; Perheentupa, 2006). Malabsorption may be related to a great variety of disorders: celiac disease, cystic fibrosis, pancreatic insufficiency, intestinal infections from *Candida albicans* or *Giardia lamblia*, intestinal lymphangectasia or cholecystokinin deficiency (Betterle et al., 1998; Högenauer et al., 2001). In about half of the patients, malabsorption is idiopathic and autoantibodies to tryptophan hydroxylase (TPHAbs) (Ekwall et al., 1998) or to histidine decarboxylase (Scoldberg et al., 2003) have been identified. Type 1 DM is rare, and autoantibodies to endocrine pancreas are usually present (Tuomi et al., 1996; Gylling et al., 2000). Pancreatic autoantibodies are frequently detectable also in patients with type 1 MAS without type 1 DM (Tuomi et al., 1996; Betterle et al., 1998; Gylling et al., 2000). However, the great majority of these patients do not develop diabetes. Ectodermal dystrophy occurs, with nail dystrophy, defects in the formation of tooth enamel, bad implantation of teeth, and phlyctenular conjunctivitis (Ahonen et al., 1990; Perniola et al., 1998; Collins et al., 2006). Gallstones and acquired asplenia can also be present (Friedman et al., 1991; Perheentupa, 2006). Finally, patients with type 1 MAS can develop mucosal cancer (Betterle et al., 1998; Perheentupa, 2006).

2.2.1.1. Genetics of APECED. Type 1 MAS is an autosomic recessive disease related to mutations in the AIRE gene (Nagamine et al., 1997; The Finnish-German APECED Consortium, 1997). Fifty-eight different mutations associated with type 1 MAS were identified by 2006 (Perheentupa, 2006). The R257X mutation on exon 6 is the most common mutation in patients from Finland, Northern Italy, Switzerland, England, Germany, and New Zealand (The Finnish-German APECED Consortium, 1997; Peterson et al., 1998; Scott et al., 1998; Wang et al., 1998; Heino et al., 2001; Trebusak Podkrajsek et al., 2005; Stolarski et al., 2006). The del13 bp is the commonest mutation in British (Pearce et al., 1998), Irish (Dominguez et al., 2006), North American (Wang et al., 1998; Heino et al., 1999), Norwegian (Wolff et al., 2007), and Polish patients (Stolarski et al., 2006). The Y85C mutation is typical of Iranian Jews (Bjorses et al., 2000). In Italy, patients from Sardinia show a distinctive mutation on exon 3 defined R139X (Clemente et al., 1998), while those from Apulia show the mutation W78R on exon 2 and Q358X on exon 9 (Meloni et al., 2002). In Veneto, patients have mutations similar to that found in Finnish population (Betterle et al., 2002). The AIRE gene is involved in the negative selection and induction of self-reactive thymocyte anergy (Pitkänen et al., 2000). This gene has a high concentration in epithelial cells and thymic cells of the monocytic–dendritic line (both are antigen-presenting cells) and a low concentration in the spleen, lymph nodes, pancreas, adrenal cortex, and peripheral blood mononuclear cells (The Finnish-German APECED Consortium, 1997).

2.2.2. Type 2 APS or MAS

Schmidt's syndrome (Carpenter et al., 1964), type 2 APS (Neufeld and Blizzard, 1980), or type 2 MAS as we prefer is characterized by AD associated with AITD and/or type 1 DM. Type 2 MAS is rare, with an incidence of 1.4–4.5 cases per 100,000 (Chen et al., 2001), and affects mainly adult women (Neufeld et al., 1981; Betterle et al., 2004). The characteristics of this MAS in 392 described cases is summarized in Table 6. AITD occurred in 69–88% and type 1 DM was found in 23–52% of cases. Other minor autoimmune diseases can develop, although they are found less frequently compared to type 1 MAS. Of 146 cases in our series, 88.4% had AD with AITD or type 1 DM, and only 11.6% had all three major components. Table 7 reports the combinations of the main endocrine autoimmune diseases found in our patients. The mean age at the onset of AD was 35 years (range 1–85), type 1 DM 28 years (range 2–63), and Graves' disease 31 years (range 7–58). ACA/21-OHAbs were detected in more than 90% of patients with AD, pancreatic autoantibodies in 70–80% of those with type 1 DM, and thyroid antibodies in 80–97% of those with AITD (Betterle et al., 2004). These prevalences were calculated at the clinical onset of the relevant diseases. Symptoms and signs, laboratory findings, imaging and pathology of AD in type 2 MAS are

Table 6
Features of type 2 multiple autoimmune syndrome (MAS)

	Neufeld et al. (1981)	Papadopoulos and Hallengren (1990)	Betterle et al. (2004)	All cases
Patients (numbers)	224	22	146	392
F/M	n.d.	2.7	4	2.7–4
Family history of MAS 2	n.d.	n.d.	0	0
Adult/children	n.d.	n.d.	10/1	10/1
Major diseases	%	%	%	%
Addison's disease	100	100	100	100
Autoimmune thyroid disease	69	73	88	69–88
Type 1 DM	52	41	23	23–52
Minor diseases	%	%	%	%
Vitiligo	5	4.5	12	4.5–12
Atrophic gastritis	n.d.	n.d.	11	11
Hypergonadotropic hypogonadism	4	9	10	4–10
Alopecia	1	n.d.	4	1–4
Chronic hepatitis	n.d.	n.d.	3	3
Cancer	n.d.	nd	2	2
Pernicious anemia	<1	4.5	2	<1–4.5
Seronegative arthritis	n.d.	n.d.	2	2

n.d., not defined.

Table 7
Combinations of the main autoimmune diseases in 146 Italian patients with type 2 MAS

Endocrine combinations	Cases number	Frequency (%)
AD + chronic thyroiditis	82	56.1
AD + Graves' disease	31	21.2
AD + type 1 diabetes mellitus	16	10.9
AD + chronic thyroiditis + type 1 diabetes mellitus	14	9.6
AD + Graves' disease + type 1 diabetes mellitus	3	2.0

indistinguishable from AD of type 1 MAS, with the only difference being the age of the patients (Betterle et al., 2002).

2.2.2.1. Incomplete type 2 MAS: a poorly understood entity. Patients with complete type 2 MAS are quite rare, but there are many other cases of incomplete forms of type 2 MAS that can be identified by an autoantibody screening in patients with one or more major diseases. The different combinations of incomplete type 2 MAS are summarized in Table 8. Determination of TSH is recommended in the presence of thyroid autoantibodies, the intravenous glucose tolerance test in the presence of pancreatic autoantibodies, and the ACTH stimulation test in the presence of ACA/21-OHAbs (Betterle et al., 2004; Coco et al., 2006).

2.2.2.2. Genetics. In patients with type 2 MAS, an increased prevalence of HLA-DR3 and/or DR4 has been reported (Maclaren and Riley, 1986). Several studies have confirmed the association with HLA-DR3, particularly with the DRB1*0301, DQA1*0501, DQB1*0201 haplotype, whereas the association with HLA-DR4 has not been confirmed (Latinne et al., 1987; Böehm et al., 1991; Weetman et al., 1991; Partanen et al., 1994; Badenhoop et al., 1995; Gambelunghe et al., 1999). Huang et al. (1996) showed that the

Table 8
Possible combinations of incomplete type 2 MAS

Clinical diseases		Serology	Function
Addison's disease	+	Thyroid Abs (30%)	Normal or subclinical thyroid dysfunction
Addison's disease	+	ICA/GAD Abs (10%)	Normal or impaired glucose tolerance
AITD	+	ACA/21-OH Abs (1%)	Normal or subclinical adrenal function
Type 1 DM	+	ACA/21-OH Abs (0.6–1.6%)	Normal or subclinical adrenal function
AITD + type 1 DM	+	ACA/21-OH Abs (1–2%)	Normal or subclinical adrenal function
None		ACA/21-OH Abs	Normal or subclinical adrenal function
		+	
		Thyroid Abs	Normal or subclinical thyroid dysfunction
None		ACA/21-OH Abs	Normal or subclinical adrenal function
		+	
		ICA/GAD Abs	Normal or subclinical thyroid dysfunction

Abbreviations: ACA, adrenal cortex antibodies; 21-OH Abs, 21-hydroxylase autoantibodies; AITD, autoimmune thyroid diseases; DM, diabetes mellitus; ICA, islet-cell autoantibodies; GAD Abs, glutamic acid decarboxylase autoantibodies.

subtype HLA-DR3, DQB1*0201 was increased in American patients with type 2 MAS, whereas HLA-DR4, DQB1*0302 was increased in subjects with associated type 1 DM. An increased frequency of DR3-DQ2 and DR4-DQ8 independent of type 1 DM has been described in Norwegian patients (Myhre et al., 2002). We studied class II HLA genes in 54 patients with type 2 MAS and found that DRB1*03, DQB1*02, DRB1*04, DQB1*03, and DRB1*03,04 were significantly associated with type 2 MAS, independent of the coexistence of type 1 DM or type of AITD. Furthermore, we observed an inverse association with DR1B1*01, DR1B1*05, and DR1B1*013, which would imply that these genes are protective (Betterle et al., 2004). In patients with type 2 MAS, other HLA-related genes have been studied, such as the tumor necrosis factor gene belonging to class III (Partanen et al., 1994) and the MIC-A gene belonging to HLA class I (Gambelunghe et al., 1999). However, because of the tight HLA linkage of these genes, it is difficult to determine the direct role of each one.

The antigen-4 gene on cytotoxic T lymphocytes (CTLA-4) on chromosome 2 encodes a co-stimulatory molecule, which is an important negative regulator in the activation of T cells (Waterhouse et al., 1995). Studies in German patients with type 2 MAS suggested that the ala17 allele of CTLA-4 is associated significantly only in a subgroup of patients with the HLA-DQA1*0501 allele (Donner et al., 1997). A study on type 2 MAS patients from different European countries showed that only English patients had an association with CTLA-4 (Kemp et al., 1998). Patients with type 2 MAS were investigated for del13 on the AIRE gene (typical of English with type 1 MAS), but the frequency of this mutation was the same as the normal population (Vaidya et al., 2000).

2.2.3. Type 3 MAS: association between autoimmune thyroid diseases and other autoimmune diseases, excluding Addison's disease

In the original classification proposed by Neufeld and Blizzard (1980), type 3 APS was defined as the association between AITD (Hashimoto's thyroiditis, idiopathic myxoedema, asymptomatic thyroiditis, Graves' disease, endocrine ophthalmopathy, or pretibial myxoedema) and type 1 DM (type 3a), chronic atrophic gastritis, pernicious anemia (type 3b), vitiligo, alopecia, and myasthenia gravis (type 3c). AD was obviously excluded from this group. In the following years, AITD appeared to be associated with many other autoimmune diseases that had not been included in Neufeld's original classification (e.g., AITD and celiac disease, AITD and multiple sclerosis, etc.). Furthermore, in patients with apparently isolated AITD, organ- and non-organ-specific autoantibody screening

Table 9
Revised classification of type 3 MAS according to preferential clinical associations

Autoimmune thyroid diseases			
Hashimoto's thyroiditis–idiopathic myxedema–symptomless autoimmune thyroiditis–Graves' disease–endocrine exophthalmos–pretibial myxedema			
Type 1 DM Hirata's syndrome Premature ovarian failure Adenohypophysitis Neurohypophysitis Chronic hypoparathyroidism	Chronic atrophic gastritis Perncious anemia Celiac disease Inflammatory bowel diseases Primary biliary cirrhosis Autoimmune hepatitis Sclerosing cholangitis	Vitiligo Alopecia Bullous diseases Chronic idiopathic urticaria Myasthenia gravis Dermatomiositis Stiff-person syndrome Multiple sclerosis Autoimmune anemia Autoimmune thrombocytopenia Autoimmune leukopenia	Systemic lupus erythematodes Discoid lupus erythematodes Rheumatoid arthritis Mixed connective tissue disease Seronegative arthritis Systemic sclerosis Sjögren syndrome Vasculitis Anti-phospholipid syndrome
Endocrine glands	Gastrointestinal tract	Skin Muscles Nervous system Blood system	Connective tissues Vascular system
3A	3B	3C	3D

frequently revealed one or more of these autoantibodies, thus revealing "incomplete" type 3 MAS. Thus, type 3 MAS appears to be a syndrome more complex and heterogeneous than that initially reported by Neufeld. In 1999, a first attempt of reviewing clinical, genetic, and immunological aspects of this syndrome was made (Muir and She, 1999), but it was not exhaustive. Therefore, we proposed four subtypes of type 3 MAS on the basis of the organs or the tissues primarily implicated (Table 9). It is important to consider that all the autoimmune diseases listed in Table 9 may be associated with AITD in potential, subclinical, or clinical forms. Moreover, taking into account that AITD (potential, subclinical, or clinical) is the most common autoimmune disease, being present in 7–8% of the general population (10% in women and 3% in males) (Dayan and Daniels, 1996; Weetman, 2000), and that about one-third of these patients can be affected by type 3 MAS (complete or incomplete) one can estimate that type 3 MAS is the most frequent MAS being present in around 2–3% of the general population.

2.2.4. Type 4 MAS: autoimmune diseases associated with other diseases not included in other categories

Type 4 MAS is a syndrome, which includes all the clinical combinations that cannot be included in one of the previously described categories (Neufeld and Blizzard, 1980). For example, AD and chronic gastritis or pernicious anemia, celiac disease and type 1 DM, myasthenia gravis and vitiligo, type 1 DM and hypogonadism, etc., can be encompassed in this MAS.

3. Conclusions

In recent years, the study of MAS has received great interest thanks to the growing number of the diseases recognized as autoimmune and the knowledge of their natural history. Clinically overt disorders are considered only the tip of the autoimmune iceberg, since latent forms are much more frequent (Fig. 1). Autoantibody

determination in patients with a single disease undoubtedly facilitates the recognition of those with the autoimmune form, but in patients with one autoimmune disease the enlarged autoantibody screening undoubtedly identifies patients with potential or subclinical MAS. This has allowed clinicians to identify patients with MAS in a preclinical stage and, consequently, to initiate early treatment. Hopefully, advancement in understanding the inner immunologic mechanisms involved in these conditions should address common treatments to prevent, or at least dampen, progression to irreversible multiple organ damage.

Key points

- Endocrine autoimmune diseases may have privileged associations with other organ- and non-organ-specific autoimmune diseases in the context of MAS.
- Four main types of MAS may be recognized according to the preferential disease association.
- In patients with one clinical autoimmune disease an autoantibody screening may discover those with potential or subclinical MAS.

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CHAPTER 13

Endocrine Manifestations of the Antiphospholipid Syndrome

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1. Introduction

Antiphospholipid syndrome (APS) is characterized by a state of hypercoagulability potentially resulting in thrombosis of all segments of the vascular bed (Hughes, 1983; Harris et al., 1986; Hughes et al., 1986; Khamashta et al., 2004). The spectrum of clinical manifestations in APS is constantly growing to involve almost every organ system in the body. Since endocrinologic complications of APS are unusual, the objective of this chapter is to define and classify these manifestations by reviewing published papers and case reports on this topic.

2. Results

Table 1 summarizes the major endocrinologic manifestations associated with APS.

2.1. Adrenal

Adrenal involvement was the first reported endocrinologic manifestation of APS. The earliest report suggesting a relation between antiphospholipid antibodies (aPL) and primary adrenal insufficiency (Addison's disease) described a young woman with SLE who developed Addison's disease. She had a false-positive test for syphilis and prolonged partial thromboplastin time, suggesting a circulating anticoagulant (Eichner et al., 1973). As APS became better defined in the early 1980s, a strong association between Addison's disease and APS was found (Grottolo et al., 1988; Asherson and Hughes, 1989; Carette and Jobin, 1989; Asherson and Hughes, 1991). Asherson and Hughes (1991) published the first review of 19 patients with primary adrenal insufficiency associated with APS. In a subsequent report, Espinosa et al. (2003) described 86 patients (71% had primary APS (PAPS)), and in 31 (36%) patients, adrenal insufficiency was the first clinical manifestation of APS. Following the publication of these series, further cases were reported in the literature (Arnason and Graziano, 1995; Vlot et al., 2001; Berneis et al., 2003; Takebayashi et al., 2003; Presotto et al., 2005; Ringkananon et al., 2005). Gerner et al. (2005) reported the case of a child with PAPS who developed adrenal failure followed by status epilepticus and hemolytic anemia and identified five additional cases reported in the literature.

Adrenal insufficiency may be the first manifestation of APS. Presotto et al. (2005) have also recently described a case of PAPS who presented with acute adrenal failure, and identified 20 cases of primary adrenal failure as the first-recognized expression of PAPS in the literature. The majority of them were males (75%) with a mean age of 42 years. The symptoms of adrenal insufficiency

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Table 1
Summary of the endocrinologic manifestations associated with the antiphospholipid syndrome

Endocrine organ	Manifestations
Adrenal	Hypoadrenalism presenting with abdominal pain and undue weakness or asthenia
Thyroid	Circulating aPL detected in autoimmune thyroid disease of undetermined clinical significance
Pituitary	Few cases of hypopituitarism reported, including a case of Sheehan's syndrome Non-pathogenic aPL in hyperprolactinemic patients
Diabetes mellitus	An increased frequency of low aCL titers which may relate to macrovascular disease Asymptomatic IgG aCL in first-degree relatives of diabetic patients aPL in diabetic sera, particularly anti-phosphatidylinositol and anti-phosphatidylcholine, probably associated with some macroangiopathic complications
Parathyroid	One report only describing regression of aCL antibodies, normalization of Ca × P product, and healing of the skin lesions after parathyroidectomy in a dialyzed patient with hyperparathyroidism and calcific-uremic arteriolopathy
Ovaries	Asymptomatic ovarian vein thrombosis
Testes	Testicular thrombosis Neurogenic bladder

associated with aPL are classical. In the series reported by Espinosa et al. (2003), abdominal pain was present in 55% of patients, followed by hypotension (54%), fever (40%), nausea or vomiting (31%), weakness or fatigue (31%), and lethargy or altered mental status (19%).

The main morphological findings by computed tomography or magnetic resonance imaging were consistent with bilateral adrenal hemorrhage in around 59% of these patients (Espinosa et al., 2003; Presotto et al., 2005). The pathologic mechanisms involved in the production of the adrenal hemorrhage are still unclear. The anatomic structure of adrenal glands with a rich arterial supply but a limited venous drainage by a single vein (Rao et al., 1989) may predispose patients to thrombosis, following which hemorrhagic infarction of the adrenal glands often occurs (Fox, 1976; Espinosa et al., 2003). Other presumed mechanisms by which adrenal insufficiency may occur in these patients include the development of adrenal hemorrhage following surgery or anticoagulant therapy (Walz et al., 1990; Arnason and Graziano, 1995; Papadopoulos et al., 1995). A recent and novel explanation is based on the accumulation in the adrenals cells of late endosomes, which are important organelles participating in cholesterol trafficking and protein sorting within cells that express epitopes recognized by aPL (Berneis et al., 2003).

Adrenal involvement has also been frequently observed in the course of the catastrophic APS (CAPS), as part of the multiorgan failure characteristic of this condition. In two series by Asherson, comprising 130 CAPS cases (Asherson et al., 1998, 2001), adrenal failure was present in 19 (26%) of the first series of 50 patients (Asherson et al., 1998), and in the second series of 80 patients (Asherson et al., 2001), it was detected in 8 (10%) patients.

PAPS should be suspected in patients with adrenal failure, even in the absence of previous history of thromboembolic disorders. Screening for lupus anticoagulant (LA) and anticardiolipin antibodies (aCL) should be performed in all cases of adrenal hemorrhage or infarction (Oelkers, 1996; Espinosa et al., 2003). Adrenal insufficiency should also be ruled out in patients with PAPS and acute abdominal pain (Marie et al., 1997).

2.2. Thyroid

Autoimmune thyroid disease is associated with circulating autoantibodies that are reactive with

epitopes on thyroid tissue. However, other autoantibodies, including aPL, that are not thyroid specific, have been detected in these patients (Baethge et al., 1988). The clinical significance of these antibodies is uncertain. Some authors have reported that while patients with autoimmune thyroid disease may have circulating aPL they do not manifest any of the clinical abnormalities associated with APS, while others have claimed that the presence of aPL may have clinical significance (Marongiu et al., 1991; Paggi et al., 1994; Hofbauer et al., 1996; Takahashi et al., 2002). Paggi et al. (1994) found aPL in 17 of 31 patients with autoimmune thyroid disease. The antibody titer was highest in patients with Graves' disease. Likewise, Marongiu et al. (1991) found an increased incidence of aPL positivity in Graves' disease patients compared to healthy controls. In these patients, the antibody was of the IgG isotype. In contrast, Petri et al. (1991) were unable to replicate these results in a cohort of patients with Graves' disease and Hashimoto's thyoiditis and concluded that aPL is no more common in autoimmune thyroid disease than in healthy individuals. Nabriski et al. (2000) studied the prevalence of aPL in a retrospective survey of 130 patients with autoimmune thyroid disease (84% had chronic thyroiditis and 16% had Graves' disease). An overall rate of 43% aPL positivity was found among patients with autoimmune thyroid disease. Of the 56 patients that were aPL positive, 48 (86%) had aPL of the IgG isotype, 4 (7%) had IgM antibodies, and 9 (16%) had both IgG and IgM antibodies. None of the patients had clinical evidence of APS. It was concluded that the prevalence of aPL in autoimmune thyroid disease is increased compared to healthy individuals but that this is likely to be an epiphenomenon. Osundeko et al. (2001) studied the presence and significance of aCL in 19 patients with Hashimoto's disease. Of the 19 patients studied, 4 (21%) tested positive for aCL (IgG in 2 and IgM in 2). None of the patients with high levels of IgG or IgM aCL had clinical features of APS. These findings support the notion that nonspecific autoantibody production, secondary to 'overstimulation' of autoreactive B cell clones, may accompany the synthesis of thyroid-specific immunoglobulin in autoimmune disease (Nabriski et al., 2000). Therefore, patients with ATD need not be examined routinely for the presence of aPL. On the other hand, a number of reports have analyzed the possibility that patients with APS may have thyroid disease. Nakamura et al. (1993) reported a patient with hypothyroidism, thrombocytopenia, and APS, and suggested that aCL may be a factor in inducing the thrombocytopenia observed in ATD. Innocencio et al. (2004) in a recent study to determine the prevalence of thyroid function in patients with different autoimmune diseases, reported a high prevalence of silent autoimmune thyroid diseases in association with systemic sclerosis and rheumatoid arthritis, but not with APS.

Primary Sjögren's syndrome (pSS) is an autoimmune exocrine disorder characterized by lymphoid infiltration and functional deterioration of the exocrine glands, especially the lachrymal and salivary glands, and by B cell hyper-responsiveness and an increase of antibodies against nuclear antigens, including SS-A and SS-B. The association between thyroid disease and pSS has been analyzed in different studies, Ramos-Casals et al. (2000) studied 160 patients with pSS to determine the prevalence and clinical significance of thyroid disease and compared them with 75 individuals without pSS, thyroid disease occurred in more than one-third of patients with pSS; the main cause was autoimmune thyroid disease, which was present in 20% of the patients studied. The prevalence of aCL in pSS ranges from 2% to 37%, most frequently IgG or IgA isotypes (Fauchais et al., 2004). Asherson et al. (1992) studied blood samples from 65 patients with pSS, and found predominantly non-pathogenic IgA aPL in 20% of these patients. Also in 1992, Jedryka-Goral et al. (1992) found non-pathogenic aCL in 5 out of 31 patients (16%) with pSS, and in 7 out of 32 patients (22%) with secondary SS. Cervera et al. (1997) in a study to determine the prevalence and clinical significance of aPL, prospectively studied 80 patients with pSS. Only 11 (14%) of these patients were found to have non-pathogenic aPL (aCL or LA, or both) in their sera. More recently, Fauchais et al. (2004) studied a cohort of 74 patients with pSS; aPL

were found in 25 (34%) patients. The presence of aCL was significantly associated with hypergammaglobulinemia. Only two patients with aPL had recurrent venous thrombosis, and one patient with moderate titers of aCL exhibited recurrent spontaneous fetal losses. In conclusion, patients with pSS tend to have low or moderate titers of aCL. These antibodies are non-pathogenic, and frequently associated with hypergammaglobulinemia suggesting that aCL are simply part of the natural repertoire of antibodies in the syndrome characterized with hyperresponsive B cells.

2.3. Pituitary

Only a few cases of hypopituitarism related to APS have been reported. In 1997, Pandolfi et al. (1997) reported the first case of global anterior pituitary insufficiency which developed soon after cerebral ischemic stroke in a 62-year-old female with LA and large atrial thrombosis. In 1998, Andre et al. (1998) described the case of a patient with PAPS who developed hypopituitarism secondary to hypothalamic dysfunction. The first case of Sheehan's syndrome associated with APS was reported by Ikeda et al. (2000), thus suggesting a role for aPL in postpartum hypopituitarism. Hence, hypopituitarism should be considered as a possible cause of hypoadrenalism in APS and LA and aCL should be searched for in the evaluation of patients with hypopituitarism, especially when a history of thrombosis is present.

Hyperprolactinemia has been reported in many rheumatic diseases, most commonly SLE (Walker and Jacobson, 2000), probably related to the presence of antiprolactin antibodies in the sera of SLE patients (Leanos et al., 1998). Only a few studies have investigated the frequency of aCL in patients with hyperprolactinemia. Buskila et al. (1995) measured circulating autoantibodies in the serum of 33 hyperprolactinemic women and in 19 healthy women with normal prolactin levels. Twenty-five of 33 (75.7%) hyperprolactinemic women were found to have at least one autoantibody, while none were found in the 19 women with normal prolactin. The autoantibodies that were more frequently expressed were: anti-single and double-stranded DNA, anti-Sm, and anti-SS-A/Ro antibodies. Toubi et al. (1997) reported the presence of aCL in 5 out of 23 (22%) of patients with hyperprolactinemia. In conclusion, it appears that the relationship between hyperprolactinemia and aPL is non-pathogenic probably related to the non-specific stimulation of the immune system by prolactin.

2.4. Diabetes mellitus

Only a few reports have addressed the relationship between APS and diabetes mellitus (DM). In 1989, we tested the hypothesis that the excess risk of myocardial infarction in diabetic subjects relates to the presence of aCL by measuring the frequency and titer of aCL in two groups of diabetic subjects and in 2500 healthy controls. One non-diabetic subject (0.04%) had low (5–20 units) IgG aCL titers. Seven out of 126 diabetics had no cardiovascular disease (5.6%) and 9 out of 79 diabetics who were either myocardial infarction survivors or who had angiographically proven coronary artery disease (11.4%) had low aCL titers ($p<0.01$ for comparison of either diabetic group with controls, and $p<0.1$ for comparison between diabetic groups). One subject in each diabetic group, but no non-diabetics, had moderate IgM aCL titers. No subjects had high aCL titers. We concluded that diabetics have an increased frequency of low aCL titers which may relate to macrovascular disease (Hendra et al., 1989). d'Annunzio et al. (1998) in a study on first-degree relatives of diabetic patients found positive levels of aCL IgG in 8/42 relatives and none in control subjects ($p=0.04$). Conversely, aCL-IgM values were similar in relatives and controls. However, no first-degree relative showed any feature of APS. Gin et al. (2002) in a comparative study of diabetic serum antibody binding to phospholipids, found aPL in diabetic sera, particularly anti-phosphatidylinositol and anti-phosphatidylcholine. These antibodies also appeared to be associated with macroangiopathic complications.

2.5. Parathyroid

In our review of the literature, we found one report describing the impact of parathyroidectomy on the spontaneous healing of necrotic lesions of the skin of the lower leg and on rapid aCL regression in a 68-year-old female dialyzed patient with hyperparathyroidism and calcific-uremic arteriolopathy. The authors concluded that the regression of aCL, normalization of Ca × P product, and healing of the skin lesions after parathyroidectomy all pointed to the elevated PTH level as a crucial factor in the pathogenesis of calcific-uremic arteriolopathy (Sefer et al., 2001).

2.6. Ovaries

In 2004, Andre et al. (2004) reported asymptomatic ovarian vein thrombosis by computed tomography scan in two female patients with APS, and suggested that in view of the lack of symptoms, this complication may be under-diagnosed.

2.7. Testes

Testicular thrombosis secondary to APS was described in few case reports in the literature. Leder et al. (2001) described the case of a 24-year-old male with acute HIV infection and elevated IgG aCL and who developed necrotic lesions on the lower extremities together with testicular thrombosis necessitating orchiectomy. Gobel et al. (2002) reported another patient with systemic lupus erythematosus and APS with thrombosis of a testicular artery. In a recent review, Fernandez Rosado et al. (2004) reported the case of a patient with neurogenic bladder secondary to PAPS.

3. Summary

We have reviewed the major endocrinologic manifestations associated with the APS. Adrenal insufficiency is the most common endocrinologic manifestation and can be the presenting symptom of APS. In patients with autoimmune thyroid disease circulating aPL have been detected; however, no clinical manifestations of APS have been described. As for the pituitary involvement, a few cases of hypopituitarism have been reported, including a case of Sheehan's syndrome. aPL has been detected in the sera of diabetic patients, probably associated with some macroangiopathic complications. Finally only very few cases of ovarian and testicular involvement have been reported.

Key points

- Adrenal insufficiency is the most common endocrinologic manifestation and can be the presenting symptom of APS.
- Circulating aPL detected in patients with autoimmune thyroid disease are not associated with clinical manifestations of APS.
- aPL in the sera of diabetic patients, may be associated with some macroangiopathic complications.

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CHAPTER 14

Autoantibodies and Infertility in Autoimmune Diseases

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Abstract

The main function of the immune system is to protect against pathogens, and carry out surveillance against cells which have become malignant. However, certain autoantibodies which are found in autoimmune diseases can impair fertility. Additionally, there are autoantibodies which cause infertility, but are unrelated to autoimmune diseases. Impairment of fertility may present as infertility if the patient does not conceive or if there is pregnancy loss or if the pregnancy fails to develop after conception. This review summarizes the possible influences of autoimmune factors on infertility. At present, it seems that three conditions responsible for infertility such as, premature ovarian failure, anti-sperm antibodies, and endometriosis, may be autoimmune in origin. Additionally, the presence of autoimmune antibodies such as, anti-phospholipid antibodies, anti-thyroid antibodies, and anti-nuclear antibodies may be associated with infertility, as well as pregnancy loss. Systemic lupus erythematosus, and diabetes mellitus are two autoimmune diseases which have been associated with infertility.

1. Introduction

There are numerous autoimmune diseases which have been suspected to cause infertility or pregnancy loss such as the anti-phospholipid syndrome (APS) (Hughes, 1983; Sher et al., 2000), juvenile onset diabetes mellitus (Greene et al., 1989; Ballester et al., 2004), and lupus (Li et al., 2002; Geva et al., 2004). Additionally, the involvement of autoimmune mechanisms has been described in infertility whether due to premature ovarian failure (POF) (Forges et al., 2004), polycystic ovary syndrome (Bannatyne et al., 1990; Suh, 1992), or endometriosis (Matarese et al., 2003), or of male origin involving anti-sperm antibodies (ASAs) (Hjort, 1999). However, the exact role of autoimmunity in the pathophysiology of these conditions remains controversial. Certain autoantibodies have been associated with infertility and pregnancy loss. Some antibodies such as anti-phospholipid antibodies (aPL) have even been reported to be pathogenic for pregnancy loss (Blank et al., 1991; Bakimer et al., 1992). Anti-thyroid antibodies have been reported to be significantly associated with infertility (Geva et al., 1997) and pregnancy loss

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(Stagnaro-Green et al., 1990). Recently our team (Shoenfeld et al., 2006) has summarized the prevalence of autoantibodies in infertility, recurrent pregnancy loss (RPL), and autoimmune diseases in a large cohort of 269 patients from 5 different centers. In autoimmune diseases, the prevalence of anti-prothrombin, anti-annexin, anti-phospholipid, and anti-nuclear antibodies was significantly higher than in the control group, OR 11.0 [CI, 3.5–35.2], 33 [CI, 7.2–174.2], 13 [CI, 1.4–309.7], and 16.1 [CI 2.4–122], respectively. In infertility, the antibodies with significantly higher levels than controls were: anti-phospholipid antibodies OR 5.11 [CI, 1.2–25.4], and anti-prothrombin antibodies, OR 5.15 [CI, 2.1–12.7]. In RPL, ASAs, anti-prothrombin, and aPL were more prevalent than in controls, OR 3.9 [CI, 1.5–10.6], 5.4 [CI, 2.4–12.5], and 4.8 [CI, 1.2–22.2] for each antibody respectively. Anti-prothrombin antibodies and aPL were more significantly associated with late pregnancy losses than early losses.

Similarly, medications used for autoimmune diseases, such as steroids, heparin, and low molecular weight heparins and intravenous immunoglobulin (IVIg), have been used on an empirical basis for enhancing fertility, in long standing infertility in general, repeated implantation failure in in vitro fertilization (IVF) in particular, and for the prevention of pregnancy loss. However, other drugs used for the long-term treatment of autoimmune diseases, such as cyclophosphamide, may impair fertility. This review discusses the effect of some of these interrelationships.

2. Autoimmune conditions associated with infertility

2.1. Systemic lupus erythematosus (SLE)

SLE does not usually affect fertility (Khamashta and Hughes, 1996). A pregnancy rate of 2.0–2.4 pregnancies per patient has been described, both during remission and during active disease (Kaufman and Kitridou, 1982; Nossent and Swaak, 1990). However, the fertility rate may be lowered in some patients, by associated clinical features such as menstrual irregularities and anovulatory cycles during active disease and high dose steroid administration (Buyon and Wallace, 1997). End stage renal failure may follow lupus nephritis, and may result in amenorrhea. One hundred sixteen autoantibodies have been described in SLE patients (Sherer et al., 2004), including autoantibodies that target nuclear antigens, cytoplasmic antigens, cell membrane antigens, phospholipid-associated antigens, blood cells, endothelial cells, and nervous system antigens, plasma proteins, matrix proteins, and miscellaneous antigens. Hence, it is inconceivable that there are no antibodies which affect fertility. Thirty-four per cent of women with SLE are seropositive aPL (Bizzaro et al., 2005), which may explain the association between SLE and pregnancy loss.

In addition, the drugs used in the treatment of lupus may impair fertility. High dose steroids may cause menstrual irregularities and ovarian failure follows cyclophosphamide treatment (Langevitz et al., 1992).

In the presence of SLE, the risk of pre-eclampsia is higher than that seen in patients without SLE (Kitridou, 1997), and the concomitant presence of aPL increases the likelihood of early onset pre-eclampsia (Dekker et al., 1995). Lupus flares are relatively common in pregnancy, necessitating careful follow up. In pregnancy lupus flares are usually mild and may consist of arthritis and cutaneous manifestations (Nossent and Swaak, 1990; Khamashta and Hughes, 1996; Buyon and Wallace, 1997), fever, fatigue, serositis, and thrombocytopenia. Major flares may involve the kidneys and the central nervous system, in 46 and 5%, of patients respectively (Khamashta and Hughes, 1996).

2.2. Diabetes

Type 1 diabetes is the result of an autoimmune process on the islet cells of the pancreas. The resulting hyper- or hypoglycemia has not been found to affect fertility per se (Cohen et al., 1995). However, fluctuations in glucose levels can induce large variations in insulin levels, and lead to ketosis. Both insulin and ketones have adverse

effects in animal models and may affect the human placenta. Insulin decreases human chorionic gonadotrophin (hCG) and progesterone secretion from placental explants (Barnea et al., 1993). Hence, it is logical to assume that diabetes may be associated with pregnancy loss. However, well-controlled diabetes mellitus does not seem to be associated with RPL (Mills et al., 1988; Clifford et al., 1994). The increased incidence of abortion and congenital malformations seems to be increased in poorly controlled or uncontrolled diabetes when blood sugar levels exceed a certain threshold (Rosenn et al., 1994), or when glycosylated hemoglobin (HbA1C) levels are raised (Greene et al., 1989).

In men erectile and ejaculatory difficulties have been reported due to vascular and neuropathic problems (Glenn et al., 2003). Although these have not been shown to affect fertility directly, they may result in reduced frequency of ejaculation with subsequent deterioration in sperm quality. There is also evidence that spermatogenesis is affected by diabetes and that patients have a reduced sperm motility and semen volume. Assisted reproductive techniques and intracytoplasmic sperm injection (ICSI) have improved the outlook for these patients.

The effect of diabetes on IVF has been studied on five insulin-dependent diabetic patients (Dicker et al., 1992). These women achieved strict preconception glycemic control. All patients had a high estradiol response, in nine IVF cycles. An adequate number of pre-ovulatory oocytes were retrieved and normal fertilization and cleavage rates ensued; one patient conceived. Hence, well-controlled insulin-dependent diabetics seem to have conventional responses to gonadotrophin stimulation and the other features of IVF.

2.3. Premature ovarian failure

POF (ovarian failure before age 40) is often associated with autoimmune adrenal disease, or autoimmune processes involving other glands. The evidence for POF having an autoimmune basis is due to the autoantibodies to steroid-producing cells in over 80% of patients (Sotsiou et al., 1980; Betterle et al., 1993), and a lymphatic and plasma cell oophoritis (Hoek et al., 1997). In this condition, the ovarian follicles are infiltrated by lymphocytes, plasma cells, and macrophages. The inflammation mainly affects pre-ovulatory follicles and corpora lutea, rather than the primordial and primary follicles (Coulam et al., 1981; Wolfe and Stirling, 1988; Bannatyne et al., 1990; Suh, 1992). There is also T-cell infiltration (CD4+, CD8+), suggesting an autoimmune process (Sedmak et al., 1987). When POF is not associated with adrenal autoimmunity, 60% of patients show complete loss of ovarian follicles (Forges et al., 2004), suggesting a different mechanism. The vast majority of patients with POF and no adrenal autoimmunity show inflammatory oophoritis.

POF may be associated with other autoimmune conditions, such as myasthenia gravis, diabetes mellitus, alopecia areata, Crohn's disease, ulcerative colitis, celiac disease, glomerulonephritis, rheumatoid arthritis, primary biliary cirrhosis, multiple sclerosis, Hashimoto's thyroiditis, autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, pernicious anemia and vitiligo (Cohen et al., 1995; Forges et al., 2004), or may be isolated. However, in these associations, the prevalence of steroid cell antibodies is less than 10% (Betterle et al., 1993; Falorni et al., 2002; Dal Pra et al., 2003). Antibodies have been described against developing follicles—including the membrane, granulosa cells, theca interna, and lutein cells and various components of the oocyte (Damewood et al., 1986), including the ooplasm and zona pellucida, ovarian proteins (Coulam and Ryan, 1985), and against gonadotrophins or their receptors (Chiauzzi et al., 1982), phospholipids, histones, and polynucleotides.

Horejsi et al. (2000) have assessed anti-ovarian antibodies in 90 women according to the outcome of IVF. 3.7% of the women who conceived had anti-ooplasm antibodies but none had anti-zonal antibodies. In implantation failure, anti-ooplasm antibodies and anti-zonal antibodies were detected in 25 and 2.5% of women respectively. In fertilization failure, anti-ooplasm, anti-zonal antibodies, and anti-granulosa antibodies were found in 40, 20, and 66.7%, respectively. Horejsi et al. (2000) concluded that the greatest prevalence of anti-ovarian antibodies occurred in fertilization failure

followed by implantation failure, and hence that anti-ovarian antibodies may play a significant role in infertility. Mardesic et al. (2000) have shown that the effect of anti-zonal antibodies can be overcome when ICSI is performed.

The role of ovarian biopsy is limited to assessing the presence of oocytes. If oocytes are present, they could be considered for ICSI. If no oocytes are present, ovum donation seems the sole alternative, if the patient desires children. In POF the lack of ripening follicles leads to high FSH levels. Estrogen replacement has been used in order to down regulate FSH secretion, and expedite the subsequent return of fertility. However, these reports need to be interpreted in the light of the fact that in 5–10% of patients with POF, spontaneous conception may occur (van Kasteren and Schoemaker, 1999), and that not all studies of hormone replacement have shown resumption of follicular activity in POF.

In autoimmune POF, immunomodulation should theoretically have an effect. Pregnancy has been reported in POF after corticosteroids have led to normalization of serum gonadotropins, an increase in serum estradiol, and ultrasonographic visualization of follicular growth (Cowchock et al., 1988; Corenblum et al., 1993). However, in van Kasteren et al's. (1999) placebo-controlled, randomized, double-blind, multicenter study of 36 patients, steroids did not influence ovarian responsiveness to gonadotropins in patients with idiopathic POF.

2.4. Endometriosis

Endometriosis is a condition in which endometrial tissue is found in ectopic locations within the pelvic cavity. The organs which are mostly affected are the ovaries, pouch of Douglas, and uterosacral ligaments. However, the lesions can spread to adjacent organs and affect the bladder, ureter, and rectum. The lesions can cause numerous adhesions and subsequent severe pain and infertility.

The immune system is clearly involved in endometriosis, however, it is uncertain whether the condition is caused by immune dysfunction, or whether the immune changes occur subsequently to ectopic endometrial growth. Endometriotic lesions are associated with an intense inflammatory reaction, infiltration by immune cells, increased production of pro-inflammatory cytokines and angiogenic factors, mobilization of fibroblasts, and proliferation of connective tissue. There are two theories as to the immune mechanisms: deficiency in cell-mediated immunity may prevent the clearance of endometrial cells from the peritoneal cavity, leading to their implantation (Dmowski et al., 1981). Alternatively, endometriosis may be an autoimmune disease in origin (Gleicher et al., 1987).

There is much evidence for an autoimmune disease. There is an increase in B-cell reactivity (Startseva, 1980) in endometriosis. Complement 3 and IgG have been found in the endometrium of women with endometriosis (Weed and Arguembourg, 1980), which together with a reduction in serum complement levels suggest an antigen–antibody reaction. IgG and IgA autoantibodies against endometrial and ovarian tissues have been found in the sera and vaginal secretions of women with endometriosis (Mathur et al., 1982). Autoantibodies to endometrial antigens with molecular weights of 26 and 34 kDa have only been identified in endometriosis (Mathur, 2000). Other investigators have demonstrated circulating autoantibodies such as anti-nuclear antibodies, anti-DNA antibodies, aPL, or anti-laminin antibodies, as seen in women with autoimmune diseases. In addition to autoantibodies against specific antigens such as phosphatidylserine, histones, and nucleotides (Gleicher et al., 1987), women with endometriosis have a higher prevalence of anti-endometrial, anti-endothelial, anti-ovarian, and anti-thyroid autoantibodies (Dmowski et al., 1995). IgG anti-laminin-1 Abs have been reported to have a significant association with endometriosis in infertile patients. These antibodies recognize a particular domain (i.e., the laminin-alpha1 chain G domain). mRNA encoding laminin-alpha1, -beta1, and -gamma1 chains is expressed in 90% of endometriotic lesions (Inagaki et al., 2003). Laminins critically contribute to cell differentiation, shape, movement, maintenance of tissue phenotypes, and promotion of tissue survival, and hence are essential during the development of early pre-implantation embryos, during implantation, and organogenesis in post-implantation embryos. The presence of

these antibodies may well present clinically as infertility or pregnancy loss. It remains to be determined whether these autoantibodies represent a loss of self-tolerance or an acquired sensitization to antigenic determinants not normally expressed. Loss of a cell's tolerance could be secondary to the loss of suppressor T cells or exposure to hyperstimulatory B cell activators. New antigenic determinants might represent "altered-self" or foreign antigens that are similar to endometrial antigens in an example of molecular mimicry, as found in other autoimmune diseases. Additionally, it has been shown that decreased apoptosis leads to the development of autoimmunity. Apoptosis is decreased in the uterine endometrium in women with endometriosis and further decreased in the endometriotic deposits (Gebel et al., 1998).

Endometriosis has been reported to affect most stages of reproduction, folliculogenesis (Tummon et al., 1988), ovulatory dysfunction (Dmowski et al., 1986), steroidogenesis by granulosa cells (Harlow et al., 1996), and fertilization (Mahadevan et al., 1983; Wardle et al., 1985). Endometriosis is toxic to the early embryo (Damewood et al., 1990), adversely affects implantation (Simon et al., 1994), and the results of IVF (Barnhart et al., 2002).

There is no hard evidence for an association between endometriosis and RPL (Vercammen and D'Hooghe, 2000).

3. Autoantibodies associated with infertility

3.1. Anti-sperm antibodies

ASAs are found in approximately 10% of infertile couples (Ayvaliotis et al., 1985; Collins et al., 1993), however, the antigens to which sperm antibodies are directed are still undefined. The antigens may be derived from secretions of the epididymis which are present in the seminal plasma, antigens of the sperm's plasma membrane which appear in sperm maturation (Cooper and Bronson, 1990), oligosaccharides on the sperm surface, or to laminins which are found in the testicular basement membrane. ASAs have been reported to affect all aspects of sperm action—including penetration into the cervical mucus (Haas et al., 1983), immobilization in the cervical mucus (Menge and Beitner, 1989), capacitation (Benoff et al., 1993), the acrosome reaction (Myogo et al., 2001), migration through the tube and motility.

ASAs affect fertilization (Chiu and Chamley, 2004), including the binding of sperm to the zona pellucida (Bronson et al., 1982; Mahony et al., 1991), penetration of the zona pellucida, zona reaction, and gamete fusion (Kutteh, 1999a). ASAs have also been reported to be associated with later effects on the embryo as fertilized ova may express sperm antigens on the zygote membrane (Gaunt, 1983). There may be both a lower cleavage rate (Tian et al., 1999), and abnormal cleavage (Naz, 1992), and a higher incidence of miscarriage has been reported.

However, the tests used to detect ASAs cannot discriminate between antibodies impairing fertility and epiphenomena. The mixed agglutination assay, immunobead test, gelatin agglutination test, and tray agglutination test only detect the gross binding of antibodies to sperm and do not examine specific antigens. Treatment regimens have included decreasing the concentration of ASAs by steroids (Keane et al., 1995). In Keane et al.'s (1995) study, four pregnancies ensued from treatment of the 10 patients. Other approaches have included removing the ASAs that are already bound to the sperm by washing or IgA protease treatment, and ICSI as part of assisted reproductive technology. However, the literature is divided on the efficacy of these treatment modalities.

3.2. Anti-thyroid antibodies

The two main types of thyroid autoantibodies (ATAs) which have been related to reproductive failure are anti-thyroglobulin and anti-thyroid peroxidase antibodies. The function of thyroglobulin is to store and synthesize thyroid hormones. Both molecules can be autoantigens in autoimmune thyroid disease. Although thyroid dysfunction could explain the association of antibodies with infertility and miscarriages, miscarriages are often encountered in the presence of ATAs and normal thyroid function (Dendrinos et al., 2000; Matalon et al., 2001). Hence, the higher rate of

miscarriages observed in women with anti-thyroid antibodies may represent an autoimmune phenomenon, rather than or in addition to thyroid dysfunction, or inability to meet the increased demand for thyroid hormones in early pregnancy.

Kutteh et al. (1999b) evaluated the prevalence of ATAs in women with RPL and in women with infertility, undergoing assisted reproduction. ATAs were more prevalent in women with RPL (22.5%), but not in women undergoing assisted reproduction treatment when compared to controls. The author's multicenter study of autoantibodies in reproductive failure (Shoenfeld et al., 2006) did not find a higher prevalence of ATAs in patients with infertility. However, certain subsets of women undergoing IVF with high ATAs may have a worse prognosis. In RPL, the association between recurrent miscarriages and thyroid antibodies may be a result of: (i) a direct effect of ATAs on fetal tissue; or (ii) the thyroid antibodies representing an underlying more generalized defect in autoimmunity. The prognostic value of thyroid autoantibodies remains uncertain.

3.3. Anti-phospholipid antibodies

aPL have been shown to cause pregnancy loss directly. Injection of serum from mice with a high titer of aPL to naive mice induces resorbtion of pregnancies in the recipient (Blank et al., 1991), and active immunization with human pathogenic monoclonal anti-cardiolipin antibody (aCL) induced the clinical manifestations of APS in BALB/c mice (Bakimer et al., 1992). It has been shown that the serum from women with APS is highly teratogenic to rat embryos in culture and also affects embryonic growth (Ornoy et al., 1998). Moreover, purification of the IgG faction of the sera of women with APS directly affected the embryo and yolk sac, reducing their growth (Ornoy et al., 2003). It has long been known that aPL require a co-factor (apolipoprotein H or β2GP1). Today this co-factor is thought to be the antigen to which aPL bind. Binding of aPL to the β2GP1 forms divalent IgG–β2GP1 complexes that have increased affinity for membrane phospholipid (Rand, 2003).

The binding of aPL–β2GP1 to cell membranes including trophoblast results in injury and/or activation. Activation may be cytokine mediated. Interleukin-3, a cytokine which is involved in implantation is decreased in APS (Shoenfeld et al., 1998). The balance of Th-1/Th-2 cytokines may be altered in APS (Krause et al., 1999). TNF-α levels have been found to be significantly higher in patients with APS than healthy controls (Bertolaccini et al., 2001).Cellular activation is thought to increase the expression of cell adhesion molecules (Meroni et al., 2000), which may promote leukocyte adhesion to the endothelial surface. Adhesion is mediated by intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1, and P-selectin (Pierangeli et al., 2001). These molecular actions are responsible for the pathological effects of aPL, thrombosis, and alteration of the thromboxane prostacycline balance.

More recently, it has become clear that aPL may also affect the adhesion molecules between the elements of syncytiotrophoblast. Cytotrophoblast cells express phospholipid on their surface, and aPL may damage the trophoblast unrelated to thrombosis. This concept is supported by histological evidence from patients with aPL and fetal death. Women with aPL have been found to have decreased vasculosyncitial membranes, increased synctial knots, and premature aging of the villi with necrosis upon exposure to anti-β_2GP1 (Piona et al., 1995; Di Simone et al., 2000). The fibrosis, hypovascular villi, and infarcts occur with a significantly higher frequency than in women without APS (Out et al., 1991). It is easy to see how thrombosis in decidual vessels, vasoconstriction of decidual arteries due to excessive thromboxane, and the trophoblastic effects of aPL can cause pregnancy loss. Indeed the incidence of pregnancy loss has been reported to be as high as 90% in APS (Rai et al., 1995), although Empson et al. (2002) have disputed this high figure in a systematic review of the literature.

aPL seems to be associated with missed abortions in which a fetal heart was previously detected. Lockshin (1992) has described a typical form of pregnancy loss in which pregnancies start normally, and a fetal heart is detected early in the first trimester. Similarly, Carp et al. (1997) have

assessed the prevalence of second trimester miscarriages in APS and reported an increased number of second trimester miscarriages compared to women with unexplained RPLs. Oshiro et al. (1996) have also reported that women with APS tend to lose their pregnancies a mean of 4 weeks later than controls. More recently, a study of 366 women with two or more pregnancy losses (Loizou et al., 1988) showed that in women with aCL at medium to high titers and/or LA, 50% of losses were fetal deaths, in contrast to 10% in women who were aPL-negative. In first trimester pregnancy losses, particularly those which present as blighted ova, the role of aPL is less clear, particularly as 30% of first trimester miscarriages are due to major chromosomal rearrangements (Takakuwa et al., 1997; Ogasawara et al., 2000). The role of aPL is even more controversial in infertility or failed IVF. aPLs have been reported to affect implantation, placentation, and early embryonic development Shurtz-Swirsky et al., 1993; Di Simone et al., 2000). In an experimental model, Bakimer et al. (1992) immunized BALB/c mice with human monoclonal antibody. A lower fecundity rate was observed in the immunized females (21% vs. 48%) ($P<0.005$). Although, there was no decrease in the number of vaginal plugs (indicating mating), there was a high percentage of resorbed pregnancies in the immunized animals (25 ± 13 vs. 3 ± 5 in non-immunized animals). Sher et al. (2000) reported a direct relationship between anti-phosphatidylethanolamine and anti-phosphatidylserine antibodies, and increased natural killer (NK) cell activity among non-male-factor infertile women undergoing IVF. Eighty-eight per cent of patients who were positive for phosphatidylethanolamine and phosphatidylserine had increased NK cell activity, compared with 12–25% in controls. Hence, anti-phosphatidylethanolamine and anti-phosphatidylserine antibodies may be more relevant markers of infertility than aCL and lupus anti-coagulant which are associated with pregnancy loss.

Numerous authors have tried to study the prevalence of aPL in women with various forms of reproductive failure. Although the literature is divided on this issue, there does not seem to be an increased prevalence, indicating that aPLs have little influence on fertilization. There is also controversy in the literature regarding the role of aPL on subsequent conception in women with failed IVF. The American Society for Reproductive Medicine (1999) has carried out a systematic review on the issue of aPL and IVF, and published a subsequent practice committee bulletin. The clinical pregnancy and live birth rates were 57 and 46% respectively, in the aPL-positive patients, compared with 49.2 and 42.9%, respectively, in the aPL-negative patients. The bulletin concluded that aPL testing is not warranted in patients undergoing IVF, and treatment is not indicated in seropositive patients.

4. Drugs used for autoimmunity and their effects on fertility

Many of the drugs used for autoimmunity have also been used empirically as fertility enhancing agents, or may have effects on fertility directly, overshadowing the autoimmune mechanisms. Some examples are given below.

4.1. Steroids

Steroids have often been used as an adjunct to ovulation-inducing agents, mainly in order to suppress adrenal secretion of androgens. Steroids activate the cytosolic glucocorticoid receptor that leads to activation or repression of protein synthesis, including cytokines, inflammatory enzymes, and adhesion molecules. Corticosteroids only reach the fetus in low amounts (Dalle and Delost, 1979), due to the expression of 11β-hydroxysteroid dehydrogenase in the placenta (Benediktsson et al., 1997), which inactivates cortisol.

There is some evidence from case control studies, but not from prospective cohort studies that high doses of steroids if taken in pregnancy, may increase the rate of cleft palate (Gur et al., 2004), but no association has been found between the long-term use of glucocorticosteroids and infertility (Jansen and Genta, 2005), or of later effects such as reduced fetal growth (Gur et al., 2004; Jansen and Genta, 2005).

4.2. Non-steroidal anti-inflammatory drugs

Aspirin is in wide use in the APS and is even officially recommended for use in pregnancy by the Royal College of Obstetricians and Gynaecologists (2003) and the American College of Obstetricians and Gynecologists (2005). However, there are now three papers which have compared the use of aspirin to placebo (Cowchock and Reece, 1997; Tulppala et al., 1997; Pattison et al., 2000). None of the three papers showed aspirin to have any effect on the subsequent live birth rate. The three papers have been combined in a meta-analysis (Empson et al., 2002), which also showed aspirin to have no effect on the live birth rate. Aspirin has also been assessed in unexplained pregnancy loss. Although there are no randomized trials, two observational studies (Rai et al., 2000; Daya, 2003) showed no reason for using aspirin.

NSAIDS including aspirin have been assessed as fertility inducing agents. The literature is divided on the issue with reports that aspirin improves uterine blood flow (Kuo et al., 1997), and improves clinical pregnancy rates in an oocyte donation program (Hsieh et al., 2000). However, not all authors have reported a beneficial effect on implantation (Urman et al., 2000), or following frozen embryo transfer (Check et al., 1998). A recent meta-analysis by Daya (2006) stated that, "Given the lack of efficacy and the potential for harmful effects to both the patient and her offspring, low-dose aspirin should not be administered to infertile women undergoing treatment with assisted reproduction." The side effects include two case reports of anovulatory infertility due to luteinized unruptured follicles (Akil et al., 1996; Smith et al., 1996). NSAIDS were administered for ankylosing spondylitis, rheumatoid arthritis, and inflammatory polyarthritis. Normal ovulation followed drug withdrawal.

Both aspirin and naproxen significantly reduce prostaglandin production both in vivo and in vitro in hCG-treated rabbits. Carp et al. (1988) found diclofenac to decrease the implantation rate from 72% in controls to 40% when rat blastocysts were transferred to host mothers. Indomethacin, nimesulide, and celecoxib, have been assessed regarding pre-implantation loss, post-implantation loss, and duration of gestation in Wistar rats. Higher doses of the three drugs significantly increased pre-implantation loss, all doses of the three drugs significantly increased post-implantation loss. There was no significant difference among the three drugs (Shafiq et al., 2004).

4.3. Chemotherapeutic and immunosuppressive drugs

4.3.1. Cyclophosphamide

Cyclophosphamide is used for the treatment of several auotimmune diseases especially for the severe manifestations of lupus such as lupus nephritis. Cyclophosphamide is used for its immunosuppressive effect which includes interfering with DNA synthesis and induction of apoptosis. Cyclophosphamide crosses the placenta (Matalon et al., 2004), and is teratogenic in most animals species.

Cyclophosphamide can induce irreversible amenorrhea and male infertility (Fine, 2005) The occurrence of this complication depends on the dose, duration of treatment, and the patient's age. Low doses of cyclophosphamide as used for immunosuppression, rarely induces infertility in young women, and if infertility occurs, it is often reversible (Langevitz et al., 1992). In males, cyclophosphamide induces oligospermia and even azoospermia in 50–90% of treated patients (Raptopoulou et al., 2004).

4.3.2. Azathioprine (AZP) and 6-mercaptopurine (6MP)

AZP is an immunosuppressive agent which is metabolized to its active compound—6MP (Matalon et al., 2004), which inhibits de novo purine synthesis. AZP is widely used in organ transplantation, inflammatory bowel disease, and autoimmune diseases. It is weakly teratogenic in man. Fertility is not affected, as most women on this drug remain fertile, and have no increase in spontaneous abortions (Jansen and Genta, 2005). There seem to be no prospective studies reporting AZP-induced infertility in men.

4.3.3. Methotrexate (amethopterine)

Methotrexate, being a potent folic acid antagonist, is teratogenic in large doses. If used in a large

bolus dose for the medical treatment of tubal pregnancy, subsequent fertility is not affected (Gervaise et al., 2004). Infertility has been reported in both men and women following chemotherapy with relatively large doses, and after use in combination with other chemotherapeutic agents (French et al., 2003). For immunosuppression, low doses are given which are apparently not teratogenic and do not interfere with fertility (Jansen and Genta, 2005).

4.3.4. Hydroxychloroquine

This anti-malarial agent is used in rheumatoid arthritis and SLE. There is insufficient literature regarding its safety in pregnancy. There are no reports of any injurious effects on male or female fertility (Jansen and Genta, 2005).

4.3.5. Mycophenolate mofetil

This immunosuppressive drug has recently been used for the treatment of lupus nephritis with relatively good results (Jansen and Genta, 2005) and also as maintenance therapy. There seem to be no reports on possible interference with fertility, despite the teratogenic effect in animals (Frieling and Luger, 2002).

4.4. Intravenous immunoglobulin

IVIg is used for numerous autoimmune diseases, due to its many immunomodulatory effects. These have been summarized by Carp et al. (2005), but include: interference with antigen presentation (Thornton et al., 1994), modulation of B (Coggeshall, 1998; Terness and Opelz, 1998), and T lymphocyte function (Simon and Spath, 2003), inhibiting the action of pathological antibodies by either the interaction of the Fc portion of immunoglobulin with Fc receptors, or the Fab receptors, or by passively acting as anti-idiotypic antibodies (Brand et al., 1988; Shoenfeld et al., 2002). Additionally IVIg modulates cytokine effects (Sherer et al., 2001; Graphou et al., 2003) and depresses the killing activity of NK cells (Ruiz et al., 1996; Szereday et al., 1999).

In RPL, there is much evidence that immunological mechanisms both alloimmune and autoimmune may be causitive, and IVIg has been used in anti-phospholipid-related pregnancy loss, and in unexplained pregnancy loss. In APS, IVIg inhibits the action (Caccavo et al., 1994) and production of aPL (Sherer et al., 2000). IVIg reduces the number of fetal resorptions in mice, in which APS had been induced by immunization with aPL (Bakimer et al., 1993). Caccavo et al. (1994) have reported the inhibition of binding of aCL to cardiolipin by the F(ab′)2 fragment from IVIg in a dose-dependent manner. Galli et al. (1991) have demonstrated the inhibition of lupus anti-coagulant activity from the F(ab′)2 fragment of IVIg. Additionally, IVIg lowers the levels of aCL after each infusion (Kwak et al., 1995). However, IVIg seems to have no advantage over heparins in respect to previous live births (Branch et al., 2000; Vaquero et al., 2001). However, the incidence of late complications of pregnancy such as intrauterine growth restriction, pre-eclampsia, and prematurity seem to be reduced with IVIg (Carp et al., 2001a).

In unexplained pregnancy loss, two meta-analyses have not shown IVIg to confer benefit (Daya et al., 1998; Porter et al., 2006). However, neither of these meta-analyses have corrected the results for fetal chromosomal aberrations. IVIg could not to be expected to benefit patients losing chromosomally abnormal embryos, or if administration commenced after fetal demise had occurred, which is the case in some of the randomized trials. However, when patients are selected for a poor prognosis, either by immune treating (Coulam et al., 1995) or a greater number of miscarriages (Carp et al., 2001b) the benefit reaches statistical significance.

Many workers have considered that in repeated implantation failure similar mechanisms may operate as in RPL. Hence IVIg has been used in an attempt to increase the pregnancy rate in IVF with implantation failure. Coulam et al. (1995) have reported a 50% pregnancy rate after intravenous immunoglobulin. This is an excellent result, but could not be confirmed by Balasch et al. (1996), who have not found this therapy to be beneficial. However, the patient selection criteria were very different for both these trials making it difficult to

compare the results. As in RPL, in vitro karyotyping (pre-gestational diagnosis, PGD) of the embryos of couples with implantation failure has shown that up to 66% may be chromosomally abnormal (Rubio et al., 2005). As in RPL, IVIg cannot be expected to enhance the implantation of chromosomally abnormal embryos. IVIg has been used on a more selective population with implantation failure. Sher et al. (1998c) administered IVIg with heparin and aspirin to 89 women with at least 4 cycles of implantation failure. Fifty-two women were positive for aPL. They had a 42% live birth rate. Thirty-seven were aPL negative. They had a 19% live birth rate. The authors concluded that IVF outcome is significantly improved in aPL-positive patients when treated with heparin/aspirin and IVIg, but this regimen did not improve the pregnancy rate in aPL-negative patients. In a subsequent paper, Sher et al. (1998b), the same team reported that heparin/aspirin improved the IVF birth rate in cases of aPL antibodies, but that this regimen was insufficient if the aPL was either IgG of IgM directed against phosphatidylethanolamine or phosphatidylserine. In these cases IVIg was also required. In the case of ATAs (anti-thyroglobulin or anti-microsomial antibodies, IVIg also improved the IVF pregnancy rate from 27 to 51% (Sher et al., 1998a). Immunoglobulins have not been reported to cause infertility or any obvious damage to the developing embryo.

The place of IVIg in infertility associated with the conditions described above remains obscure. There are virtually no studies on IVIg in POF, ASAs, etc. It appears that there may be a place for IVIg in patients with long standing persistent autoimmune infertility which is refractory to other forms of treatment.

5. Conclusions

Autoimmune diseases mainly affect females, and usually during the child bearing years. However, the presence of autoimmune diseases as such do not usually affect fertility except in certain conditions, e.g., in lupus, the associated anti-phospholipid antibodies may cause pregnancy loss, and even possibly infertility. Moreover, there may be other autoantibodies at subclinical levels, whose only expression may be reproductive failure. Anti-thyroid, anti-ovarian, anti-zona pellucida and anti-corpus luteum, and ASAs are such examples. Various medications which are in use for autoimmune conditions have been used on an empirical basis for enhancing fertility; however, the results are controversial. The medications described above, may act on autoimmune mechanisms, rather than on all patients with infertility. Hence a trial of any of the medications described above should analyze for subgroups of patients including the subgroup with autoimmune disease or subclinical autoimmunity.

Key points

- Three causes of infertility, premature ovarian failure, anti-sperm antibodies, and endometriosis, may be autoimmune in origin.
- Certain autoantibodies such as, anti-phospholipid antibodies, anti-thyroid antibodies, and anti-nuclear antibodies may be associated with infertility, in addition to pregnancy loss. In the case of lupus, it is associated features, rather than the disease per se which is associated with infertility.
- Systemic lupus erythematosus, and diabetes mellitus are two autoimmune diseases, which have been associated with infertility.
- Many of the drugs used for autoimmunity have been used as fertility enhancing agents, or may have effects on fertility directly (e.g., steroids, intravenous immunoglobulin, etc.).

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CHAPTER 15

Systemic Lupus Erythematosus and Endocrine Disorders

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1. Introduction

In patients with systemic lupus erythematosus (SLE), disease activity is clearly influenced by hormonal variations that are linked to gender, aging, and changes in reproductive status. Substantial progress has been made in clarifying the signaling molecules and receptors involved in these processes and understanding the immunomodulatory effects of numerous endocrine mediators, such as estrogen or cortisol. Many other hormones regulate immune responses, including thyroid hormone, prolactin, androgens, corticotrophin-releasing hormone (CRH), and insulin-like growth factor I. Evidence shows that the immune system and its products modulate neuroendocrine functions. A disturbance in this delicate balance between soluble mediators released by activated cells of the immune system and products of the neuroendocrine system may be involved in the pathogenesis of the autoimmune diseases. In this chapter we will review the links between SLE and endocrine diseases, focusing on epidemiology, pathophysiology, and clinical implications. The overlap of SLE with thyroid disease has been frequently described, but there is relatively less information available regarding the association of SLE with other endocrine disorders. Furthermore, some endocrine conditions have never, or virtually never, been described in the context of SLE. We have not considered the polyglandular autoimmune syndromes here as they have been covered in another chapter, in this book.

2. Thyroid disease

Since Roitt et al. first noticed in 1956 that of 27 patients they were studying with Hashimoto's disease, 3 had rheumatoid arthritis, considerable interest has focused on the association of thyroid disease and other autoimmune diseases (Roitt et al., 1956). It is now well recognized that thyroid disease is associated most commonly with Sjögren's syndrome, but also with rheumatoid arthritis, SLE, and systemic sclerosis, among other autoimmune conditions. The first reported cases of coexistence of thyroid disorders with SLE were published in 1961 (White et al., 1961), followed by large-scale controlled studies in the next decades. The results of these investigations showed an increased incidence of autoimmune thyroid disease in lupus patients, particularly in Hashimoto's thyroiditis (HT). Boey et al. (1993) studied a cohort of 129 patients with SLE and found an overlap with Hashimoto's disease in 3.9% of them. In a major study, Biró et al. (2006) recently reported the prevalence of the two major forms of autoimmune thyroid disease (HT and Graves' disease (GD)) in 1517 patients with different

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autoimmune diseases (SLE, rheumatoid arthritis, systemic sclerosis among others). Among the 482 patients with SLE, a prevalence of 2.3% for HT and 2.9% for GD was reported, significantly higher than the prevalence in their general population, reported as 30/100,000 for HT (0.0003) and 15/100,000 (0.00015) for GD (Petrányi et al., 2000). A mismatch between this clinical range of autoimmune thyroid disease and the laboratory abnormalities is evident. The prevalence of antithyroid antibodies in patients with SLE has been reported to range between 15 and 50% (Tsai et al., 1993; El-Sherif et al., 2004), much higher than the prevalence of overt autoimmune thyroid disease in these patients, as discussed before. The majority of these abnormalities appear to be clinically silent. In fact, these antibodies may be present in patients without clinical thyroid disease. We know from population studies that antithyroid antibodies are present in many healthy euthyroid subjects but not to this extent. In women, for example, the prevalence of antibodies against thyroid peroxidade (anti-TPO) is about 10–15% at the age of 20 (Prentice et al., 1990). The reason for the higher prevalence of antithyroid antibodies in SLE remains poorly understood, but perhaps represents silent thyroiditis associated with systemic inflammation. It is also possible that the frequent abnormal thyroid test results are not all caused by primary thyroid pathology, but rather, may represent the result of the production of thyrotrophin by activated lymphocytes (Smith et al., 1983), a peculiar metabolism of thyroid hormones in SLE or autoantibodies directed against the thyroid, its hormones, or receptors (Sakata et al., 1985). The prevalence of hypothyroidism in SLE has also been shown to be different than in the general population. The British National Health Service's estimate of the incidence of hypothyroidism is 1% for the general population (Bajaj et al., 1998). In females, the Whickham study (Vanderpump et al., 1995) showed a prevalence of 1–1.5% for clinical hypothyroidism in women from an English community, similar to the prevalence of 1.4% found in a population of hospitalized female patients in a general medical department in Switzerland (Riniker et al., 1981). In lupus patients, the reported prevalence for this condition varies widely between studies but it is consistently higher than in the normal population. In small series with less than 100 lupus patients, Eberhard et al. (1991) and Weetman and Walport (1987) reported prevalence of 11.4 and 24%, respectively. However, in larger series the reported prevalence is about 4% (Boey et al., 1993). Thus, Pyne and Isenberg (2002) reported on a cohort of 300 lupus patients among whom 5.7% had hypothyroidism. The reported prevalence of hyperthyroidism in SLE is less consistent, and there is a disagreement as to whether it is really more frequent in these patients. The largest cohort lupus studies (Pyne and Isenberg, 2002; Boey et al., 1993) reported no increase in the prevalence of hyperthyroidism in SLE compared with the normal population, where the prevalence of hyperthyroidism is about 1.9%. Smaller series have quoted higher rates in their lupus patients that could range up to 11% (Byron and Mowat, 1987).

Different mechanisms have been proposed to explain the pathogenesis of these associations. Among genetic factors, the HLA type B8, DR3 occurs more commonly both in patients with SLE and autoimmune thyroid disease than in general population (Miller et al., 1987). Cross reactivity of autoantibodies with thyroid antigens and cytokine imbalance was also suggested as a contributing mechanism (Masuko-Hongo and Kato, 1999). As SLE may precede or follow the thyroid disorder by years, there may be two-way interactions between the pathogenic factors leading to the development of both conditions.

The wide range of somewhat conflicting data makes it difficult to be certain about the real clinical value of some of the associations between thyroid disorders and SLE. There are various problems encountered by epidemiological studies on thyroid diseases which can make the comparison between them erroneous: (a) racial differences and patient selection; (b) the definition of the disorders may not be the same (e.g., overt hypothyroidism and subclinical hypothyroidism); (c) few studies have had an age- and sex-matched population for comparison. The group most affected by SLE, predominantly young-to-middle aged women, is similar to the group which develops autoimmune thyroid disease and whether

the associations observed are real or coincidental remains debatable; (d) thyroid disease is often difficult to diagnose clinically in a general population. Some of its non-specific complains can be attributed to SLE, testing for thyroid function was not common until recently and the diagnostic methods for detection of thyroid disorders may vary between studies; (e) the results of endocrinological investigations are not always reproducible as the hormone levels may not be stable. Kausman and Isenberg (1995) also showed that the serological status of some patients fluctuates and they may become thyroid antibody negative over time. This subgroup is unlikely to develop clinical thyroid disease. The authors reviewed the follow-up data of 31 SLE patients that were positive for thyroid antibodies. Over an average of 7.9 years, 40% of them became thyroid autoantibody negative on at least one occasion during follow-up. Also, it was observed that all the cases of clinical thyroid disease were developed only in patients persistently positive for thyroid antibodies. In summary, patients with SLE frequently have abnormal results of thyroid function and antibodies tests. Some of these have no implications for clinically overt thyroid disease; however, a significant subset of lupus patients seems to be predisposed to the development of autoimmune thyroid disorders and especially hypothyroidism. These thyroid disorders may precede the development of other lupus signs by years or develop many years afterward and this should be considered in the clinical evaluation of these patients and their complaints. A periodic biochemical thyroid screening is recommended, particularly in those with subclinical disease./subclinical disease.

3. Parathyroid gland

The disorders of the parathyroid glands described in patients with SLE are mainly secondary to lupus nephropathy. The secondary hyperparathyroidism is common in patients with renal involvement who develop organ failure and need dialysis. This has been associated with an increased incidence of Jaccoud's arthropathy (Babini et al., 1989). Other rare disorders were described in this study. Concomitant hypoparathyroidism has been reported in patients with SLE (Gazarian et al., 1995). A few cases of severe hypercalcemia on the onset of SLE have also been reported, with calcium levels normalizing with the treatment of lupus (Gazzaruso et al., 2000). The pathophysiology of hypercalcemia in SLE is not completely understood. In some cases, it is associated with elevated levels of parathyroid-related peptide (PTHrP). Deftos et al. (1996) described one patient with SLE, lymphadenopathy, and hypercalcemia in which immunohistological studies of a biopsied lymph node revealed the abundant expression of PTHrP in the absence of malignant transformation. In other patients the level of PTHrP is normal and it has been hypothesized that it could be caused by the presence of stimulatory anti-PTH receptor antibodies (Berar-Yanay et al., 2001). Whatever its pathogenic mechanism, SLE should be considered among the unusual causes of hypercalcemia and a good response to immunosuppressive therapy can be anticipated.

4. Adrenal gland

4.1. Adrenal cortex insufficiency

4.1.1. Addison's disease

Primary adrenocortical insufficiency, also known as Addison's disease, is the end stage of a destructive process involving the adrenal cortex. The precise cause of this progressive destruction is unknown but it is suspected of being autoimmune in origin (Betterle et al., 2002). The condition has been described in association with other autoimmune disorders, particularly with primary antiphospholipid syndrome and there are some rare cases described in patients with SLE. The clinical manifestations of primary adrenal insufficiency relate to the low to undetectable levels of aldosterone and cortisol in the blood. Weaknesses, fatigue, nausea, hyperpigmentation of the skin and mucous membranes, hypotension, fever, or weight loss are common findings. Because some of these manifestations are also present in lupus, the

endocrine disorder may be underdiagnosed and some manifestations may be concealed by the use of steroids. Unlike the primary condition, secondary adrenal insufficiency is common in patients with lupus, as a result of corticoid therapy. The long-term administration of corticosteroids blunts the pituitary–adrenal function, primarily due to inhibition of the synthesis and secretion of corticotrophin. The degree of such suppression is not predictable in individual patients and cannot be reliably estimated from the daily dose of glucocorticoid, duration of therapy, or the basal plasma cortisol concentration (Schlaghecke et al., 1992). We know, however, that this suppression is related to the time of the day the steroid is administered. Because of the diurnal rhythm of cortisol secretion, morning regimens of corticoid administration have shown to be less suppressive than divided doses. If tablets are taken early in the morning, when the pituitary is ready for its next diurnal source of ACTH secretion, the corticosteroid will no longer be circulating in concentrations high enough to block it. On the contrary, evening administration is the most suppressive. An optional regimen is the alternate-day therapy, in which twice the usual daily dose of corticoid is administered every other morning. The purpose of this regimen is to minimize certain undesirable effects, including pituitary–adrenal suppression, and it is primarily designed for patients in whom long-term pharmacologic corticoid therapy is anticipated. Short-acting corticoids, including methylprednisolone, hydrocortisone, and prednisolone are recommended for alternate-day therapy, contrary to long-acting corticoids (dexamethasone) because of their prolonged suppressive effect on adrenal activity. Morning regimens or alternate-day therapy are usually suitable for less severe lupus disease. More severe states usually will require daily divided high-dose therapy and in these situations, the initial control of the disease process is the priority. The ideal corticoid regimens are not yet defined as they vary widely between patients and severity of the conditions. Our practice is to use steroids on an everyday basis and usually first thing in the morning. If large doses of oral steroids are required we may, for convenience, split the dose into two or three as too much steroid at night tends to cause the patient problems in sleeping. Intravenous steroids may be used for very active patients and/or for those in whom non-adherence to treatment is considered to be a concern. In secondary adrenal insufficiency, the severity of symptoms is often less marked and the manifestations are usually limited to those of glucocorticoid deficiency. The most significant acute adverse outcome of adrenal insufficiency is adrenal crisis which can sometimes be life threatening as rapid hypotension, dehydration, and shock can develop. This crisis can be triggered by stress (e.g., due to surgery or infection) or by the abrupt cessation of corticoid therapy. As normal mineralocorticoid function is preserved in secondary adrenal insufficiency, it is less likely for patients to experience an adrenal crisis compared with the primary condition but this possibility should be taken into consideration in all patients with lupus treated with steroids, particularly those whose condition is worsening or who may not be adhering correctly to the treatment regiment. A stimulation test with CRH may be warranted so that impaired pituitary–adrenal function is not missed. This test could also be useful in identifying patients with no impaired function, in whom glucocorticoid therapy could be discontinued rapidly. If a stressor is predicted for patients under chronic corticotherapy, a surgical procedure for example, ‘stress coverage’ can be provided in the perioperative period. The recommendations indicate replacing glucocorticoids only in an amount equivalent to the normal physiological response to surgical stress. For minor surgery, about 25 mg of hydrocortisone equivalent on the day of the surgery; for moderate surgical stress, the glucocorticoid target is about 50–75 mg/day of hydrocortisone equivalent before surgery and for up to 1–2 days; for major surgical stress, the target is 100–150 mg per day of hydrocortisone equivalent for 2–3 days. A preoperative steroid dose should be taken within 2 h of surgery (Salem et al., 1994).

4.1.2. Hypoaldosteronism

Rare case reports have described the existence of a relative hyporeninemic hypoaldosteronism state in patients with SLE. These observations were confirmed by Lee et al. (1988) among a group of

142 lupus patients, where less than 10 had unexplained hyperkalemia with impaired renin and aldosterone response to stimulation.

4.2. Adrenal cortex hyperfunction

4.2.1. Cushing's syndrome

Secondary Cushing's syndrome is a well-known complication of steroid therapy in patients with SLE, but far more rare is the coexistence of SLE and a primary Cushing's syndrome. One of the few reported cases in the literature describing this association, concerns a 43-year-old woman with SLE who subsequently developed Cushing's syndrome due to an adrenal adenoma (Arima et al., 1998). Her lupus entered in complete remission with the onset of Cushing's syndrome but a marked exacerbation was observed after surgical removal of the tumor. In addition, there is a report on the onset of SLE after pituitary adenomectomy in a patient with Cushing's syndrome (Noguchi et al., 1998).

4.2.2. Conn's syndrome

Primary hyperaldosteronism results from an excess, inappropriate production of aldosterone. It is twice as common in woman as in men and usually occurs between the age of 30 and 50. Although occurring in a similar population to patients with SLE, we have been unable to find reports describing any particular association between these two conditions.

4.3. Adrenal medulla

4.3.1. Phaeochromocytoma

Rare cases of phaeochromocytomas are described in patients with rheumatic diseases and a few have been reported in patients with SLE. Lin et al. (2002) reported the only one of these cases in which the phaeochromocytoma was discovered at the onset of SLE and not some years after the diagnosis. An interesting observation in this patient was the resolution of lupus manifestations after the surgical removal of the mass, as well as some antibodies (anti-Sm, antiribonucleoprotein, antichromatin) becoming negative at 4 and 6 months after surgery, suggesting a possible causal relationship. But whether the occurrence of these diseases in the same patient is incidental or represents a true association has yet to be determined. Phaeochromocytoma is an uncommon cause of hypertension but is, nevertheless, an important correctable condition. Hypertension, however, is common in SLE, affecting nearly a quarter of patients. A thorough investigation is omitted in most cases as the high blood pressure is usually attributed to nephritis, vasculitis, or the use of steroids. In contrast, phaeochromocytoma can manifest with no hypertension, and therefore the diagnosis can easily be missed. Although rare, this association should be excluded in patients with SLE with abnormal presentation of hypertension or resistant to therapy, especially in the absence of renal impairment.

5. Diabetes

Diabetes mellitus type 1 (DM1) is also an autoimmune disease as is confirmed by the presence of insulitis, islet cell antibodies, and T-cell responses to B-cell antigens. The pancreatic β-cell destruction that leads to impaired glucose tolerance, and then to overt diabetes is T-cell mediated. The presence of islet cells antibodies is very common in patients diagnosed with DM1, and there is a susceptibility associated with particular MCH class II alleles (HLA DQ2, HLA DQ8) (Erlich et al., 1993). A positive ANA is more common in DM1 patients than in general population and it can be present in 41% of patients with DM1 (Helmke et al., 1987). Another disorder of glucose homeostasis is called type B insulin resistance, in which autoantibodies against insulin receptor antagonize the physiologic actions of insulin, leading to severe hyperglycemia refractory to administration of high doses of insulin, or rarely, to hypoglycemia by agonist activity (Varga et al., 1990). These patients do frequently have another autoimmune disease, most commonly SLE, or have other autoantibodies. Rosenstein et al. (2001) investigated the presence of

autoantibodies against insulin receptor in patients with SLE and found a prevalence of 2.6%. However, none of the patients studied had any evidence of altered glucose homeostasis. In addition, there are descriptions of disturbed glucose metabolism associated with the presence of these autoantibodies in lupus patients (Di Paolo and Giorgino, 1991) and the measurement of these antibodies should be done only in patients that show abnormalities in glucose metabolism. A group of patients with SLE and steroid-induced diabetes that developed anti-insulin antibodies after treatment with exogenous insulin has been described (Thomas et al., 1987).

The presence of a lupus anticoagulant, an antiphospholipid antibody frequently associated with thromboembolic events, was investigated in patients with type 1 and type 2 diabetes (Dash et al., 2005); it is quite frequent in DM1 (around 20%) and this association is even more evident in the subgroup of patients with retinopathy (3 out of 9 patients with DM1 and retinopathy showed positivity for lupus anticoagulant). The presence of lupus anticoagulant has also been reported in two cases of diabetic females who presented with a rare complication of the disease, diabetic muscle infarction (Palmer and Grec, 2001). However, Palomo et al. (2005) did not find any greater prevalence of antiphospholipid antibodies in a Chilean diabetic population, than in the general population. There are some common clinical features shared by patients with diabetes and SLE that can be confusing and challenging for a physician. A good example is peripheral polyneuropathy, which is the most frequent complication of DM and its prevalence increases with disease duration. Metabolic, vascular, genetic factors, as well as protein glycosylation and neurotrophism seem to play a role in the pathogenesis. It is usually symmetrical, with a peripheral distribution and mainly sensitive. In SLE, the prevalence of peripheral neuropathy varies from 5 to 27% and usually presents as a mild sensory or sensorymotor neuropathy with evidence of a distal axonopathy in the electrophysiologic studies. Vasculitis has been reported as the pathogenic mechanism but it is still unclear (Omdal et al., 2001). Renal disease is a common feature of SLE present in at least one-third of the patients. The glomerular injury is caused by local formation of immune complexes. Diabetic patients are also prone to renal injury and diabetic nephropathy has become the single most common condition found in patients with end-stage renal disease in western countries. The pathophysiological mechanisms involved are complex and hyperglycemia plays a central role in the glomerular injury process. In both diseases the renal involvement manifests by the existence of proteinuria, which should be carefully studied in a patient with SLE–DM overlap in order to establish the injury mechanism involved. Thus, it is important to know the main risk factors for the development of diabetic nephropathy: poor glycaemic control, high blood pressure, positive family history, hyperglycemia in pregnancy, obesity, and insulin resistance.

Retinal damage can also happen in SLE and DM. In a cohort of 194 patients with SLE, retinal vascular abnormalities were found in 34.5% with retinal angiopathy being the most common (80.6%). These abnormalities were usually minor, but retinopathy was correlated with disease activity (Ermakova et al., 2001). In contrast to SLE, serious retinal involvement is quite common in DM. Retinopathy is one of the most troublesome complications of diabetes mellitus and a major cause of blindness. There are multiple risk factors in common with diabetic retinopathy, such as hyperglycemia, hypertension, and dyslipidaemia.

Premature cardiovascular disease is another major concern in SLE patients and the classic atherosclerosis risk factors do not completely explain the excess cardiovascular risk observed in these patients. The largest SLE cohort ever assembled (9547 patients) confirms an increased risk of death due to circulatory events (Bernatsky et al., 2006). Diabetes is a well-known risk factor for cardiovascular disease: there is an excess mortality due to cardiovascular causes in both DM1 and DM2 patients. It is not well established in the literature how prevalent the coexistence of DM type 1 and 2 is, in patients with SLE. Based on our long-term observational cohort at University College London Hospital, among 450 patients with SLE who have been followed up

during the period from January 1978 to December 2006, 8 patients have developed diabetes, 4 DM type 1 and 4 DM type 2. All of the patients with DM type 1, 3 females and 1 male developed this condition before SLE: 28, 20, 15 and 4 years before, respectively. One of them developed DM type 1 at the age of 58 and SLE at 62. Among the patients with DM type 2, one female patient developed both conditions at the same age (32 years old), two developed SLE before diabetes (female), and another developed SLE after DM (male).

Steroid-induced diabetes (SID) is a well-recognized possible complication of steroid therapy in SLE patients. Our literature search does not reveal an accurate prevalence of this condition in SLE patients. It is known, however, that the cumulative dose of steroids is an important risk factor for SID, so the patients on steroids should have their urine assessed for glucose regularly and if necessary fasting glucose levels and glycosilated hemoglobin levels should be obtained. The first alterations in glucose metabolism are rarely evident in the fasting glucose levels thus patients with normal or slightly elevated fasting glucose levels can have alterations in glucose metabolism, namely in the post-prandial glycaemic curve, which can only be detected by oral glucose tolerance tests. The early recognition of SID (and also DM not related to steroid therapy) or glucose intolerance (not overt DM but a predisposing condition) will allow the clinician to: (1) organize an appropriate diet for the patient in order to control glycemic levels and provide information about the risks (mainly cardiovascular) associated with both conditions (SLE and DM); (2) perform a baseline evaluation of the patient looking for the complications of DM (retinopathy, coronary heart disease, and nephropathy), which will be helpful in the future for monitoring the evolution; (3) initiate anti-diabetic pharmacological therapy if necessary; and (4) review the patient's steroid regimen. In a study (Ariza-Andraca et al., 1998), comparing patients with steroid-induced DM with rheumatic diseases ($n=27$) versus rheumatic patients on steroids but without DM ($n=27$, age and sex matched), the authors concluded that the higher cumulative dose of steroids was a risk factor for the development of DM. They did not find any correlation of family history of DM or body mass index with a higher risk of DM. In the lupus cohort of University College of London Hospital, of the 8 patients with DM (out of 450 in the cohort), only 1 has SID. There are some challenging situations that can occur when treating patients with SLE and DM. For instance, if a high dose of prednisone is needed in a lupus patient with an unstable DM1, it is wise to treat the patient with the steroids but a very careful monitoring of the glucose levels is mandatory, and the patient should be closely observed.

6. Hyperprolactinemia

Prolactin (PRL) is a lactogenic neuropeptide primarily produced not only by the anterior pituitary, but also at various extrapituitary sites including neurons, prostate, deciduas, mammary epithelium, skin, and immune cells. Its secretion is stimulated by suckling and stress and inhibited by hypothalamic dopamine. There is strong experimental evidence that PRL is an enhancer of immune responses and receptors for PRL have been found on B and T cells and monocytes (Matera et al., 1997). It is synthesized by stimulated lymphocytes and its receptor structure and signal transducing pathways are similar to cytokines. PRL acts itself like a cytokine, is a lymphocyte growth factor and induces IL2 receptors on these cells. Hyperprolactinemia (HPRL) is observed in non-organ-specific autoimmune diseases such as rheumatoid arthritis, systemic sclerosis, and Sjögren's syndrome as well as in organ-specific autoimmune diseases as DM1, GD, HT, Addison's disease, lymphocytic hypophysitis, celiac disease, and multiple sclerosis. Also, elevated serum PRL levels are frequently associated with SLE, being present in 15–31% of patients with lupus (Jara et al., 2001). In most of these patients, the cause of HPRL cannot be found but, in general, the highest levels of circulating PRL occur in association with PRL-secreting tumors. Other identifiable explanations can be encountered in a small number of patients: hypothyroidism,

chronic renal failure, or medication induced HPRL (Blackwell, 1992). An association between HPRL and anti-DNA antibodies in women under 50 years of age has been reported (Jara et al., 1992). There are several circulating PRL isoforms and most patients with HPRL have predominantly free PRL (monomeric little PRL, molecular weight 23 kDa) and lesser amounts of big and big big PRL (45–50 and >100 kDa, respectively). The clinical features of HPRL are different, depending on the predominant type of seric PRL isoform: hyperprolactinemic patients with predominance of macroprolactin (independently of the nature of big big PRL) frequently do not show classic clinical symptoms of HPRL (amenorrhea and galactorrhea in women and impotence in men). In SLE patients with HPRL, there is a high prevalence of macroprolactinemia (31.7%), and in these patients there was less clinical and serological disease activity compared to those with idiopathic HPRL but macroprolactin-negative (Leaños-Miranda et al., 2001). These data suggest that high molecular weight PRL has less biological activity in vivo, when compared to low molecular weight PRL. The relationship between PRL levels and SLE activity is not entirely clear as clinical studies have produced equivocal data (Blanco-Favela et al., 1999; Pacilio et al., 2001). More recently, Li et al. (2006) presented three patients with SLE and prolactinomas and there was no consistency in findings related to PRL excess or in the coincidence of hyperprolactinemia with flares of SLE disease activity. This issue was also investigated by Leaños-Miranda and Cárdenas-Mondragón (2006) in 259 patients with lupus, but comparing different prolactin measurements and isoforms. They concluded that elevated serum direct or total PRL levels were not associated with disease activity, but elevated serum-free PRL levels showed a positive association. Moreover, higher percentages of little PRL and lower percentages of big big PRL proved to be factors related to lupus activity. These data suggest that characterization of serum-free PRL levels and isoforms might be helpful in the evaluation of SLE patients and in understanding the clinical and pathological role of PRL in autoimmune diseases. Also, it may clarify the variable results of studies comparing PRL levels and lupus expression.

6.1. Bromocriptine in SLE

Bromocriptine is an ergot alkaloid that binds to the dopamine receptor, suppressing the pituitary PRL synthesis and release, which results in lower serum PRL concentration. Bromocriptine administration has been associated with decrease T-cell proliferation and cytokine production in animal models and in humans (McMurray, 2001). Through its suppressive properties on PRL (immunostimulatory) and direct actions on T and B lymphocytes, bromocriptine may have a role in the management of rheumatic and autoimmune diseases. In the NZB $\times$ NZW F1 mice it was effective in the treatment of lupus-like disease and protected the mice against the immunostimulating effects of estrogen (Peeva et al., 2000). In patients with SLE, treatment with bromocriptine was first reported in a patient with central nervous system (CNS) involvement (Rabinovich et al., 1990). Several other studies have been conducted with encouraging results. Walker et al. (1999) compared the efficacy of bromocriptine with hydroxychloroquine in the treatment of active lupus disease and confirmed improvement in SLE activity in both groups. Further studies with the use of bromocriptine in the treatment of SLE should be considered.

7. Hypogonadism/Klinefelter's syndrome

In addition to its striking female predominance, lupus flares have been associated with parturition and with estrogen-containing oral contraceptives. In contrast, the occurrence of SLE is rare in males and the association of male hypogonadism with SLE reinforces the role of sex hormones in the pathogenesis of SLE. Lahita and Bradlow (1987) studied several patients with SLE and Klinefelter's syndrome (KS) and concluded that metabolism of sex steroids in these patients was similar to that female patients with SLE. KS is a chromosomal disorder characterized by a 47/XXY karyotype,

and its main clinical features are hypergonadotrophic hypogonadism, gynecomastia, testicular hyalinization (Mok and Lau, 2000), and female distribution of body hair. The incidence of KS is estimated at 1.7/1000 male births (Caldwell and Smith, 1972). Its association with SLE is well recognized and has been reported several times in the literature. In a review of the published data on KS–SLE association (Gilliland and Stashower, 2000), the clinical features of these patients were similar to other SLE patients, except for the lower frequency of cutaneous symptoms (discoid lesions, photosensitivity, and oral ulcers). Interestingly, a comparison between clinical SLE features in male and female patients concluded that males tend to have more discoid and subacute cutaneous lupus and less arthritis (Font et al., 1992). Estrogens seem to have a proinflammatory and immuno-enhancing activity, while androgens seem to act as immunosuppressors (Cutolo et al., 2006). Androgen deficiency has been reported in male patients with SLE and with rheumatoid arthritis (Cutolo and Masi, 1998). Testosterone therapy in SLE–KS patients has been tried successfully in a few patients, leading to clinical and serological remission of SLE (Bizzarro et al., 1987). Also Olsen and Kovacs (1995) treated successfully a patient with testosterone replacement and obtained clinical, hematological, and serological improvement of SLE. In addition, KS and SLE with autoimmune hepatitis have been described in a patient, who was also successfully treated with testosterone (Sasaki et al., 2006). KS has been associated with other autoimmune diseases like progressive systemic sclerosis, and increased number of autoantibodies (rheumatoid factor, ANA) has also been reported. Antiphospholipid antibody syndrome has been described in patients with KS. However, the number of case reports of this interesting association is small and the hypothesis of coincidence cannot be excluded.

8. ADH and SLE

The syndrome of inappropriate secretion of antidiuretic hormone (SIADH) is characterized by hyponatremia (without edema), renal failure or adrenal and thyroid deficiency, and volume depletion. It has seldom been reported in SLE patients. The pathogenic mechanisms that lead to oversecretion of ADH in SLE patients are not entirely clear. It has been suggested that central neuroendocrine lesions due to vasculitis or focal lesions induced by specific anti-neuronal antibodies may be involved (Elisaf et al., 1999). The relationship between CNS involvement of SLE and SIADH is controversial: symptoms of active CNS lupus were detected in a few patients (Martin Santos et al., 1996; Mirsattari et al., 1998). Other patients presented with convulsions and psychosis probably related to hyponatremia, with no other evidence of active CNS lupus (Castanet et al., 1993). There was remission of these manifestations after correction of hyponatremia. Patients with SLE and SIADH and no evidence of CNS involvement are presented in other case reports (Ben Hmida et al., 1992). It should be noted that in some of these cases the onset of SLE was at an unusual advanced age (between 60 and 88 years old). There are several references in the literature regarding the occurrence of SIADH in patients receiving Cyclophosphamide, especially in patients under chemotherapy for neoplastic diseases (Björck and Samuelsson, 1996; Vanhees et al., 2000; Festuccia et al., 2002). The clinician should be aware of this possible complication when treating a lupus patient with this alkylating agent.

9. Metabolic profile

Hypertriglyceridemia, sometimes discovered in a routine lipid assessment, may rarely be the initial manifestation of SLE, particularly in children.

10. Conclusions

Although the main endocrine condition to complicate SLE is hypothyroidism, as we have reviewed, the diverse nature of lupus may mask a variety of other, though less common, endocrinopathies. Physicians looking after patients with lupus must constantly be aware of these potential complicating conditions.

Key points

- Many endocrine disorders have been described in association with SLE, but most are uncommon.
- Hypothyroidism is the endocrine disease most frequently linked to SLE (in just over 5% of SLE patients).
- The diverse nature of SLE may conceal a concomitant endocrine disorder.

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CHAPTER 16

Pregnancy, Hormones, and Autoimmune Rheumatic Diseases

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1. Introduction

Pregnancy is a physiological condition characterized by complex molecular interactions between the mother and the embryo. Following implantation, the maintenance of pregnancy depends on a constellation of endocrinological and immunological events that will eventually lead to the successful growth and development of the fetus. Immune and endocrine alterations could potentially lead to recurrent pregnancy loss. Inadequate progesterone secretion, luteal phase deficiency, hyperprolactinemia, thyroid disease, hypoparathyroidism, uncontrolled diabetes, decreased ovarian reserve, and polycystic ovarian syndrome are some examples of conditions that affect the outcome of pregnancy. Cytokines make important contributions to successful pregnancy. Both Th1 and Th2 cytokines play roles at different stages of pregnancy. Changes in patterns of local cytokines during pregnancy correspond to neuroendocrine changes in which hormones act as powerful regulators of cytokine expression (Arredondo and Noble, 2006; Rai and Regan, 2006).

During pregnancy, experimental animals and patients with autoimmune diseases develop abnormal immune–neuroendocrine responses. In rheumatic diseases with a predominance of Th1 immune response, a shift to the Th2 response during pregnancy is regarded as beneficial. Pregnant patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) have shown significant differences in a few cytokines, related to the activity of the underlying disease (Ostensen et al., 2006). Estrogens (E), progesterone (P), androgens (A), and prolactin (PRL) have the potential to predispose to either successful or pathologic pregnancies (Jara et al., 2006). The aim of this chapter is to analyze the influence of the immune–neuroendocrine system on autoimmune disease during pregnancy.

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2. Hormones, the immune system, and pregnancy

2.1. The first days of pregnancy

Implantation of the blastocyst into the endometrium involves a series of steps leading to effective "crosstalk" between invasive trophoblast cells and the maternal endometrium. This dynamic process involves the coordinated effects of endocrine, paracrine, and autocrine factors. Therefore, successful implantation depends on development of the embryo to the blastocyst stage, followed by its invasion into the decidualized endometrial stroma. In humans, decidualization is initiated in the luteal phase of the menstrual cycle. The disruption of certain pathways results in fertility defects. Uterine differentiation to support blastocyst implantation is coordinate by P and E. These ovarian hormones produce molecular and morphological changes of the endometrium and development of large ectoplasmic projections called pinopodes (markers of endometrial receptivity) during a limited period of time (window of implantation). In humans the receptive window is days 20–23 of a typical 28-day menstrual cycle (Makrigiannakis et al., 2006). The window of uterine receptivity remains open for an extended period at lower E levels, but closes rapidly at higher levels (Ma et al., 2003). Increases and decreases of P levels and P receptor B were closely associated with the formation and regression of pinopodes respectively (Stavreus-Evers et al., 2001). Corticotrophin-releasing hormone (CRH) is produced in several organs of the female reproductive system, including the endometrial glands, decidualized stroma, and the trophoblast. The gene encoding the CRH receptor type 1 (CRHR1) is expressed in human endometrial and myometrial cells, due to a local effect of uterine CRH. In this regard, a new role in implantation has been described for CRH, which has been shown to inhibit the invasion of extravillous trophoblasts. This action of CRH was controlled through the type 1 CRH receptor (CRHR1) by means of inhibition of carcinoembryonic antigen-related cell adhesion molecules by extravillous trophoblasts. Interestingly, maternal plasma concentrations of CRH are elevated and there is a concomitant reduction in CRHR1 expression in pregnancies complicated by pre-eclampsia. Therefore, it has been suggested that a defective CRH/CRHR1 system is involved in the pathophysiology of placental ischemia in pre-eclampsia (Bamberger et al., 2006).

Different isoforms of PRL receptors may be present in various stages of development of the mouse preimplantation embryo and may play an important role in controlling its growth and development (Kiapekou et al., 2005). Other factors and genes are involved in implantation, including chorionic gonadotropin, leukemia inhibitor factor, cytokines and growth factors, matrix metalloproteinases (MMPs), adhesion molecules. Numerous gene networks participate in the endometrial responses to ovarian stimuli, and further analysis is required to understand the molecular pathways leading to successful implantation (Makrigiannakis et al., 2006).

2.2. HLA, endocrine–immune response, and pregnancy

In order for gestation to be maintained, it is important to have immunological recognition between the mother and the fetus, by fetal antigen presentation and by recognition and reaction to these antigens by the maternal immune system. A variety of hormonal and immunologic events occurring during pregnancy could modulate maternal immunity against fetal antigens. Therefore, more than one mechanism appears to induce tolerance and immunological privilege. These mechanisms occur not because the uterus is the site of the pregnancy, but because of the combined functions of trophoblast cells at the materno–fetal interface and maternal immunoregulatory processes that control responses to fetal alloantigens (Simpson, 2006).

The placenta plays a key role in the maintenance of local tolerance and allows the mother to accept the embryo until completion of pregnancy. Regulation of the expression of HLA antigens, such as HLA-G, HLA-C, and HLA-E by the trophoblast, and the virtual absence of HLA class I proteins,

favors the induction of maternal tolerance. HLA-G class Ib is expressed in extravillous cytotrophoblast and also in endothelial cells of fetal vessels in the chorionic villi, amnion cells, and amniotic fluid. Progesterone regulates HLA-G expression through P receptor activation, followed by binding to a novel P response element in the HLA-G promoter region. HLA-G presents antigens for gamma/delta T cells and at the same time defends the trophoblast from cytotoxic effector mechanisms. Previous studies indicate that soluble isoforms of HLA-G have immunosuppressive properties (Yie et al., 2006). Normal human pregnancy is characterized by low peripheral natural killer (NK) cell activity, whereas increased NK activity seems to play a role in spontaneous abortions. Uterine NK (uNK) cells are under hormonal control, and increased uNK have been found in sites where fetal trophoblasts infiltrate the decidua, suggesting that one of the functions of these cells is control of placentation. Another protective mechanism operating in favor of pregnancy is progesterone-dependent immunomodulation. Due to stimulation by fetal-derived antigens, pregnancy lymphocytes develop P receptors and in the presence of P produce a mediator that alters the cytokine balance, inhibits NK activity, and exerts an effect that suppresses abortion in mice (Szekeres-Bartho, 2002).

Global crosstalk between the trophoblast and decidua was detected in an in vitro study, using a functional genomics approach. Products secreted by the trophoblast induced pro-inflammatory cytokines and chemokines, as well as angiogenic/static factors, in decidualized endometrial stromal cells. The data suggest that the trophoblast alters the local immune environment of the decidua to facilitate the process of implantation and ensure an enriched cytokine/chemokine environment. At the same time, trophoblast limits the mitotic activity of stromal cells during the invasive phase of implantation (Hess et al., 2007).

2.3. Hormones, innate immunity, and pregnancy

During normal pregnancy, cellular immunity and Th1 cytokines—which are potentially harmful to the fetus—are inhibited, whereas humoral immunity, autoantibody production, and Th2 cytokines are enhanced (Ostensen et al., 2006). Although the exact mechanism is unclear, high levels of E, P, and PRL may be responsible for these immune profile changes. The changes of local and systemic cytokine patterns during pregnancy correspond to neuroendocrine changes, with hormones as powerful modulators of cytokine expression. During the third trimester of gestation, high levels of cortisol (C), E, P, and 1,25-dihydroxyvitamin D3 suppress Th1-mediated immune responses and stimulate Th2-mediated responses. Ex vivo monocytic IL-12 production was about threefold and tumor necrosis factor (TNF) production was approximately 40% lower than postpartum values. At the same time, urinary C and norepinephrine excretion and serum levels of 1,25-dihydroxyvitamin D3 were two- to threefold higher compared to postpartum values. These hormones can directly suppress IL-12 and TNF production by monocytes/macrophages in vitro, and P and E up-regulate the production of IL-4 and IL-10 by Th2 cells in vitro. These findings suggested that C, norepinephrine, and 1,25-dihydroxyvitamin D3 induced inhibition. Subsequent postpartum rebound of IL-12 and TNF production may represent a major mechanism by which pregnancy and postpartum alter either susceptibility to autoimmune diseases or the course of these disorders (Kanik and Wilder, 2000; Elenkov et al., 2001).

Moreover, cytokines such as IL-10 and IL-6 rise during pregnancy, but IL-15 and IL-18 also have a role in different stages of gestation. IL-15 has been implicated in differentiation and proliferation of uNK cells, while IL-18 enhanced innate immunity and both Th1- and Th2-driven immune responses depending on the cytokine milieu (Laskarin et al., 2005). On the other hand, the trophoblast expresses Fas ligand, thereby conferring immune privilege: maternal immune cells expressing Fas will undergo apoptosis at the placenta/decidua interface. The cytolytic mediators, perforin and Fas/Fas ligand (FasL), are found at the maternal–fetal interface, where they may affect the immunological interrelations between maternal tissues and trophoblast cells (Bogovic Crncic et al., 2005). In support of these findings, decidual lymphocytes

have the characteristics of lymphokine-activated killer (LAK) cells and are able to use both the perforin and the FasL cytolytic pathways effectively (Crncic et al., 2007).

Experimental models and clinical studies show that the innate immune system is enhanced and the adaptive immune response is suppressed during pregnancy. Maternal plasma concentrations of complement proteins (an important component of innate immune response) are increased, and alterations of total complement hemolytic activity (CH50), and C3a, C4a, and C5a have been demonstrated (Richani et al., 2005). In addition, active complement proteins are also present in the placenta. Eight complement proteins (factor B, C3, C1r, C1s, C1 inhibitor, factor H, C4, C2) were detected in chorionic tissue, and complement synthesis was regulated by IL-1 beta, TNF-α, and IL-6. On the other hand, interferon (IFN)-gamma increased the synthesis of C1s, C1r, C1 inhibitor, C4, and factor H in chorion-derived cells. The fact that the latter two complement proteins have opposing effects on immune activation of the complement cascade demonstrates the complex balance required to protect both the fetus and the mother against infectious and other toxic agents while, at the same time, the immune response is suppressed to enable tolerance of the allograft fetus (Goldberg et al., 2007). Protection against the undesired effects of complement activation products is achieved by the surface expression of complement regulators acting at different steps of the complement sequence, such as decay accelerating factor (DAF), membrane cofactor protein (MCF), and CD59. However, excessive complement activation could potentially harm the developing fetus (Girardi et al., 2006). Recent evidence in murine models suggests that Crry (a protein that belongs to a family of molecules and regulates complement activation, protecting tissues from complement-mediated damage) deficiency, a C3 convertase inhibitor, and C3 products are implicated in the mechanisms of pregnancy loss (Molina, 2005; Richani et al., 2005). Therefore, it has been proposed that inhibition of the complement system is an absolute requirement for normal pregnancy. As fetal tissues are semi-allogeneic and alloantibodies commonly develop in the mother, the placenta is potentially subject to complement-mediated immune attack at the fetal–maternal interface with the risk of fetal loss. However, the total inhibition of complement activation may limit defense mechanisms against infection.

The regulation of complement proteins by steroid hormones and the role of complement in host defense in the uterus are not clearly defined. A recent study demonstrated that the estrogen, 17 beta-estradiol (E2), and the glucocorticoid, dexamethasone, had major and opposing effects on the amount and latent activity of complement effectors in the uterus. E2 increased the amount and latent activity of complement proteins in the rat uterus, and simultaneous dosing with dexamethasone completely blocked this increase (Rhen and Cidlowski, 2006).

In conclusion, a complex interaction is established during pregnancy between the maternal endocrine system and immune system and fetal cells to allow survival and normal growth of the fetus. Clinical observations are needed to provide a better understanding of the fetal–maternal interaction in normal and pathologic conditions of pregnancy (Fig. 1).

3. Systemic lupus erythematosus

3.1. Obstetrical systemic lupus erythematosus (O-SLE)

SLE is an autoimmune disease that affects predominantly women during their reproductive years and its course is altered by menses, menopause, the use of oral contraceptives, and especially pregnancy. These observations suggest a role for endogenous sex hormones in disease predisposition. Here, we will explore the relationship between SLE, hormones, the immune system, and pregnancy.

Despite previous controversial reports, the present consensus is that pregnancy could exacerbate lupus activity, perhaps by hormonal shifts required to maintain pregnancy. Rates of pregnancy or postpartum flares of SLE are in the range of 15–63%. Other frequent maternal complications in pregnant patients with SLE include

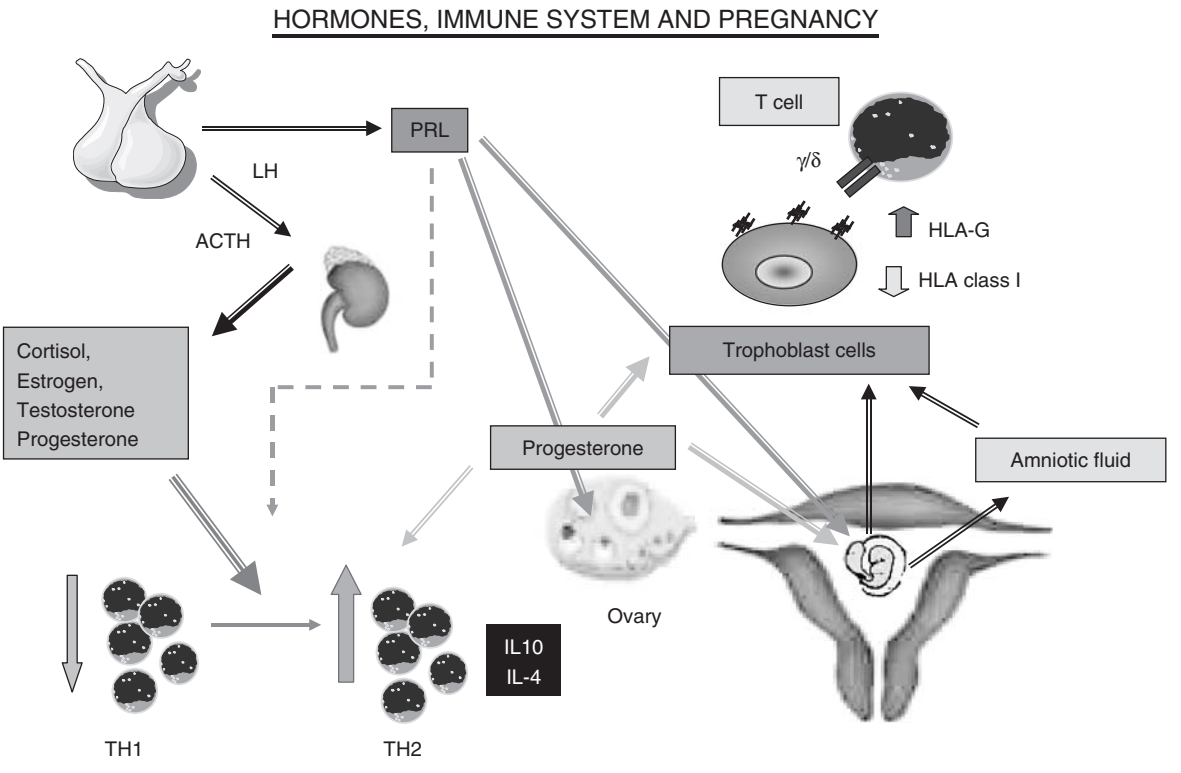

Figure 1. From the first days of pregnancy, hypothalamic-pituitary-adrenal and gonadal axis, and PRL secretion interact locally with HLA, T cells, trophoblast cells, innate, and adaptive immune response, producing the Th1/Th2 shift, in order to maintain tolerance and to assure fetal survival.

pre-eclampsia and hypertension, especially in patients with active renal disease. Fetal adverse outcome in O-SLE frequently includes fetal loss (spontaneous abortion and intrauterine fetal death), intrauterine growth restriction (IUGR), premature birth, premature rupture of membranes, neonatal lupus, and perinatal mortality. Fortunately, the majority of pregnancies in women with SLE are successful. However, the interaction between pregnancy and SLE activity can lead to maternal–fetal complications (Warren and Silver, 2004; Clowse, 2007).

3.2. Human studies

Hormones such as P, E, C, and PRL play an important role in the immune response, and hormonal–immune system interactions are crucial during SLE pregnancy (Szyper-Kravitz et al., 2005). An early report showed high levels of PRL and low levels of E and T in pregnant SLE patients in comparison to healthy pregnant women and women with RA (Jara-Quezada et al., 1991). These changes may be related to fetal wastage and disease activity. Doria et al. (2002) confirmed the variation of steroid hormone levels during pregnancy in patients with SLE. Serum levels of E, dehydroepiandrosterone sulfate (DHEAS) and P were decreased throughout pregnancy, especially in the last trimester of gestation, probably as a result of placental insufficiency. These findings could explain why some studies showed a low percentage of lupus flares in the third trimester of gestation. A prospective study was performed to analyze immune and neuroendocrine changes in pregnant women with RA or SLE. Serum levels of P and E were increased during pregnancy and diminished

in the postpartum period. In pregnant lupus patients, C was decreased significantly compared to healthy pregnant women, and production of IL-10 was increased, possibly as a result of treatment with prednisone (Muñoz-Valle et al., 2003).

PRL is a peptide hormone that acts as a cytokine and is critical for maintaining pregnancy and lactation. It is produced by the anterior pituitary gland and in various extra-pituitary sites, such as neurons, prostate, decidua, mammary epithelium, skin, and immune cells. PRL production in lymphocytes and the expression of PRL receptors in immune cells suggest that PRL affects the immune system (Szyper-Kravitz et al., 2005). Hyperprolactinemia (HPRL) has been described in about 25% of patients with SLE, and high PRL levels during pregnancy in SLE patients correlate with disease activity (Jara et al., 2001).

Recent data suggests that PRL complexed with IgG has a biological role in O-SLE. In this regard, a woman with SLE and increased circulating 150-kDa PRL (big big PRL) and remission of disease during pregnancy was studied before, during, and after pregnancy. The circulating form of PRL (IgG-23 kDa bioactive complex) and remission of SLE persisted during pregnancy, a finding suggesting that these autoantibodies contributed to morbidity (Leaños-Miranda et al., 2001). A controlled study in 99 consecutive SLE pregnant women confirmed this interesting observation. In fact, an adverse outcome of pregnancy was more frequent in SLE women without anti-PRL autoantibodies than those who had anti-PRL autoantibodies. The frequency of anti-PRL autoantibodies in lupus pregnancy was 13.1% (Leaños-Miranda et al., 2007).

Of interest, bromocriptine (BRC), an ergot derivative that inhibits secretion of PRL, showed efficacy in preventing postpartum flares in lupus patients (Yang et al., 2003). More recently, BRC was used during SLE pregnancy in a pilot clinical trial. Our results suggested that BRC plays a role in the prevention of maternal–fetal complications such as premature rupture of membranes, preterm birth, and active disease (Jara et al., 2007a).

3.3. Experimental models

There are few reports of the effects of pregnancy in SLE animal models. McMurray et al. (1993) showed that female autoimmune B/W mice, which are excellent models of hormonally influenced SLE, were subject to sustained HPRL in the pseudopregnant state and had significant acceleration of multiple variables of autoimmune disease activity such as anti-DNA antibodies, antibodies against the viral protein gp70, and hypergammaglobulinemia. Another study analyzed the effects of treating pregnant dams with either T or the androgen blocker, flutamide, to examine the effects on autoimmune B/W fetuses and non-autoimmune C57BL/6 fetuses. The alterations of the hormonal environment in late gestation produced significant depression of serum E in male B/W fetuses, and these fetuses had reduced placental testosterone content. It was concluded that placental androgen control was regulated differently in the autoimmune vs. non-autoimmune maternal-placental-fetal unit (Keisler et al., 1995).

4. Rheumatoid arthritis

4.1. Obstetrical rheumatoid arthritis (O-RA)

RA is an inflammatory and autoimmune disorder that is more common in women than men. A significant body of evidence implicates gender-specific factors in facilitating the development of RA. E-containing oral contraceptives can modify the disease course or onset of RA, a finding that supports a role for this hormone in disease pathogenesis. Pregnancy has an ameliorating effect on disease activity, while the disease tends to flare in the postpartum period. Breast feeding appears to increase the risk of RA, possibly through the actions of PRL (Jara et al., 2006).

4.2. Experimental models

Pregnancy influences the course of experimental RA, such as type II collagen-induced arthritis in

DBA/1 mice. A characteristic feature is remission during gestation and exacerbation during the postpartum period, with the postpartum flare possibly due to decreased steroid hormone levels and HPRL. In this regard, treatment with E actually protected against postpartum flares (Mattsson et al., 1991). Postpartum exacerbations of arthritis were found within 30 days of parturition in 68% of MRL-lpr mice, a model of accelerated autoimmunity with features of RA and SLE. Microscopic examination of synovial tissue showed a significant increase of subsynovial inflammation and synovial hyperplasia, without changes in the level of cartilage and bone erosion. Injection of physiological levels of E postpartum delayed and reduced the flare to 23% of the animals (Ratkay et al., 1994).

In another experimental study, the prolactin suppressor BRC suppressed the postpartum exacerbations of collagen-induced arthritis in mice. Approximately 50% reduction in severity of disease was achieved with BRC. The effect was due to suppression of the maternal PRL release that normally occurs following parturition (Whyte and Williams, 1988). The successful use of E and BRC supports the protective role of E supplementation and PRL suppression in suppression of disease following pregnancy in RA.

4.3. Human studies

Hormonal changes during pregnancy and the postpartum period have profound effects on RA incidence and activity. The effect of pregnancy on RA activity is actually greater than the effect of some of the newer therapeutic agents. The striking increase in C, E, and P during pregnancy may suppress RA onset or activity through the regulation of production or action of cytokines such as TNF-α, IL-1, IL-6, IL-12, and IL-10 (Kanik and Wilder, 2000; Muñoz-Valle et al., 2003).

Pregnancy is a period of transient relative hypercortisolism. Activation of the hypothalamic-pituitary-adrenal axis during pregnancy has been proposed to function as a biological clock. The placenta is perceived as a stress-sensitive organ and placental CRH as a timing starter that determines preterm, term, or post-term labor. In pregnancy and the immediate postpartum period, maternal hypothalamic CRH secretion is suppressed because of circulating levels of C. This transient postpartum maternal hypothalamic CRH suppression, lasting 12 weeks, together with the steroid withdrawal that follows parturition, might be causally related to the vulnerability to RA often observed during the postpartum period (Mastorakos and Ilias, 2000). In fact, the ameliorating effect of pregnancy on RA has been well known since 1938 and confirmed for 75% of RA pregnancies. Improvement of symptoms usually occurs in the first trimester and increases as pregnancy progresses. A flare of RA is observed within 6 months after delivery in most patients. However, some studies did not find a correlation between C levels and disease activity in pregnancy and the timing of gestational improvement and postpartum flares does not coincide with the rise and fall of C. New research has disclosed neuroendocrine disturbances in RA, including a relative glucocorticoid deficiency. Therefore, pregnancy may modify the neuroendocrine defects in RA patients (Ostensen, 2000).

5. Future directions

The risk of complications and adverse fetal outcome in pregnant women with O-SLE is high. Complications of pregnancy, particularly pre-eclampsia, can be difficult to distinguish from symptoms of lupus making diagnosis and treatment challenging. Elevation of total serum inhibin A and activin A (placental hormones) has been interpreted as evidence of placental dysfunction in women who develop pre-eclampsia. The possible role of inhibin A and activin A in SLE pregnancy has not been studied (Hamar et al., 2006).

Relaxin is a 6-kDa polypeptide hormone of pregnancy that has been implicated in decreased immune responsiveness. Elevated serum relaxin levels have been reported in pregnant women with type I diabetes, but the significance of this change has not been explained (Whittaker et al., 2003). Relaxin and estradiol valerate therapy ameliorate adjuvant-induced arthritis. The roles of relaxin in

human O-SLE and O-RA are worthy of further study (Santora et al., 2005).

A recent study of Th1/Th2 cytokine balance during and after pregnancy in patients with RA, juvenile idiopathic arthritis (JIA), and ankylosing spondylitis (AS) yielded results that were in conflict with earlier reports. Concentrations of IL-10 were low, and IFN-gamma and IL-1beta were not detected. Increases of IL-1Ra and sTNFR from the second to the third trimester correlated with improvement of disease activity in both RA and AS, and it was proposed that these anti-inflammatory mediators affected disease activity (Østensen et al., 2005). The hormonal and cytokine environments have not been studied in pregnant women with other autoimmune diseases, such as systemic sclerosis, Sjögren's syndrome, and adult-onset Still's disease.

6. Antiphospholipid antibody syndrome

6.1. Obstetrical antiphospholipid syndrome

Obstetrical antiphospholipid syndrome (O-APL), described initially in the 1950s, included recurrent pre-embryonic and embryonic miscarriage, fetal loss, pre-eclampsia, IUGR, and possibly placental abruption in association with lupus anticoagulant. At present, live birth rates of approximately 70–80% are reported when O-APL is treated with low-dose acetylsalicylic acid and heparin. Despite these encouraging results, the incidence of severe maternal and fetal complications remains high (Wu and Stephenson, 2006).

The mechanism by which antiphospholipid antibodies (APA) lead to pregnancy loss is unclear, and studies have been performed in animal models and humans to resolve this question. Traditionally, pregnancy loss associated with APA was ascribed to thrombosis and infarction of the uteroplacental vasculature (Rai and Regan, 2006). However, these findings are neither universal nor specific to APS and other mechanisms are believed to be involved. This discussion will focus on the important roles of endocrine and immunologic factors in experimental and human O-APS.

6.2. Experimental obstetrical antiphospholipid syndrome

Evidence from animal models strongly suggests that APA have direct effects on fecundity and the outcome of pregnancy (Blank et al., 1991), and hormonal interactions with APA may play a role in maintaining pregnancy. An analysis of placental explants showed that mouse monoclonal antibodies to cardiolipin (ACL) increased the pulsatility of beta human chorionic gonadotropin (βhCG). In contrast, human polyclonal ACL were inhibitory. These results showed that ACL antibodies may have an effect on placental hormone secretion and thus could affect the outcome of pregnancy (Shurtz-Swirski et al., 1993). The increase in βhCG production was inhibited under phospholipase A2 and phospholipase C stimulation. These observations suggested that aPL antibodies exerted their adverse effect on reproductive processes through the interception of signal transduction processes (Gleicher et al., 1992). In fact, β2GPI binds to trophoblast in vitro through its fifth domain and can be recognized by anti-beta 2GPI antibodies. The antibody binding down regulates trophoblast hCG synthesis and secretion. This mechanism might explain defective placentation in women with APS (Di Simone et al., 2000; Di Simone et al., 2005). There is evidence that aPL antibodies stimulate premature onset of cytotrophoblast proliferation and syncytial fusion, leading to loss of trophoblast function and increased risk of pregnancy failure (Bose et al., 2006). A study performed using human placental explants showed that aPL antibodies could damage the placenta directly in patients with APS by inhibiting βhCG secretion without affecting E or P secretion. Therefore, circulating βhCG levels are a predictive marker for placental damage and pregnancy loss in women with APS (Schwartz et al., 2007).

PRL has emerged as a factor that interacts with APA and is involved in recurrent miscarriage. ACL inhibited the expression of decidual markers such as PRL and insulin-like growth factor-binding protein 1 (IGFBP-1) in endometrial cultures (Pierro et al., 1999). Anti-β2GPI antibodies have the same effect, inhibiting the expression of PRL, signal

transducers, and activators of transcription 5 (Stat5) (Mak et al., 2002). Expression of the endometrial PRL gene, Stat5, and complement regulatory proteins was significantly lower in samples obtained from aPL (+) patients with recurrent pregnancy loss in comparison with women who were negative for APL before conception (Francis et al., 2006). The molecular relationship between PRL and APL at the level of endometrial stromal cells is an area that requires further exploration.

6.3. Human studies

Lockshin et al. (1985) were the first investigators to suggest an interaction between APL and hCG during pregnancy in SLE/APS patients. They found that hCG levels were abnormal for the stage of gestation when there were alterations in fetal heart rate. These hormonal changes could be due to placental insufficiency. Another clinical observation in women with lupus anticoagulant showed an association between disproportionately elevated maternal serum hCG and severe IUGR, without Down's syndrome. Elevated hCG on prenatal screening should prompt consideration of maternal testing for lupus anticoagulant (Clark et al., 1995). In this regard, a recent study suggested that high PRL levels were associated with LA, active SLE, and poor outcome of pregnancy in SLE/APS patients (Jara et al., 2007b). Therefore, disproportionately high levels of PRL seem to be a new risk factor for poor pregnancy outcome in SLE/APS.

During human pregnancy, the placenta produces a variety of proteins for the establishment of the fetoplacental unit, including inhibins and activins. Inhibins suppress and activins increase gonadotropin-releasing hormone (GnRH)-induced hCG release with stimulation of P release (Petraglia et al., 1989; Mylonas et al., 2006). Prakash et al. (2006) did not find any alteration in βhCG, inhibin A or activin A in APS women from the time of conception to 11 weeks compared with a control group. Another study found low, normal, and high levels of hCG in patients with anti-β2GPI during the first and second trimester of pregnancy, with a negative correlation between anti-β2GPI antibodies and α1-fetoprotein. High levels of α1-fetoprotein suggested an immunosuppressive effect on anti-β2GPI biosynthesis (Fialova et al., 2002).

6.4. Treatment

The conventional therapy for pregnant women with APS focuses on anticoagulation. Heparin has anti-complementary effects at various points in the classical, alternative, and terminal pathways (Girardi et al., 2006) and prevents obstetrical complications by blocking activation of complement induced by APL targeted to decidual tissues (Girardi et al., 2004). On the other hand, low-dose aspirin improves pregnancy outcome in women with aPL by irreversibly blocking the action of cyclooxygenase in platelets, inhibiting platelet thromboxane synthesis, acting as a potent stimulator of interleukin-3 (IL-3), raising leukotriene production and preventing thrombosis of the placental vasculature (Fishman et al., 1995). Combination of heparin and low-dose aspirin is superior to aspirin alone in achieving successful pregnancies in women with recurrent pregnancy losses and non-thrombotic APS (Rai and Regan, 2006). Evidence of inflammatory-mediated tissue damage in placentas of APS patients suggests that therapy should also include prevention of inflammation (Salmon et al., 2007). Recent studies have suggested that heparin may exert direct effects on placental trophoblast, independently of its anticoagulant activity. Heparin abrogates apoptosis of primary first trimester villous trophoblast in response to treatment with the pro-inflammatory cytokines IFN-gamma and TNF-α (Hills et al., 2006). Heparin treatment did not produce a significant change in activin and inhibin levels, suggesting that the beneficial effect of heparin treatment was unlikely associated with significant alteration of these hormones in early APS pregnancy (Prakash et al., 2006). Di Simone et al. (1997) observed in trophoblast cells that a pharmacological dose of low molecular weight heparin significantly reduced APL and restored

GnRH-induced hCG secretion. Low aspirin doses also restored, at least partially, GnRH-induced hormone secretion. These findings provide another explanation of beneficial effects of both treatments in women with recurrent miscarriage and APS.

7. Future directions

Even when interventions toward inhibition of complement activation prevent thrombosis and miscarriage, conclusive data on the mechanisms of action in humans are lacking. It is possible that women with recurrent miscarriage, fetal losses or thrombosis belong to different APS subsets, making it necessary to develop new targeted therapies to address different forms of APS (Ruiz-Irastoza and Khamashta, 2005). Understanding the molecular pathways in endocrine–immune interactions in the human endometrium is crucial to understanding events such as blastocyst implantation and developing new therapies that can be used during pregnancy (Kayisli et al., 2004). In this context, compounds that promote endometrial differentiation such as P, hCG, and phosphodiesterase inhibitors may be useful in the management of recurrent pregnancy loss associated with APS, especially if conventional anticoagulation therapy has been ineffective (Francis et al., 2006). Of interest, combination treatment with prednisone, aspirin, folate, and P was associated with a higher live birth rate compared with no treatment in women with idiopathic recurrent miscarriages. There were no cases of IUGR or Cushing's syndrome (Tempfer et al., 2006). The combined therapy of P, folate, aspirin, and low weight heparin has not been tried in APS. Nowadays, prednisone use in APS patients is more harmful than beneficial in preventing pregnancy loss.

At the level of the decidua, P, E, and PRL have potential roles on proliferation, promotion, differentiation, and maturation of uNK cells. Disruption of any of these mechanisms may result in pregnancy loss (Dosiou and Giudice, 2005). Restoration of hormonal balance may improve pregnancy outcome in APS patients. In a murine model, pregnancy failure resulted from impaired P synthesis by the corpus luteum of the ovary associated with ovarian resistance to PRL effects with participation of TNF-α. Such links between innate immune activation (activated uNK cells, T-cells, aPL antibodies, complement, systemic immune activation by CD40 ligation, TNF-α) and reproductive endocrine dysfunction involving the hypothalamic-pituitary-gonadal axis with ovarian insufficiency may be relevant to pregnancy failure without inflammatory injury in decidual tissues. New therapies directed at innate immune and hormonal mediators are needed (Erlebacher et al., 2004; Salmon, 2004).

8. Conclusion

1. Experimental studies and clinical observations strongly suggest an abnormal immune–neuroendocrine interaction in rheumatic autoimmune diseases: SLE, RA, and APS.
2. The most important immunological modification during normal pregnancy, SLE and RA, are the Th1/Th2 shift. Th1, Th2 cytokines, and hormones have not been studied in APS.
3. Th1-mediated diseases such as RA improve and Th2-mediated diseases, like SLE, worsen during pregnancy due to Th1/Th2 shift. Decreased gonadal hormones and increased PRL have roles in activation of autoimmune diseases (Table 1).

Table 1
Immune–neuroendocrine alterations in pregnancy SLE, APS, and RA

Disease	Innate immune	Adaptive immune	Th1	Th2	C	E	T	P	PRL	hCG
Normal pregnancy	↑	↓	↓	↑	↑	↑	↑	↑	↑	↑
SLE-pregnancy	?	?	↓	↑	↓	↓	↓	↓	↑	↓
APS-pregnancy	?	?	?	?	?	?	?	?	?	↓
RA-pregnancy	?	?	↓	↑	↑	↑	↑	↑	↑	?

Key points

- During normal pregnancy, high levels of estrogen, progesterone, and prolactin participate in Th1 cytokines inhibition and Th2 cytokines enhancement.
- Abnormal immunoneuroendocrine interactions in pregnancy and SLE, APS, or RA may lead to adverse maternal–fetal outcome.
- New therapies directed toward immune response and hormonal mediators are needed in SLE, APS, and RA pregnancy.

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CHAPTER 17

Autoimmune Hypothyroidism and Hyperthyroidism in Systemic Autoimmune Disease

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Autoimmune disease can be divided into systemic diseases in which there are autoantibodies binding ubiquitously expressed antigens such as double stranded DNA in systemic lupus, tRNA synthetases in dermato- and polymyositis, or the spliceosome complex in mixed connective tissue disease and systemic lupus erythematosus (SLE). Except for Sjögren's syndrome, which is present in about 20% of persons with another systemic autoimmune disease, systemic autoimmune diseases do not tend to occur in the same individuals. This is in contrast to organ-specific autoimmune diseases, which occur together more than expected by chance. The presence of two or more organ-specific autoimmune diseases is generally considered a polyglandular autoimmune syndrome (Eisenbarth, 2004). In organ-specific autoimmune disease, there are circulating antibodies binding antigens generally expressed in only the affected organ.

Among the most common of organ-specific autoimmune diseases is autoimmune thyroid disease. Graves' hyperthyroidism is mediated by autoantibodies that bind and activate the TSH receptor on the surface of thyroid cells (Davies et al., 2005). This results in autonomous production of thyroid hormone and hyperthyroidism. Meanwhile, autoimmune thyroid disease can also result in hypothyroidism in the form of Hashimoto's thyroiditis in which there is destructive lymphocytic infiltration of the thyroid gland. In both Graves' and Hashimoto's disease, antibodies binding thyroid peroxidase or thyroglobulin are commonly found.

Autoimmunity and autoimmune disease are usually defined as such by the presence of antibody binding self-structures or the presence of lymphocytic infiltrates without infection. When considered together, autoimmune diseases are extremely common in the human population. However, the population is not uniformly affected. Young adult to middle-aged women are much more likely to have autoimmune disease than men with some diseases such as SLE, Sjögren's syndrome, and autoimmune thyroid disease having female to male ratios ranging from 10:1 to 15:1.

Thus, because of these shared demographic features, autoimmune thyroid disease and autoimmune rheumatic diseases are likely to be found together in adult women. This fact begs the question as to whether or not autoimmune thyroid disease occurs in systemic autoimmune rheumatic disease more often than expected, or simply occurs in rheumatic disease based on a similar target population. There are many studies confirming that thyroid disease is common among patients with systemic autoimmune diseases such as SLE, Sjögren's syndrome, scleroderma, or rheumatoid arthritis. However, there are only few data comparing

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patients to controls properly matched for age and gender.

1. Systemic lupus erythematosus

SLE is a prototype systemic autoimmune disease with protean clinical manifestations ranging from life-threatening renal, neurologic, hematologic, pulmonary, or heart disease to skin or mucous membrane involvement (Tan et al., 1982). Virtually all patients have antinuclear antibodies in their sera, while the majority of patients can be demonstrated to have antibodies that bind specific nuclear antigens (Kurien and Scofield, 2006). Young adult women are affected about 10 times more often than adult men.

Several uncontrolled studies have demonstrated that autoimmune thyroid disease is commonly found among patients with SLE (reviewed in Scofield, 1996). These publications documented that both hyperthyroidism and hypothyroidism occurred frequently in patients with SLE (Table 1), but did nothing to determine whether autoimmune thyroid disease occurs more commonly in SLE than in an age- and gender-matched population. For example, in one large and early study of 332 SLE patients, 22% had hypothyroidism while 0.9% had already been diagnosed with hyperthyroidism (Miller et al., 1987). In another study, 5% of patients with SLE, without known thyroid disease, had hypothyroidism upon screening (Kohno et al., 1989). Thus, despite the lack of controls, this body of work does emphasize that SLE patients often have autoimmune thyroid disease. Numerous case reports underscore the diagnostic difficulties found in patients with SLE who develop thyroid disease, or present with simultaneous SLE and hypo- or hyperthyroidism.

A few studies have examined thyroid disease among SLE patients and compared these findings to a control group. One study recruited 100 SLE patients and 100 age- and gender-matched controls. Among the patients, 6 had hypothyroidism while 2 had hyperthyroidism. Among controls, four and one had hypothyroidism and hyperthyroidism, respectively. These differences were not significantly different (Vianna et al., 1991). In another study from the United Kingdom of 41 SLE patients, 10 (24%) had hypothyroidism, but none had hyperthyroidism. Among 41 controls, 5 had hypothyroidism (Weetman and Walport, 1987). Again, SLE patients did not differ significantly from the controls. In one study of pediatric SLE with a control population, no differences were found in serum levels of T4, T3, or TSH (Ronchezel et al., 2001).

Thus, there is little evidence that SLE patients have an excess of autoimmune thyroid disease. The reverse, that is, whether or not patients with autoimmune thyroid disease have excess SLE, has been studied less often. Two older studies suggested there was no association of SLE with autoimmune thyroid disease when cohorts of the latter are studied for SLE (Masi et al., 1965; Mulhern et al., 1966).

SLE and autoimmune thyroid disease are associated in another interesting way. Treatment of Graves' hyperthyroidism can consist of anti-thyroid drugs such as methamizole or propylthiouricil

Table 1
Studies of thyroid disease in patients with SLE in which a control population was not studied

References	Patients	Hypothyroid	Hyperthyroid
Miller et al. (1987)	332	22 (22.0%)	3 (0.9%)
Kohno et al. (1989)	175	9 (5.0%)	0 (0.0%)
Vianna et al. (1991)	100	6 (6.0%)	2 (2.0%)
Park et al. (1995)	63	6 (9.5)	3 (4.8%)
Mihailova et al. (1999)	12[a]	0 (0%)	0 (0%)
McDonagh and Isenberg (2000)	215		
Pyne and Isenberg (2002)	300	17 (5.7%)	5 (1.7%)

[a] This study was of pediatric SLE patients.

(PTU), collectively known as thioamides. Both these drugs block uptake and organification of iodine by the thyroid gland, and their use is first line therapy for Graves' disease in most parts of the world (Pearce, 2006). An adverse effect of these medications is the induction of a lupus-like illness. A recent review described 12 reports of drug-induced lupus along with 30 cases of ANCA-positive vasculitis as a result of anti-thyroid medications. The drug-induced lupus patients were younger than the vasculitis patients, and renal involvement in the thioamide-induced lupus was uncommon (Aloush et al., 2006). There may be a familial component to the risk of developing this complication of PTU or methamizole. A teenage girl with Graves' disease, whose sister had SLE, developed drug-induced lupus after PTU (Yamada et al., 2002). In addition, Searles et al. (1981) reported a father–son pair who both developed drug-induced lupus with thioamide treatment. The mechanism by which thioamides induce a lupus-like illness is not known, but like other drugs that cause drug-induced lupus, the thioamides are substrates of neutrophil myeloperoxidase (Jiang et al., 1994).

Clinical manifestations in SLE patients have been associated with autoimmune thyroid disease. Spence et al. (2006) showed that among 102 infants born to mothers with anti-Ro and anti-La, 7 of 8 had neonatal lupus when the mother also had hypothyroidism, while only 45 of 78 had neonatal lupus when the mother was euthyroid. Thus, anti-Ro/La-positive hypothyroid mothers had a ninefold increased risk of their infants having neonatal lupus than did anti-Ro/La-positive euthyroid mothers (Spence et al., 2006). Mitral valve prolapse was more common among SLE patients with thyroid autoantibodies than among SLE patients without these autoantibodies (Evangelopoulos et al., 2003).

Finally, SLE and hypothyroidism may have a common pathogenic feature. A genetic linkage study of families with two or more SLE patients in which at least one of the SLE patients also had hypothyroidism has shown linkage with a marker on chromosome 5 at q14.3-15 (Namjou et al., 2005). This same marker also shows genetic linkage among Amish families with hypothyroidism, but no SLE (Allen et al., 2003). An independent study found a similar result for a different gene. A specific allele of a protein tyrosine phosphatase (PTPN22) was transmitted more frequently to offspring with both SLE and autoimmune thyroid disease than to controls (Wu et al., 2005). These findings suggest common genetic susceptibility genes for SLE and autoimmune thyroid disease. One possibility is that this putative gene predisposes individuals to autoimmune disease in general, while other genes or environmental factors determine the specific disease that is manifested. As yet, the gene or genes leading to SLE and/or hypothyroidism in the genetic interval at 5q14.3-15 is not identified.

2. Sjögren's syndrome

Sjögren's syndrome is a common systemic autoimmune disease in which the principal clinical features are dry eyes and dry mouth, associated with lymphocytic infiltration of the salivary and lacrimal glands. These patients can also have interstitial kidney disease, fibrotic lung disease, and vasculitis. Most patients with the disease have antibodies in the sera that bind the Ro (or SSA) and La (or SSB) proteins.

Sjögren's syndrome predominantly affects middle-aged to older women, the population also most affected by autoimmune thyroid disease (Vanderpumo et al., 1995). Thus, perhaps even more than SLE, one would expect autoimmune thyroid disease and Sjögren's syndrome to occur together more frequently than by chance alone. On the other hand, Sjögren's syndrome can be considered as an autoimmune epithelialitis in which polarized epithelial cells, such as those found in salivary gland and kidney interstitial tissue, are targeted (Mitsias et al., 2006). Thus in this regard, the thyroid could be considered a specialized polarized epithelial gland, and it could be hypothesized that the thyroid is a target of autoimmunity in patients with Sjögren's syndrome.

Similar to SLE, there are a number of studies documenting the degree of thyroid disease in patients with Sjögren's syndrome, but without a control population (Karsh et al., 1980; Lovisella et al., 1988; Hansen et al., 1991; Foster et al., 1993;

Perez et al., 1995). These studies used various classification or diagnostic criteria to determine the presence of Sjögren's syndrome, including older classifications such as the Copenhagen (Manthorpe et al., 1986) and the Fox criteria (Fox et al., 1986), as well as the newer Combined American-European Combined Classification Criteria (Vitali et al., 2002). Based on the varying sensitivity and specificity of the classifications, there may be up to a 10-fold difference in the number of identified cases of Sjögren's syndrome. Thus, comparisons across these studies are difficult. Given the idea that autoimmune thyroid disease is highly prevalent in older women, there is no surprise in the finding that up to a quarter of Sjögren's syndrome patients have autoimmune thyroid disease (see Table 2). For example, Karsh et al. (1980) found that 6 of 24 Sjögren's syndrome patients had previously diagnosed hypothyroidism, while another 7 had TSH elevation indicative of undiagnosed hypothyroidism. Another study found that 8 of 33 Sjögren's syndrome patients were hypothyroid and 2 had hyperthyroidism (Perez et al., 1995).

Four studies have examined Sjögren's syndrome and a control population for autoimmune thyroid disease. In one of these studies, 42 patients and 207 family members were studied along with 2779 historical controls. Four of 42 (9.5%) patients had autoimmune thyroid disease while 16 of 207 (11.4%) relatives did so (Foster et al., 1993). Compared to gender- and age-matched, but non-concurrent controls, there was significantly more autoimmune thyroid disease in the Sjögren's patients as well as their first-degree relatives (Foster et al., 1993). A confounding factor in this study was the possibility that investigation for thyroid disease was more intense in the Sjögren's patients and their relatives compared to the controls. A study from Turkey examined 53 primary Sjögren's syndrome patients and 53 age- and gender-matched controls for thyroid antibodies and thyroid disease. Neither antibodies nor thyroid disease were found in excess among the Sjögren's syndrome patients (Tunc et al., 2004).

In two of the studies with concurrent controls, opposite results were found. In one of these studies, 41 of 137 (30%) of Sjögren's syndrome patients had autoimmune thyroid disease, while only 5 of 120 (4%) age- and gender-matched controls had thyroid disease (D'Arbonneau et al., 2003). As discussed below, the control population had a remarkably low rate of thyroid disease. The other study with a matched control population found 36% of 120 Sjögren's syndrome patients and 27% of 75 age- and gender-matched controls had thyroid disease (Ramos-Casals et al., 2000). This difference was not statistically different.

Table 2
Studies of thyroid disease among patients with primary Sjögren's syndrome

References	Patients	Anti-thyroid Ig	AITD	Criteria	Controls
Karsh et al. (1980)	24	5 (21%)	13 (54%)	NA	ND
Loviselli et al. (1988)	8	NG	2 (25%)	NA	ND
Kelly et al. (1991)	100	40 (40%)	14 (14%)	Fox	ND
Bouanani et al. (1991)	26	26 (100%)	8 (31%)	Fox	ND
Hansen et al. (1991)	28	10 (36%)	5 (18%)	Copenhagen	ND
Perez et al. (1995)	33	16 (48%)	11 (33%)	NG	ND
Punzi et al. (1996)	119	22 (19%)	16 (13%)	ESCG	$n=27$, ?match
Foster et al. (1993)	42	14 (33%)	4 (9.5%)	Fox	Age, gender[a]
Ramos-Casals et al. (2000)	160	25 (165)	32 (20)	ESCG	Age, gender
D'Arbonneau et al. (2003)[b]	137	ND	41 (30%)	ESCG	Age, gender, neg serology
Tunc et al. (2004)	53	4 (8%)	2 (4)	ESCG	Age, gender

NG, not given; ND, not done; AITD, autoimmune thyroid disease, that is, both hypothyroidism and Graves' disease.
[a] Controls were not concurrent, but historical.
[b] Five of 120 (4%) age- and gender-matched controls had thyroid disease but controls were ANA-negative, likely eliminating many with autoimmune thyroid disease (see text for complete discussion).

These studies, which came to opposite conclusions, are different in several aspects. In the study that found a difference, the controls were not healthy but had either osteoarthritis or sciatica, and did not have autoantibodies (ANA, rheumatoid factor, anti-Ro, and anti-La all negative). In the study of Ramos-Casals et al. (2000), the controls were healthy and had no symptom that could be attributable to Sjögren's syndrome. In fact, the elimination of controls with a positive ANA may account for the very low prevalence of autoimmune thyroid disease in that many patients with either hypothyroidism or hyperthyroidism have a positive ANA. A summary of the findings of five studies of rheumatic disease serology in patients with autoimmune thyroid disease showed that 45 of 108 such patients were ANA-positive (see Scofield, 1996; Katakura et al., 1987; Baethge et al., 1988; Petri et al., 1991; Loviselli et al., 1992; McDermott, 1990). Thus, in the author's opinion, D'Arbonneau et al. (2003) found a low incidence of autoimmune thyroid disease in the control population because of the strategy of excluding controls with a positive ANA.

Sjögren's syndrome is found in about 20% of patients with SLE, rheumatoid arthritis, scleroderma, dermato/polymyositis, or primary biliary cirrhosis, and in this setting is referred to as secondary Sjögren's syndrome. One small study found that among 62 SLE patients, 7 of 8 with autoimmune thyroid disease had secondary Sjögren's syndrome and a total of 13 patients had secondary Sjögren's syndrome (Jonsson et al., 1987, 1988). In a more recent and much larger study of 1138 SLE patients, 2291 SLE-unaffected relatives and 581 unrelated controls, 169 SLE patients had a diagnosis of secondary Sjögren's syndrome, of whom 50 (29.6%) also had autoimmune thyroid disease. Among SLE patients without secondary Sjögren's syndrome, only 12.7% also had autoimmune thyroid disease (Scofield et al., 2007). Thus, the relative risk of autoimmune thyroid disease was 2.3 among those with secondary Sjögren's syndrome compared to those without secondary Sjögren's syndrome. Among relatives of the SLE patients, thyroid disease was found in excess in those diagnosed with primary Sjögren's syndrome with a relative risk of over 4.0 (Scofield et al., 2007). These studies demonstrated that in patients with SLE, secondary Sjögren's syndrome is associated with autoimmune thyroid disease. Secondary Sjögren's syndrome in the setting of a primary disease other than SLE has not been studied to the author's knowledge.

3. Rheumatoid arthritis

Rheumatoid arthritis is a common illness, affecting about 1% of the population worldwide. The disease has a female preponderance, but at three to four women for one man, this is not as great as that found in SLE or Sjögren's syndrome. The disease is characterized by symmetrical inflammatory polyarthritis, involving the metacarpophalangeal and metatarsophalangeal joints as well as the large joints. Patients can also have extra-articular manifestations including lung disease and vasculitis. About 80% of patients have rheumatoid factor (generally IgM antibodies that bind IgG) and/or antibodies directed against peptides containing citrullinated arginine (van Venrooij et al., 2006).

Autoimmune thyroid disease has been studied in multiple case series of rheumatoid arthritis patients (Buchanan, 1965; Caron et al., 1992; Bianchi et al., 1993; Chan et al., 2001; El-Sherif et al., 2004), and these studies were more likely to have control populations than those in cohorts with SLE or Sjögren's syndrome (see Table 3). The data are contradictory in some instances, but the preponderance of evidence shows that autoimmune thyroid disease is more common among patients with rheumatoid arthritis than among controls matched for race, age, and gender. For example, both Shiroky et al. (1993) and Al-Awadhi et al. (1999) found significantly more hypothyroidism in rheumatoid arthritis patients, 30 and 11% respectively, compared to 15 and 0%, respectively, in matched controls.

Family studies of rheumatoid arthritis have also examined the association of autoimmune thyroid disease with rheumatoid arthritis. For example, Thomas et al. (1983) studied the family history of 295 patients with rheumatoid arthritis as well as

Table 3
Controlled studies of autoimmune thyroid disease among patients with rheumatoid arthritis

References	Patients			Controls		
	n	Hypothyroid	Hyperthyroid	*n*	Hypothyroid	Hyperthyroid
Shiroky et al. (1993)[a]	91	29 (30%)	NG	93	10 (11%)	NG
Andonopoulos et al. (1996)	101	4 (4%)	6 (6%)	70	4 (5.7%)	NG
Al-Awadhi et al. (1999)	48	7 (15%)	0 (0%)	90	0 (0%)	0 (0%)
Caron et al. (1992)	131	44 (33.8%)	0 (0%)	27	??	0 (0%)

OA, osteoarthritis; AITD, autoimmune thyroid disease.
[a] All RA patients were females in this study; age- and gender-matched controls.

307 controls with osteoarthritis and found that 17.5% of first-degree relatives and 7.5% of second-degree relatives of the rheumatoid arthritis patients had autoimmune thyroid disease. Family histories of the osteoarthritis patients showed thyroid disease in only 2.2% of first degree and 1.6% of second-degree relatives. The differences were significant for both types of relationships (Thomas et al., 1983). This interesting study suggested that rheumatoid arthritis patients are at excess risk for autoimmune thyroid disease, and so are their close relatives. Several other studies examined autoimmune thyroid disease in families with rheumatoid arthritis (Grennan et al., 1986; Silman et al., 1989; Deighton et al., 1992; Taneja et al., 1993), but none included controls; and therefore, the studies did not confirm the findings of Thomas et al. (1983).

TNF-suppressing drugs, which have dramatically changed the therapy of rheumatoid arthritis, are associated with a lupus-like illness (De Bandt et al., 2005) as well as new-onset or worsening multiple sclerosis (Mohan et al., 2001). There have been two case reports of silent thyroiditis in rheumatoid arthritis patients treated with etanercept (Allanore et al., 2001; Andres et al., 2002). On the other hand, silent thyroiditis has been reported in association with rheumatoid arthritis without TNF-inhibiting treatment (Sakata et al., 1992). Thus, it is not known if suppression of TNF induces thyroiditis.

Rheumatoid arthritis and Graves' disease have both been linked or associated with the PTPN22 gene that encodes for a tyrosine phosphatase (Kyogoku et al., 2004; Velaga et al., 2004; Criswell et al., 2005; Skorka et al., 2005). The genetic associations of single nucleotide polymorphisms within the PTPN22 gene were compared recently between the two diseases (Heward et al., 2007). In the new study, haplotype analysis suggested that the genetic association within this gene differs between rheumatoid arthritis and Graves' disease. This led the authors to the conclusion that there are distinct mechanisms by which PTPN22 leads to susceptibility for either disease (Heward et al., 2007). To further complicate matters, PTPN22 has been associated with not only rheumatoid arthritis and Graves' disease, but also with SLE, hypothyroidism, type 1 diabetes mellitus, juvenile idiopathic arthritis, and vitiligo.

Several investigations have examined either thyroid autoantibodies in rheumatoid arthritis or the relationship of autoimmune thyroid disease to autoantibodies. Rheumatoid arthritis patients with a positive ANA test are likely to have hypothyroidism (Capsi et al., 2001). This is not surprising, given the fact that ANA-positivity is common among patients with either hypothyroidism or hyperthyroidism (reviewed in Scofield, 1996). These authors found an association between autoimmune thyroid disease and ANA, and not between thyroid disease and rheumatoid arthritis. Other reports found antibodies to thyroid hormones (Ruggeri et al., 2002) and thyroid-stimulating antibodies (Kirkegaard et al., 1987), but there thyroid abnormalities related to the presence of the autoantibodies. These findings were not confirmed by other investigators (Strakosch et al., 1978), and it is possible that the presence of rheumatoid factor in the tested sera led to false positive results in some immunoassays.

4. Scleroderma

Scleroderma, or systemic sclerosis, is an uncommon inflammatory disease that primarily affects the skin, lungs, and kidneys. The characteristic clinical feature of the disease is thickened, hidebound skin. Fibrosis of the lungs is a significant cause of morbidity and mortality in scleroderma, while renal crisis is largely prevented by present medical therapy. Middle-aged women are most commonly affected, but the ratio of women to men is not as great as that seen in SLE or Sjögren's syndrome. Circulating antibodies directed against topoisomerase I (anti-Scl70) may be found in patients with this disease.

There is ample evidence that autoimmune thyroid disease occurs commonly in scleroderma, but controlled studies are infrequent. As early as 1895, fibrosis of the thyroid gland was reported at autopsy of a patient with scleroderma (Singer, 1895). Subsequent pathological studies confirmed thyroid fibrosis in scleroderma (Rake, 1931; D'Angelo et al., 1969; Gordon et al., 1981), but the relationship of such fibrosis to thyroid dysfunction has not been defined.

Thyroid function has been examined in scleroderma, and the results resemble SLE. Hypothyroidism was common, and 26–65% of scleroderma patients were affected (Gordon et al., 1981; Serup and Hangdrup, 1986; Kahl et al., 1986; Schwartz et al.; De Keyer et al., 1990; Kucharz, 1993; Shalin et al., 2002; Biró et al., 2006). None of these studies, however, included a proper control group. As late as 2006, Biró and colleagues compared hypothyroidism in patients with scleroderma, but the controls consisted of the general population, matched for neither age nor gender. There have also been multiple case reports of Graves' disease in patients with scleroderma, also (see Kucharz, 1993; Anzai and Tajima, 1996).

Several studies have examined the relationship of serum thyroid hormone levels with clinical manifestations of scleroderma. Levels of serum T4 correlated with diffusion capacity (Shalin et al., 2002), and altered serum lipids, in particular elevated triglycerides, were present in scleroderma patients with hypothyroidism (Kotyla et al., 2006). This finding underscored the known effect of hypothyroidism on lipid metabolism and the high degree of hypothyroidism among scleroderma patients, but did not provide insight into pathogenic mechanisms.

Farzati et al. (2005) studied immune activation and the pathogenesis of scleroderma complicated by hypothyroidism. Peripheral blood mononuclear cells in 6 hypothyroid scleroderma patients had increased numbers of T helper cells producing interferon and IL-4, while 14 euthyroid patients were more likely to have increases of only interferon-producing CD4+ T cells. Another study examined polymorphisms in the AIRE gene among 19 patients with scleroderma, 22 patients with scleroderma and hypothyroidism, and 100 controls (Ferrera et al., 2007). An intronic polymorphism was correlated with both diseases, compared to controls. Mutations in the AIRE gene cause type 1 polyglandular autoimmune failure. The significance of the findings of this small study is not known.

5. Dermato/polymyositis

Dermatomyositis and polymyositis are inflammatory autoimmune diseases of the muscle with characteristic skin involvement in dermatomyositis. Patients commonly present with muscle weakness involving the proximal muscles and elevated muscle enzymes. These diseases can also involve the lung with fibrosis and restrictive airway disease. The lung disease is strongly associated with autoantibodies that bind tRNA transferases (Targoff, 2006).

Proximal muscle weakness is also common among patients with either hypothyroidism or hyperthyroidism. Furthermore, the presenting feature of hypothyroidism can be muscle weakness with elevated muscle enzymes, and hypothyroidism can be easily confused with polymyositis (Ciompi et al., 1994; Ohtsuka et al., 1999; Madariaga, 2002). More than 30 hypothyroidism patients have been reported with a polymyositis-like presentation (see Madariaga, 2002). On physical examination, delayed tendon reflex relaxation phase is a clue to hypothyroidism but this finding is not universal in hypothyroidism with a polymyositis-like

presentation, being found in only 41% of patients (Madariaga, 2002). Creatine kinase was elevated to greater than 2000 U/L on average in these patients, and thus did not distinguish hypothyroidism from true idiopathic inflammatory myositis (Madariage, 2002). However, measurement of myositis-specific antibodies and TSH should sort out diagnostic difficulties in such patients. Madariaga (2002) and others propose measuring TSH in all patients who present with muscle weakness and/or elevated muscle enzymes. Thus, hypothyroidism should be ruled out in all patients in whom inflammatory myositis is a consideration.

One condition may be confused with the other, but there are few data that support an association between inflammatory muscle disease and hypothyroidism. Several case reports have documented coexisting idiopathic inflammatory myositis with either Graves' disease (Kobayashi et al., 1997; Kamei et al., 2002) or hypothyroidism (Gamsky and Chan, 1988; Charalabopoulos et al., 2006), but there are no series that show the extent of autoimmune thyroid disease in idiopathic inflammatory muscle disease, much less studies with a control group for comparison. In other words, the association of inflammatory myositis and autoimmune thyroid disease is virtually unstudied.

6. Antiphospholipid syndrome

Individuals may have antiphospholipid antibodies in the setting of SLE or other rheumatic disease, or these antibodies may be found without other disease. Antiphospholipid syndrome is found in patients with these autoantibodies who also have hypercoagulation with either venous or arterial blood clots or recurrent fetal loss (Asherson et al., 2006).

There are only a few case reports of patients with both autoimmune thyroid disease and antiphospholipid antibodies or antiphospholipid syndrome (Dagenais et al., 1992; Nakamura et al., 1993; Hofbauer et al., 1996; Li et al., 1999; Takahashi et al., 2002). Like SLE, antiphospholipid syndrome can be seen as a complication of anti-thyroid drug therapy (Gaburri et al., 2005). No study of autoimmune thyroid disease in a cohort of patients with antiphospholipid syndrome has been reported. One study found no excess antithyroid antibodies in patients with antiphospholipid syndrome (Innocencio et al., 2003). On the other hand, examination of patients with thyroid disease for antiphospholipid antibodies has been performed (Nabriski et al., 2000). Of 130 patients studied, 109 had chronic hypothyroidism and 21 had Graves' disease. A total of 43% of each group had antiphospholipid antibodies but none had evidence of the antiphospholipid syndrome with the majority (48) having antibodies of the IgG class, while 9 had both IgG and IgM antibodies and 4 had only IgM antibodies (Nabriski et al., 2000).

7. Conclusion

In general, autoimmune thyroid disease and autoimmune rheumatic disease occur in the same demographic segment of the population. Thus, thyroid disease is seen frequently in patients with SLE, Sjögren's syndrome, rheumatoid arthritis, and scleroderma. However, convincing data that the degree of thyroid disease is above that expected by chance alone is scant.

Key points

- Autoimmune thyroid disease and rheumatic autoimmune disease occur in the same demographic population—namely, adult women.
- Therefore, autoimmune thyroid disease is found together with autoimmune rheumatic disease in many individuals.
- Patients with SLE and secondary Sjögren's syndrome are at risk for autoimmune thyroid disease compared to SLE patients without Sjögren's.
- Hypothyroidism with muscle disease and elevated CK can be confused with polymyositis.

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CHAPTER 18

Type 1 Diabetes Mellitus at the Crossroad of Polyautoimmunity

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1. Introduction

1.1. Definition

Type-1 diabetes mellitus (T1D) is a disease that affects all age groups, in contrary to the common conception of being a disease of the young. Its definition encircles two facts that are closely related. One is the destruction or dysfunction of β-cells mediated mostly by T lymphocytes (Foulis and Stewart, 1984). The other is a consequence of the previous, and consists in the absence of insulin in the blood and its direct metabolic outcomes (hyperglycemia, ketosis, gluconeogenesis, and wasting). Patients that suffer from this disease are known as insulin-dependent diabetics (IDD), thus requiring regular doses of exogenous insulin to replace the absence of their own.

The American Diabetes Association (ADA) since 1997 divided type-1 diabetes in 1a or immune mediated, and 1b or non-immune mediated, in order to combine both clinical findings and etiopathology (ECDCDM, 1997). Recent studies have divided this type of diabetes in two subgroups; one characterized by an early-rapid onset, while the other, frequently confused with type-2 diabetes, shows a late-slow onset (LADA) affecting people above their 30s (Narendran et al., 2005). We will thus focus on type-1a diabetes, reviewing its epidemiology in the world and especially in Latin America while evaluating the evidence in favor of an autoimmune origin and its relationship with other autoimmune diseases (ADs).

1.2. Epidemiology

Because of its highly heterogeneous epidemiology, it is preferred to describe the distribution of T1D according to geographic regions rather than to consider its incidence in the world (Karvonen et al., 2000). The DiaMond project developed by the WHO between 1990 and 1994 revealed epidemiologic information useful for the understanding of the disease; for example, the geographical clustering of T1D in isolated regions of Europe such as the Island of Sardinia or Finland, and in other regions around the world like Kuwait in the Middle East or New Zealand in Oceania. According to the report, the first 10 regions with the highest incidences of T1D were Sardinia [IT] (36.8 per 10^5), Finland (36.5 per 10^5), Sweden (27.5 per 10^5), Canada (24.2 per 10^5), Canterbury [NZ] (21.9 per 10^5), Norway (21.2 per 10^5), Portalegre [Portugal] (21.1 per 10^5), Northern Ireland [UK] (19.7 per 10^5), Kuwait (18.3 per 10^5), and Puerto Rico [US] (17.4 per 10^5). Besides the fact that some of these regions are considered or belong to developed countries, the other feature they have in common, excluding Kuwait and Puerto Rico, is their Caucasian

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inheritance. Owing to the fact that geographically all these regions are distant from one another and at some degree of isolation, it is reasonable to infer that there are indeed both genetic and environmental components explaining the development of the disease. Whether this hypothesis is supported by emigrational patterns or biologic plausibility requires more insight (Fig. 1).

In Latin America the incidence of T1D falls in a lower limit range with great heterogeneity from country to country. Again, ethnicity plays a key role in the explanation of this phenomenon, for countries with a higher percentage of Amerindians in their population present both lower incidence and prevalence of T1D (Collado-Mesa et al., 2004). In Latin American countries the lowest incidence of T1D are in Venezuela [Caracas] (0.1 per 10^5), Peru [Lima] (0.4 per 10^5), Paraguay (0.9 per 10^5), Mexico [Veracruz] (1.5 per 10^5), Chile [Santiago] (1.6 per 10^5), Cuba (2.9 per 10^5), and Colombia [Bogotá] (3.8 per 10^5). The ones with the highest incidence were Puerto Rico (17.4 per 10^5), Uruguay [Montevideo] (8.3 per 10^5), Brazil [Sao Paulo] (8.0 per 10^5), and Argentina [Avellaneda] (6.5 per 10^5). As portrayed in the previous list of incidences there are some inconsistencies with the conception that individuals from countries with a more prevalent Amerindian population are less likely to develop T1D. This could be attributed to risk factors other than ethnicity such as diet, habits, economic status, or any other environmental event previously associated with the development of the disease.

2. T1D is a predictable autoimmune disease

2.1. B-cell response

As described for many other autoimmune diseases (ADs) T1D is characterized for the presence of antibodies directed against self-antigens. However, these autoantibodies detected in T1D patients are not necessarily exclusive to the disease; they might be detected in the serum of patients with other ADs or vice versa (De Block et al., 2001a, b; Barker et al., 2005). The number of autoantibodies reported for T1D is numerous; however, they can be reduced to three highly important for their biologic plausibility and epidemiologic relevance (Pihoker et al., 2005; Wilkin, 1990). The first two, glutamic acid decarboxylase autoantibodies (GADA) and protein tyrosine phosphatase-like autoantibodies (IA-2A) correspond to a group of autoantibodies named islet cell autoantibodies (ICA) initially reported by Bottazzo and collaborators. The assay initially employed for the detection of ICA was based on indirect immunofluorescence, a semi-quantitative method (Bottazzo et al., 1978). Later on, further investigations demonstrated that a portion of ICA was specific against the small isoform of glutamic acid decarboxylase (GAD65) and against an inactive member of the protein tyrosine phosphatase family initially isolated from an insulinoma cell line (IA-2); these antigens have now been cloned and used for quantitative detection of GADA and IA-2A in T1D patients displacing the ICA test used initially (Chaillous et al., 1994; Myers et al., 1995). The third type of autoantibodies reported in T1D patients are those against insulin (IAA). They are measured by competitive radioimmunoassay or by ELISA, and differ from the first group of autoantibodies in their lack of diagnostic strength once the patient starts insulin therapy (Wilkin, 1990; Notkins and Lernmark, 2001).

There are two main implications of ICA in T1D, being the most important their importance as diagnostic tools to differentiate diabetes of an autoimmune origin from diabetes of any other cause. The recommendation is to use a combination of GADA and IA-2A for screening purposes instead of using ICA test alone for the following. The prevalence of the HLA high-risk genotype for developing T1D (DQB1 *02/0302) was found to be present in 13% of the Finnish population, but only 6% of them expressed ICA, from this small percentage only 8% progressed to clinical T1D for a total prevalence of 0.06% (Kimpimaki et al., 2001). In a population where the prevalence of T1D is close to 0.37% this test lacks efficiency. Recent studies are more interested in the unification of GADA and IA-2A for better comparisons between centers. A series of 46 laboratories in 13 countries performed an array of tests in samples

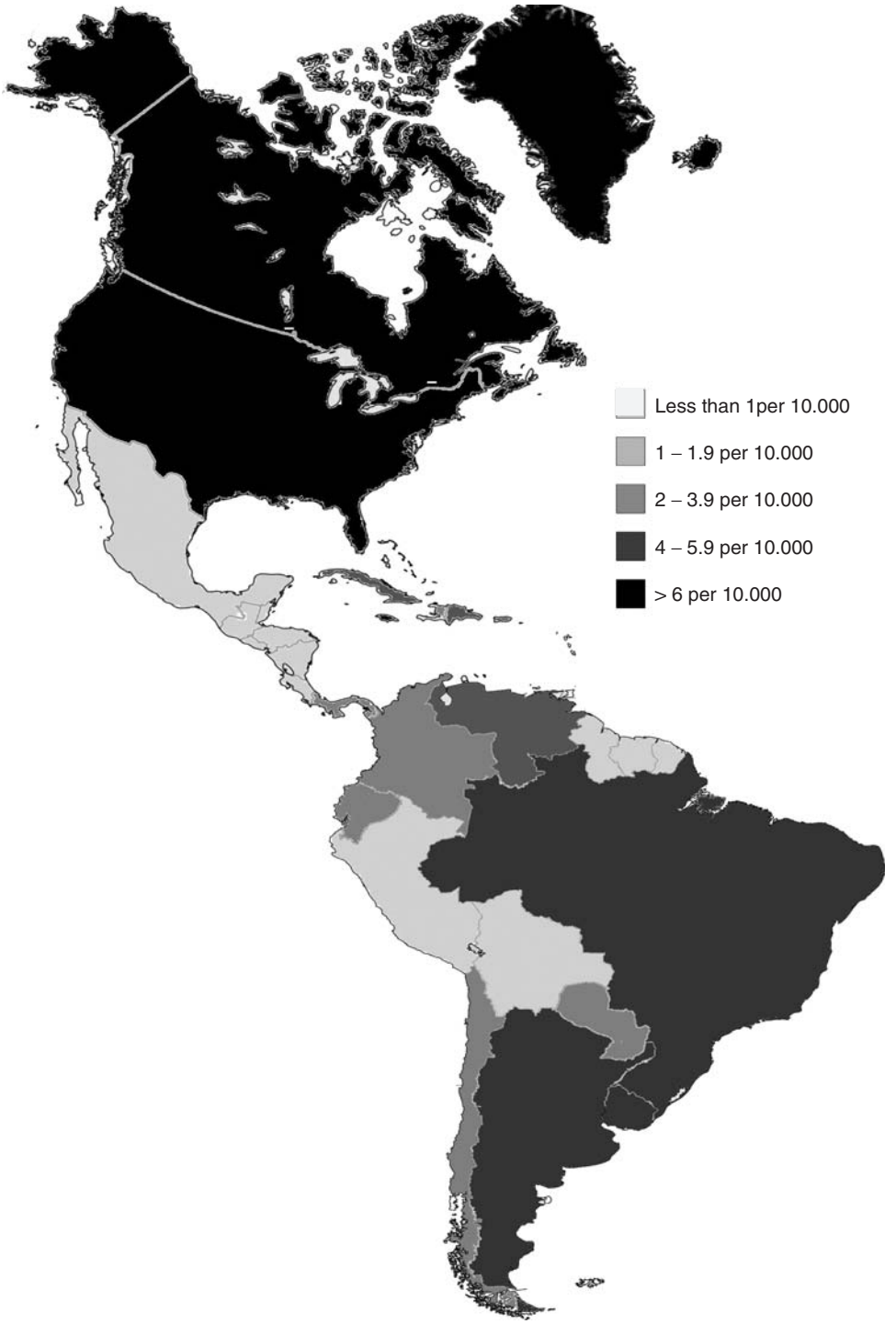

Figure 1. Map of geographical distribution of T1D in America. The countries with the highest prevalence are Canada, USA, and Puerto Rico, followed by Argentina, Brazil, and Uruguay. The ones with moderate prevalence are Venezuela, Cuba, Jamaica, and Dominican Republic. Those with lower prevalence are Chile, Paraguay, Ecuador, Colombia, and Panama. Finally, the ones with the lowest are Bolivia, Peru, Guyanas and Surinam, Haiti, Costa Rica, El Salvador, Honduras, Nicaragua Guatemala, and Mexico (Collado-Mesa et al., 2004).

from 50 newly diagnosed T1D patients and 50 controls (Bingley et al., 2003). The results showed a sensitivity for GADA of 77% ± 3 (median ± interquartile range), for IA-2A the sensitivity was 57% ± 3, and for IAA 14% ± 7. The specificity remained high among all three tests (96–100%) in spite of the differences in their sensitivity. These results suggest the use of GADA and IA-2A for the diagnosis of T1D in children with suggestive clinical features or for screening purposes in risk populations. For the question concerning the age of cut-off for testing, the consensus recommends to perform the test on patients younger than 15 years of age. This is sustained by the fact that only around 50% of patients with a positive test within the first year of age remain positive after 2 years (Kimpimaki et al., 2002).

The second important implication autoantibodies have in T1D is their importance in the prognosis of the disease. Prospective studies have proved ICA to be of poor prognostic value in T1D patients (Scholin et al., 2004). The natural course of islet cell antibodies in T1D patients is their progressive decrease in time. In the case of GADA, not only are they the most prevalent autoantibodies at baseline in T1D patients, but also have the lowest decreasing rate. Furthermore, when measuring plasmatic C-peptide, lower levels always correlate with the presence of GADA-positive sera both at diagnosis and 8 years later, making it a good candidate marker for the follow up of patients at risk for developing islet cell dysfunction (Scholin et al., 2004). On the other hand, studies on patient with type 2 diabetes (T2D) falling into the latent autoimmune diabetes in adults (LADA) variant (younger than 45, with a prevalence of GADA between 15 and 35%) revealed that 60% of them require insulin therapy within the following 10 years compared to 2% in the group of sero-negative T2D patients (reviewed in Tuomi, 2005).

2.2. T-cell response

Although T1D is characterized by the presence of antibodies against islet cell epitopes, the physiopathologic hallmark of the disease is insulitis, a T lymphocyte-mediated infiltration and destruction of β-cells (Foulis and Stewart, 1984). This fact represents a difficulty because it places an intermediate step between the estimators for β-cells destruction (i.e., markers for β-cells function (Sosenko et al., 2006), and for immunologic activity (Lethagen et al., 2002; Rosario et al., 2007)) and the true presence of insulitis. In an attempt to facilitate this approach, some authors developed minimally invasive procedures with conclusive results (Imagawa et al., 2001). In a cohort of 35 patients intervened, pancreatic biopsy was successful in 31, enough to show an association between insulitis and sero-positivity for either GADA or IA-2A (sensitivity 82.4%; specificity 66.7%). This association of insulitis and antibody sero-positivity is consistent with discoveries showing low-to-moderate affinity coefficients of synthetic 20-mer epitopes derived from GAD65, ICA65, and Proinsulin with HLA-DR4 and specially with HLA-DR3; this feature suggests an acquired property of these molecules to miss autoreactive T lymphocyte during thymic selection (Geluk et al., 1998). In agreement with this theory, HLA-A2 has shown increased reactivity to islet amyloid polypeptide, islet-specific glucose-6-phosphatase, and insulin-derived epitopes in peripheral blood mononuclear cells (PBMC) of T1D patients (Ouyang et al., 2006). Other studies on transgenic DQ8 + /mII–/mIE– mice arranged to constitutively express B7 in β-cells have shown that DQ8 in the presence of a costimulatory signal is enough to develop T1D, emphasizing its function in its development (Wen et al., 2000). In humans, PBMC from DQ8 prediabetic patients react against epitopes of Phogryn, but show no correlation with sero-positivity to IA-2A (Kelemen et al., 2004).

3. T1D and other autoimmune diseases (ADs)

3.1. Familial aggregation

From a genetic point of view, T1D is a complex disease; meaning that their inheritance does not follow a single-gene dominant or single-gene

recessive Mendelian law, and thus that it is polygenic. A primary characteristic of complex diseases such as T1D is that affected individuals tend to cluster in families (familial aggregation, also referred to as recurrence risk or λ). The aggregation of a phenotype is observed when a disease occurs at a higher frequency in the relatives of an affected individual when compared with the observed in the general population. Aggregation values $\lambda > 1.0$ are taken to indicate evidence in favor of a genetic component. In general, the higher the value is, the stronger the genetic component. The aggregation value of T1D is 15 (Wandstrat and Wakeland, 2001).

When estimating this excess in risk, two different approaches are possible. First, to treat the presence of the disease in relatives as a risk factor for the development of the disease in probands (type-I RR), or to evaluate whether the relatives of an affected index person have an excess risk of disease compared to the general population (type II RR) (Susser and Susser, 1989).

However, familial aggregation of a disease does not mean that a disease must have a genetic contribution. Non-genetic factors could have the same effect—besides sharing alleles, families share culture, behavior, diet, and environmental exposure. An alternative measure for a disease to be considered heritable is concordance, which is the probability that a pair of individuals will both have a certain characteristic, given that one of the pair has the characteristic. For example, twins are concordant when both have or both lack a given trait (Lewontin, 1982). The concordance rate for T1D in monozygotic twins ranges between 30 and 50%, while in dizygotic twins its falls to less than 13% (Wandstrat and Wakeland, 2001). This difference in the rate of concordance between monozygotic and dizygotic twins means that environmental factors play an important role in the development of disease.

Age remains an important topic in autoimmunity, not only because of the biological implications of aging on the immune system, but also because of the setback it constitutes for epidemiologic studies whose goal is the common origin of ADs (Anaya et al., 2006b). The problem with age in epidemiologic studies dwells in the fact that many ADs have different ages of onset (Anaya et al., 2007). For children, for example, the most common diseases are T1D, coeliac disease (CD), autoimmune thyroid disease (AITD), and vitiligo. The mosaic of ages constitutes one of the biggest problems in aggregation and co-occurrence studies, and can be summarized in two types of setbacks (Sloka, 2002). The first is the reduced probability of finding aggregation of ADs in affected patients when age differences are considerable; for example, a young child affected with T1D whose parents are young and restricted to a small group of ADs, the opposite scene being the old patient whose parents are already decease and whose children are too young to present a great number of these kind of pathologies. The second type of setback is the one arising when doing co-occurrence studies. The limitation arises when two diseases are so far apart on their time of diagnosis that will cause a necessary and rigorous follow-up in order to find co-occurrence in one patient (Sloka, 2002).

According to several reports the foremost AD found in relatives of T1D patients is AITD followed by T1D (Hanukoglu et al., 2003; Criswell et al., 2005; Anaya et al., 2006a). This relative risk is higher in siblings compared to other family members, a feature perfectly explained by the higher probability in siblings of sharing higher genetic information compared to parents or any other relatives (Anaya et al., 2006b, 2007). This familial predisposition to AITD and T1D can be explained by the considerable number of genetic variants shared by these two ADs. Among them the ones with the strongest association are the major histocompatibility (MHC) genotype HLA-DR3-DQ2/DR4-DQ8, PTPN22 +1858C/T, and CTLA-4 +49A/G (Vaidya et al., 2002; Criswell et al., 2005; Golden et al., 2005). Although there is no consistency in the results from one study to another, one constant characteristic they share is the strong association they have in families with multiple autoimmune phenotypes. Another important aspect of these polymorphisms is their relationship they have with systemic ADs.

For HLA the association is well known from some time now, being HLA-DR3 (DRB1*0301) in the case of systemic lupus erythematosus (SLE),

and HLA-DR4 (DRB1*0401 and *0404) for rheumatoid arthritis (RA) the groups (alleles) of risk (Criswell et al., 2005). On PTPN22, the results are variable especially in different ethnic groups, depending on the Asian ancestry. For CTLA-4, previous works have found significant differences in the +49G variant in RA patients with either T1D or AITD compared to controls (Vaidya et al., 2002), but results are variable for SLE in different ethnic groups (Heward et al., 1999; Barreto et al., 2004).

3.2. Polyautoimmunity in T1D

3.2.1. Organ-specific ADs

One of the features that shifted T1D conception toward an autoimmune origin was the increased occurrence of other ADs in this subgroup of patients. Since the relationship stated by Carpenter et al. (1964) with Addison's disease (AD) and AITD (autoimmune polyglandular syndrome type II) several others have been studied. However, although the list of diseases is enormous, three have brought new insights in the physiopathology of T1D, AITD, CD, and pernicious anemia–autoimmune gastropathy (PA-AG). Recent studies have found a close relationship between the co-occurrence of them and T1D. Commonly known as autoimmune polyglandular syndromes this mosaic of diseases set their grounds in clinical, immunologic, and genetic evidence. Therefore, in the clinical milieu the list is headed by AITD with a mean prevalence in T1D patients of 14% (Barker et al., 2005; Kordonouri et al., 2005), followed by PA-AG 8.6% (De Block et al., 1999, 2003, 2004), and finally CD with 5.8% (Barera et al., 2002; Hanukoglu et al., 2003; Mahmud et al., 2005). Immunologic evidence takes hand of the presence of multiple autoantibodies in T1D patients. As illustrated by Table 1, the most common are in order of frequency anti-peroxidase/anti-tyroglobuline (aTPO/TG), anti-parietal cell/anti-intrinsic factor (PCA/IFAbs), anti-tissue transglutaminase/anti-endomysial (tTG/EMAbs), and anti-andrenal cell/anti-25-OHase (AAA/25-OHAbs). Their frequency is in close relationship with the order of prevalence of the disease they represent making them good predictors of disease development. However, it is important to make clear that the gap between antibody positively and development of the disease remain ample, the overall ratio is between 1:5 and 1:2 (Barera et al., 2002; De Block et al., 2003; Kordonouri et al., 2005). Finally, as illustrated in Fig. 2, multiple

Table 1
Autoimmune diseases (ADs) associated with T1D

Disease	Autoantigens	Antibodies%	Disease%	RR	Genetic variants	Population at risk
AITD	TPO TG	12.9–29 7.0–14.4	26.4–28	28	HLA-DRB1*0405, CTLA-4[a], PTPN22[b], CYP27B1[c]	Women, puberty, pregnancy
PA/AG	PCA H+/K+ATPase	19.9–20.9 –	2.6–10.5	11	HLA-DQA1*0501-DQB1*0301, IL-8[d]	Children with the HLA variant
CD	EMA tTG	2.0–8.7 10	4.6–6.8	4–9	HLA-DQA1*0501-DQB1*0201, CTLA-4[a], TNFα[e], MIC-A[f]	Children with <3month of breast-feeding and/or gluten diet
AD	21-OH	1.0–1.6	<0.5	−80	HLA-DRB1*0404, MIC-A[f], PTPN22[b]	Non-determined

[a] CTLA-4 refers to the +49G allele.
[b] PTPN22 refers to the +1858T allele.
[c] CYP27B1 refers to the –1260C allele.
[d] IL-8 refers to the –251A allele.
[e] TNFα refers to the –308A allele.
[f] MIC-A refers to the 5 and 5.1 repetition of the GCT codon.

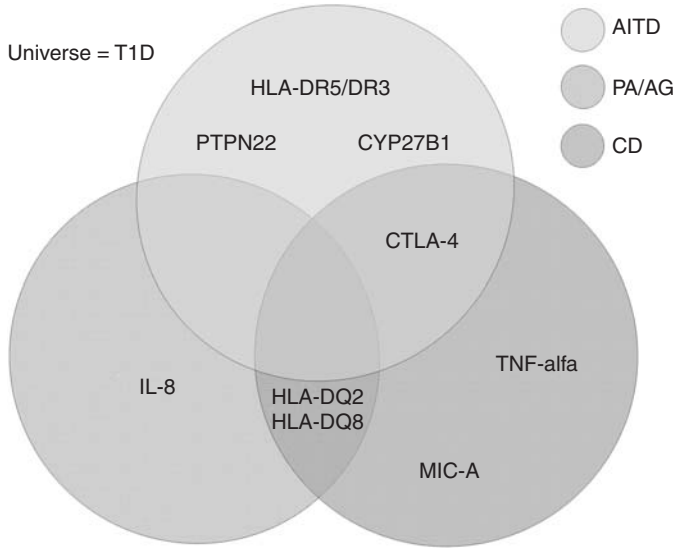

Figure 2. Genes influencing the risk to acquire T1D and other ADs.

genetic variants have been found associated with more than one disease. Merriman et al. (2001) elegantly showed that a locus (or loci) exists on human chromosome 18q12-q21 that influences multiple ADs including T1D, multiple sclerosis, and RA, and that this association might be conserved between species. The Wellcome Trust Case Control Consortium (2007) reported a common association of a single nucleotide polymorphism at 10p15 (rs2104286) with both T1D and RA mapping close to the alpha chain of the IL-2 receptor. The IL-2 receptor mediates IL-2 stimulation of T lymphocytes and is thereby thought to have an important role in preventing autoimmunity. So far the most strong genetic variants associated with co-occurrence are *HLA-DR3*, *HLA-DQ2/DQ8*, *PTPN22* 1858 T, *CTLA4* + 49G, and *TNF*-308A (De Block et al., 2003; Criswell et al., 2005; Golden et al., 2005; Sumnik et al., 2006).

Since AITD remains the most prevalent AD in T1D patients, a brief review on its clinical implications will be explained. Thus, the risk for developing AITD is associated in the general population with female gender, older age, family history of the disease, and the presence of thyroid-specific antibodies (aTPO, TGAbs) (Chistiakov, 2005). In T1D patients the same applies with the inconvenience that T1D itself is a risk factor for developing AITD. Thus, while the prevalence of AITD in the general population is close to 6.6% (Wu, 2000), in T1D patients it goes up to 12% (Barker et al., 2005). In order to identify a useful marker for the identification of this risk group, previous studies found that besides female gender and older age, GADA also are more common in patients at risk for AITD, which makes them good candidates for screening tests (De Block et al., 2001a, b; Barker et al., 2005). The recommendation is to follow GADA-positive patients with sensitive thyroid stimulating hormone (sTSH), aTPO, and aTG for they have proved close relationship with thyroid autoimmunity and ultrasonographic changes (Hansen et al., 2003; Kordonouri et al., 2005). Also, females going through critical hormonal changes in life require special consideration. In a prospective study with a mean 6-year follow-up, young females duplicated the incidence rate for developing AITD compared to young males during puberty (Kordonouri et al., 2005). Pregnant women with T1D also have increased risk for developing AITD with an estimated prevalence of 16% in the post-partum period (Gallas et al., 2002). In a recent

genome-wide scan study of T1D, several regions (including 18q22 and 18p11) showed association with autoimmune thyroid disease (Todd et al., 2007).

3.2.2. Systemic ADs

Systemic ADs seem to play almost a protective role for the development of T1D. This assumption is not arbitrary for, besides one genetic study that found T1D in 4 patients from a cohort of 123 (3%) with juvenile rheumatoid arthritis (JRA), other epidemiologic studies in search for simultaneous ADs in patients with RA, SLE, and Sjögren's syndrome (SS) were unable to find T1D in the pool of concurrent diseases (Coll et al., 1987; McDonagh and Isenberg, 2000; Vaidya et al., 2002; Lazarus and Isenberg, 2005). Although age would be a reasonable explanation for this, it is not the case, not only because the studies were performed on patients already having the old-age-related diseases, but also because some of them were retrospective which argues for completely the opposite effect (i.e., a higher chance to detect diseases prevalent at younger age). A possible explanation for this lack of association could be the size of the samples used for the studies, which never got higher than 230 patients; nevertheless, if a bigger sample is to be used, the prevalence of T1D would be close to that of the general population. On the other hand, under the immunologic point of view, the animal model for T1D, or non-obese diabetic (NOD) mouse, has shown an interesting feature that links this disease to SS. This mouse, besides developing T1D at an early age, also has a high risk for developing sialadenitis associated with a decrease in salivary gland's function, a feature reported in other murine models for T1D (Wen et al., 2000). Moreover, under the genetic point of view, associations with genetic variants in PTPN22 and CTLA-4 have turned out to be an interesting link between T1D and systemic ADs. For this reason, since both molecules are involved in immunomodulatory mechanism of tolerance, a feature that has proved to be ubiquitous throughout the immune system, there is no reason to exclude a systemic effect of these molecules in T1D patients.

Key points

- T1D is an AD that affects young patients mostly.
- There is a strong familiar correlation for the development of this disease, due to genetic and environmental factors that patients share with their relatives.
- Autoantibodies play a pivotal role in the diagnosis, prognosis, and associated comorbidities of this disease.
- There are immunologic, genetic, and epidemiological evidence in favor of an association between T1D and other ADs.
- There are four ADs that affect patients with T1D more frequently than the general population; AITD, PA-AG, CD, and AD, but the last one is uncommon. Careful scrutiny for these diseases is advised in patients with T1D and their relatives.

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PART III:

Hormonal Modulation in the Therapy of Autoimmune Diseases

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CHAPTER 19

Estrogen in Systemic Lupus Erythematosus: Lessons from the SELENA Study

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Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease with a strong female predominance. The incidence of SLE in women is 10 times higher than in men. Disease onset is more common after menarche and before menopause, implicating estrogens in the pathogenesis of SLE.

1. Estrogens in SLE

The autoimmune-prone NZB/NZW F_1 mouse is an excellent model for systemic lupus erythematosus (SLE). These mice produce autoantibodies at 2–3 months of age. These autoantibodies are deposited in the kidneys by 4–5 months of age, leading to nephritis and renal disease by 9–10 months of age (Holmes et al., 1961; Holmes and Burnet, 1963). This murine model has been used extensively to study the pathogenesis of SLE and to test potential interventions for controlling SLE. In the NZB/NZW mouse, removal of estrogen or the addition of androgens has been shown to lessen disease activity and improve survival (Roubinian et al., 1979; Steinberg et al., 1979). Other investigators have shown that exogenous estradiol given to non-autoimmune C57BL/6 mice resulted in elevated serum autoantibody titers (Verthelyi and Ansar Ahmed, 1997). Grimaldi et al. (2002) have shown that estrogen alters the survival and may increase autoreactivity of murine B-cells.

The effects of estrogen on the immune system are variable and may differ in B-cell and T-cell-mediated autoimmune responses. In another mouse model of lupus, MRL/lpr mice, a single lymphoproliferation (lpr) gene is mutated, leading to a lupus-like syndrome. Estradiol has been shown to worsen B-cell-mediated manifestations such as immune-complex glomerulonephritis in the MRL/lpr mouse model of SLE. However, T-cell-mediated presentations of renal vasculitis, periarticular inflammation, and focal sialadenitis improved (Steinberg et al., 1979).

2. Menstrual function in SLE

Menstrual irregularities are frequent in women with SLE. Menorrhagia, or increased menstrual flow, has been noted in 12–15% of patients. Associated risk factors for menorrhagia include the use of corticosteroids, nonsteroidal anti-inflammatory use, thrombocytopenia, and presence of anti-phospholipid antibodies (Harvey et al., 1954; Wallace and Dubois, 1987).

Temporary or permanent amenorrhea may be present in up to 25% of adult SLE patients. Immunosuppressive medications, particularly cyclophosphamide, may contribute to this dysfunction (see below). Increased SLE disease activity and autoimmune ovarian injury may also play a role in

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amenorrhea (LaBarbera et al., 1988; Boumpas et al., 1993).

3. Oral contraceptive use in SLE

The use of oral contraceptives in premenopausal women with SLE has been controversial. In the Nurses' Health Study, among 121,645 women who had previously used oral contraceptives, there was a small increase in the risk of developing SLE (relative risk [RR] 1.4; 95% confidence interval [CI] 0.9, 2.1) compared to women who had never used oral contraceptives (Sanchez-Guerrero et al., 1997). A case-control study found an association with the past use of oral contraceptives and risk of developing SLE (adjusted odds ratio [OR] 1.3; 95% CI 0.9, 2.1) (Cooper et al., 2002).

In retrospective studies, there was an increase in disease flares in patients treated with oral contraceptives. Jungers et al. (1982) noted flares of lupus nephritis in 9 of 26 women in the first 3 months of treatment with oral contraceptives compared to zero flares in 11 women treated with progestogen-only preparations. Other investigators noted no increase in flares in women taking oral contraceptives (Julkunen, 1991; Sanchez-Guerrero et al., 2005).

Results of the most definitive clinical trial on oral contraceptives in SLE were recently published (Petri et al., 2005). In this study, 183 women with SLE were randomly assigned to receive oral contraceptive (triphasic 35 μg ethinylestradiol/0.5–1 mg norethindrone) or placebo for 12 months. Patients were under 40 years of age if nonsmokers and under 36 years of age if smokers. All patients had clinically stable disease. Subjects were stratified by disease activity into inactive disease or stable active disease. Inactive disease was defined as a SELENA-SLEDAI score of 4 or less and a daily dose of oral prednisone ≤ 0.5 mg/kg; stable active disease was defined as a SELENA-SLEDAI score of 5–12 with a daily dose of prednisone ≤ 0.5 mg/kg. Patients were excluded if they had previously taken oral contraceptives for more than 1 month after SLE diagnosis or had a diastolic blood pressure >95 mmHg or a systolic blood pressure >145 mmHg on three measurements, history of deep venous thrombosis, arterial thrombosis, or pulmonary embolus, presence of anti-cardiolipin antibodies or lupus anticoagulant, history of gynecologic or breast cancer, history of myocardial infarction, hepatic dysfunction, hyperlipidemia, uncontrolled diabetes, migraine headaches, unexplained vaginal bleeding, or positive pregnancy test.

The severe flare rate was no different between the two groups (0.084 oral contraceptive and 0.087 for placebo, $p = 0.95$). This was less than the maximal clinically accepted difference between the groups. The rate of mild or moderate flares also did not differ between the groups. The incidence in the oral contraceptive group was 1.4 flares per person-year for the oral contraceptive group and 1.44 flares per person-year for the placebo group (RR 0.98; 95% CI 0.76, 1.26). These data are compelling and support the use of oral contraceptives in women with SLE without thrombotic risk factors who have stable SLE.

4. Hormone replacement therapy (HRT) in SLE

HRT in women with SLE is an important issue. As noted earlier, women with SLE may experience amenorrhea either secondary to the disease or to medications. The short-term use of HRT in women with SLE may be useful to alleviate symptoms such as vaginal dryness or hot flashes. The SELENA research group studied 351 menopausal women with SLE with inactive or stable disease activity (Buyon et al., 2005). Patients were assigned to receive HRT (0.625 mg of conjugated estrogen daily, plus 5 mg of medroxyprogesterone for 12 days per month) or placebo for 12 months. As in the oral contraceptive trial, patients were excluded if they had thrombotic events or lupus anticoagulant or anti-cardiolipin antibodies.

There was no difference in severe disease flare rates between groups (0.081 flare rate in HRT group, 0.049 flare rate in the placebo group, $p = 0.23$). However, mild to moderate flares were more frequent in the HRT group (RR 1.34; 95%

CI 1.07, 1.66). Thus, HRT replacement therapy may be considered in some women with SLE to alleviate menopausal symptoms.

5. Cyclophosphamide in SLE and risk of ovarian failure

Cyclophosphamide is used in the treatment of severe disease manifestations of SLE, including diffuse proliferative glomerulonephritis. Cyclophosphamide has been associated with amenorrhea and this effect seems to be dependent on cumulative dose. In a retrospective review of women treated for lupus nephritis, sustained amenorrhea developed in 9 of 23 women treated with 15 or more monthly pulses of cyclophosphamide compared to 2 of 16 with amenorrhea treated with seven monthly pulses of cyclophosphamide (Boumpas et al., 1993). The amenorrhea began in the first 7 months of treatment and even earlier in women over age 25 years. In a retrospective study of 77 pediatric patients with SLE, cyclophosphamide was associated with significantly reduced ovarian function (RR 2.8; 95% CI 1.7–4.8) (Brunner et al., 2006).

There are multiple methods for preserving ovarian function and fertility in women treated with cyclophosphamide. Oocyte cryopreservation, cryopreservation of ovarian tissue, or embryo cryopreservation are potential approaches under investigation. Patients can find information on these topics on the following website: www.fertilehope.org

Suppression of ovarian function during cyclophosphamide treatment may also reduce toxicity. This diminished ovarian function can be achieved with gonadotropin-releasing hormone (GnRH) agonists. There are multiple animal and observation studies in humans to suggest that these agonists have a protective role in ovarian preservation (Ataya et al., 1995; Blumenfeld et al., 1996; Pereyra Pacheco et al., 2001; Somers et al., 2005; Brunner et al., 2006). In a matched case-control study of 20 women treated with GnRH agonist administered monthly throughout the course of cyclophosphamide therapy, premature ovarian failure developed in 1 of 20 treated women compared to 6 of 20 controls (Somers et al., 2005). Side effects of GnRH agonist medications include hot flashes, injection site reactions, and osteoporosis.

6. Dehydroepiandrosterone (DHEA)

Androgens naturally suppress the immune system and DHEA, a weak androgen and intermediate compound in testosterone synthesis, has been evaluated in the treatment of SLE. Animal studies with NZB/NZW mice show that DHEA produces decreased antibody production and prolonged survival rates (Matsunaga et al., 1989; Yang et al., 1998). In a double-blind, placebo-controlled study of 28 SLE women given DHEA (200 mg/day) for 3 months, there were fewer flares, decreased SLE Disease Activity Index scores, decreased disease activity, and less prednisone use in the DHEA-treated patients. The main side effect was mild acne.

In a study exploring whether prednisone doses could be reduced to ≤ 7.5 mg per day for 2 months or longer while women with SLE were receiving 100 or 200 mg of DHEA, Petri et al. (2002) noted that 51% of patients responded in the 200 mg group compared to 29% in placebo ($p = 0.031$). In another study to determine whether DHEA impacts SLE disease activity, women with active SLE were randomized to 200 mg DHEA plus standard SLE treatment or placebo plus standard SLE treatment for up to 12 months. Eighty-six of one hundred forty-seven women (58.5%) in the DHEA group showed improvement or stabilization in activity indices compared to 65 of 146 in the placebo group (44.5%), $p = 0.017$ (Petri et al., 2004). However, DHEA has not been approved by the Food and Drug Administration for the treatment of SLE.

7. Summary

There is a strong female predominance in SLE, implicating estrogens in the pathogenesis of disease. However, recent studies have shown that

exogenous estrogens, given as oral contraceptives or HRT, do not increase the risk of mild or moderate flares in women with SLE. These and other studies provide physicians with new evidence for the rational use of estrogen therapy in women with SLE.

Key points

- There is a strong female predominance in SLE, implicating estrogens in the pathogenesis of disease.
- In the SELENA trail, oral contraceptives did not increase flares, but hormone replacement was associated with an increase in mild/moderate flares.
- Cyclophosphamide, used in the treatment of severe manifestations of SLE, has been associated with amenorrhea; however, suppression of ovarian function during cyclophosphamide treatment may reduce toxicity.

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CHAPTER 20

Hormonal Modulation of Autoimmune Diseases: Glucocorticoids

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1. Introduction

Glucocorticoids (GCs) are the most effective and most used immune modulators in systemic autoimmune diseases. In the context of this book four aspects are highlighted, mostly in the context of rheumatic diseases: (1) mechanisms of action; (2) the hypothalamic–pituitary–adrenal (HPA) axis in rheumatic diseases; (3) therapeutic use of GCs; and (4) adverse events of GCs.

2. Mechanisms of glucocorticoid actions

2.1. Cellular effects on immune cells

GCs mediate fascinating anti-inflammatory and immunomodulatory effects when used therapeutically (Buttgereit et al., 2005). There are many specific effects of the commonly used GC drugs, which include prednisone, prednisolone, methylprednisolone, or dexamethasone. However, for daily practice we can summarize their clinical actions as follows:

- Inhibit leukocyte traffic and access of leukocytes to the site of inflammation
- Interfere with functions of leukocytes, fibroblasts, and endothelial cells
- Suppress the production and actions of humoral factors involved in the inflammatory process.

Virtually all primary and secondary immune cells are more or less affected. A selection of the most important effects on the different cell types is listed below (Table 1 from Buttgereit et al., 2005).

2.2. Underlying molecular mechanisms

How do GCs manage to produce this broad spectrum of effects? Four different mechanisms have been identified to date: (Almawi, 2001; Buttgereit et al., 2002a, 2004, 2005; Schäcke et al., 2002; Adcock and Lane, 2003; Wikström, 2003) (Table 2 from Buttgereit et al., 2005). In the following paragraphs we present a short description of these effects. The interested reader can find more details in a recent review by Buttgereit et al. (2004).

The expression *cytosolic GC receptor (cGCR)-mediated classical genomic effects* refers to the classical mechanism by which GCs up- or downregulate the synthesis of specific regulatory proteins. To this end, the GC molecule binds to the cGCR. The activated GC/GCR complex in turn binds to specific DNA-binding sites

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Table 1
Important effects of glucocorticoids on primary and secondary immune cells

Monocytes/macrophages
↓ number of circulating cells (↓ myelopoiesis, ↓ release)
↓ expression of MHC class II molecules and Fc receptors
↓ synthesis of pro-inflammatory cytokines (e.g., IL-2, IL-6, TNFα) and prostaglandins
Increased production of anti-inflammatory cytokines (e.g., IL-10)
T cells
↓ number of circulating cells (redistribution effects)
↓ production and action of IL-2 (most important)
Granulocytes
↓ number of eosinophile and basophile granulocytes
↑ number of circulating neutrophils
Endothelial cells
↓ vessel permeability
↓ expression of adhesion molecules
↓ production of IL-1 and prostaglandins
Fibroblasts
↓ proliferation
↓ production of fibronectin and prostaglandins

Table 2
Mechanisms of glucocorticoid actions

cGCR[a]-mediated classical genomic effects
cGCR-mediated non-genomic effects
mGCR[b]-mediated non-genomic effects
non-specific non-genomic effects

[a] cGCR = cytosolic glucocorticoid receptor
[b] mGCR = membrane bound glucocorticoid receptor

(GC-responsive elements). In some cases, this results in the upregulation of the synthesis of certain proteins (Almawi, 2001; Schäcke et al., 2002). This process is called 'transactivation.' There are also negative GC-responsive elements, but inhibitory effects are rather mediated by the negative interference of the GC/GCR complex with transcription factors such as nuclear factor-kappaB (NFκB) and activator protein-1 (AP-1) (De Bosscher et al., 2000). Via this latter pathway, GCs downregulate the synthesis of, for example, pro-inflammatory cytokines such as interleukin 1 (IL-1), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNFα). This mode of action is termed 'transrepression.' It appears now that adverse effects are predominantly caused by the transactivation mechanism whereas anti-inflammatory effects are mostly mediated by transrepression mechanisms (O'Brien et al., 1995; Schäcke et al., 2002, 2004). This concept has led to a search for so-called dissociating GCs (or selective GC receptor agonists, SEGRAs) which induce predominantly the desired transrepression effects and have a diminished transactivation activity (Schäcke et al., 2002, 2004). These drugs are expected to have similarly effective anti-inflammatory actions but to be accompanied by weaker adverse effects.

Recently it became clear that GCs also mediate effects via so-called *cGCR-mediated non-genomic effects* (Croxtall et al., 2000). It has been suggested that following GC binding (i) the GC/GCR complex mediates the above-mentioned classical genomic actions, but (ii) there is also a rapid release of proteins (chaperones and co-chaperones such as Src) from the multi-protein complex that includes the cGCR (see below). These (co-)chaperones may be responsible for producing measurable effects within a few minutes (11 min). Very recently Lck and Fyn kinases have been identified as being rapid targets of GCs, mediated via a cGCR-mediated non-genomic pathway (Löwenberg et al., 2005).

It is suggested that GCs also mediate therapeutic-relevant effects via membrane-bound GCR (mGCR) (Lösel and Wehling, 2003; Bartholome et al., 2004). These effects are therefore termed as *mGCR-mediated non-genomic effects*. mGCR have been recently detected on the surface of human monocytes and B-lymphocytes. Moreover, evidence has been presented for a higher expression of mGCR on monocytes following immunostimulation with lipopolysaccharide (LPS) in vitro as well as in patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (Bartholome et al., 2004; Spies et al., 2006). In RA, mGCR are upregulated and positively correlated to disease activity (Bartholome et al., 2004). In SLE, no correlation to disease activity but a down-regulation of mGCR induced by high-dosage GC therapy was found (Spies et al., 2006). It should be noted, however, that origin and function of these receptors is still unclear and further experiments are needed to answer questions in this regard (Lösel and Wehling, 2003; Bartholome et al., 2004; Spies et al., 2006).

Finally, GCs at high concentrations are able to intercalate into cellular membranes, such as plasma and mitochondrial membrane, and change their properties (Buttgereit and Scheffold, 2002b). This is the basis for *non-specific non-genomic effects*, possibly mediated by changes in the cation transport through the plasma membrane and in the proton leak of the mitochondria. These physicochemical interactions with biological membranes are very likely to be the key to the very rapid immunosuppressive and anti-inflammatory effects of high-dose GCs (Buttgereit et al., 2004, 2005). These high GC concentrations are achieved by intra-articular GC injections or intravenous GC pulse therapy (Buttgereit et al., 2004, 2005).

3. The hypothalamic–pituitary–adrenal (HPA) axis in chronic autoimmune diseases

Several risk factors are involved in the pathogenesis of chronic inflammatory diseases, including genetics, chronic infections, sex hormones, and stress. Stress is an important risk factor in chronic autoimmune conditions. This is probably mediated by the impact of the stress-response system upon the close relationships between the HPA, the sympathetic nervous system (SNS), and the immune system (Straub and Cutolo, 2006a) (Fig. 1). Severe stress, associated with acute critical illness, activates the HPA axis and stimulates the release of cortisol from the adrenal cortex (Fig. 1). Cortisol is essential for general adaptation to stress and plays a crucial role in cardiovascular, metabolic, and immunologic/inflammatory homeostasis. The HPA axis is also activated in response to acute immune/inflammatory challenges—for example, from LPS or cytokines such as TNFα), IL-1, and IL-6 (Straub et al., 2005). After such acute challenges, there is activation at all levels of the axis, resulting in the release of GC from the adrenal cortex. These GC exert a negative feedback at the level of the pituitary, hypothalamus, and other higher brain centers to regulate further activation. In addition, GCs have a potent

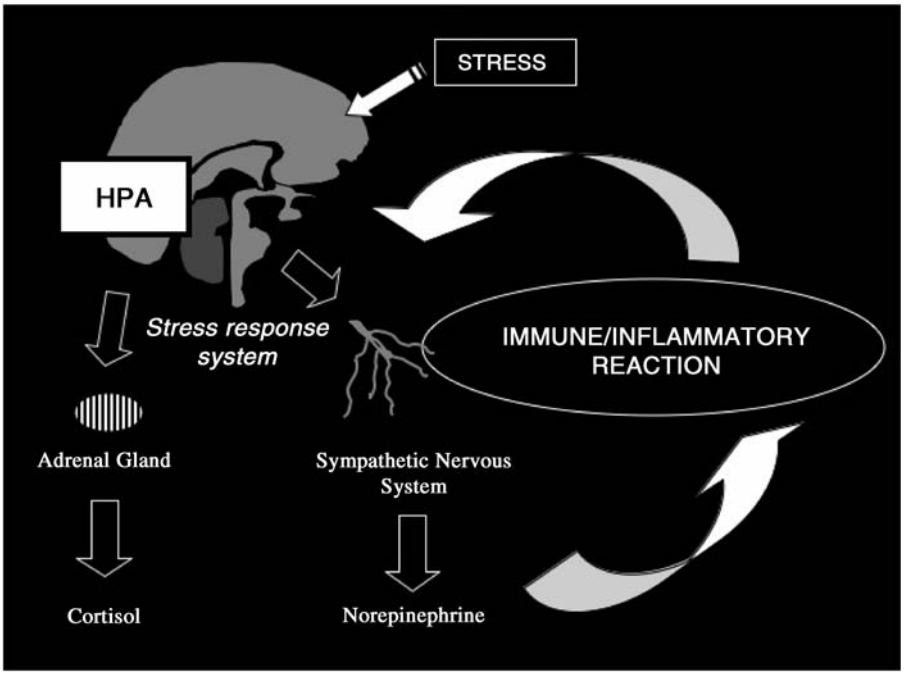

Figure 1. Severe stress, associated with acute illness, activates the hypothalamic–pituitary–adrenal (HPA) axis and stimulates the release of cortisol from the adrenal cortex. The HPA axis is also activated in response to acute immune/inflammatory challenge.

immunosuppressive effect, preventing further immune/inflammatory stimulation of the HPA axis and overactivity of the immune system.

This anti-inflammatory action is also crucial in chronic inflammatory disease conditions. However, during chronic illness, prolonged activation of the HPA axis tends to induce desensitization of the system. The end common result is a relative hypocortisolemia that can be detrimental to recovery. In polymyalgia rheumatica (PMR) and RA patients the responsivity of the HPA axis is diminished in relation to ongoing inflammation (Cutolo et al., 1999; Straub and Cutolo, 2006b). The integrity of the HPA axis in humans with chronic inflammatory rheumatic diseases such as RA is controversial, with some workers reporting major alterations, whereas others believe that dysfunction may be more subtle (Harbuz and Jessop, 1999). Arginine vasopressin (AVP) is believed to have an important role in GC regulation in RA (Chikanza et al., 2000). Recognition of adrenal dysfunction in chronically ill patients is complex, as confounding factors make laboratory results difficult to interpret.

Other hormones are also abnormally regulated in RA. In particular, levels of dehydroepiandrosterone (DHEA) and its sulphated form, DHEA-S, have been reported to be decreased in both RA and SLE (Masi et al., 1999). These adrenal steroids are the most abundant among those found in the human circulation. DHEA has been shown to inhibit secretion of pro-inflammatory cytokines such as IL-6 and TNFα in vitro (Straub et al., 1998). The naturally occurring decrease in HPA function, including androgen secretion, associated with ageing may be related to the increased incidence of autoimmune disease in aged individuals.

Another important phenomenon is the change in circadian rhythmicity in patients with RA (Cutolo et al., 2005), a feature which is considered as a marker of deleterious stress in experimental animals. In RA patients GC receptor abnormalities are also observed, with lower density of intracellular receptors and increased density of membrane-bound receptors independent of GC therapy (Bartholome et al., 2004). These abnormalities may lead to cortisol resistance, which has been proposed to play a role in some RA patients. With respect to HPA axis responsiveness, several studies reported inadequate ACTH/cortisol release after insulin-induced hypoglycemia and during corticotropin-releasing hormone test (Gutierrez et al., 1999). A subpopulation of patients with RA demonstrated impaired hypothalamic–pituitary regulation during dexamethasone test (Harbuz et al., 2003).

A recent study showed that RA patients have a subnormal ACTH response upon controlled psychological stress, as compared to controls (Dekkers et al., 2001). It has also been demonstrated that controlled exercise-induced release of cortisol was markedly decreased in patients with RA as compared to controls (Pool et al., 2004). In addition, controlled adrenaline infusion, simulating a stress response, leads to a fast decrease of cortisol serum levels in RA, but not in controls (Straub et al., 2002). Although the HPA axis is relatively robust, it seems that the activation of the stress response can lead to a paradoxical decrease of HPA axis mediators in RA patients as compared to healthy subjects. This would yield an overall pro-inflammatory situation (Fig. 1). Therefore, susceptibility to autoimmune chronic diseases may be related to an impaired responsiveness of the HPA axis; that is, an inability to mount an appropriate cortisol response to downregulate the immune system might allow the immune system to rampage unchecked and attack self.

Conversely, these observations help understand the positive effects of GC treatment in most patients with chronic immune/inflammatory diseases, as they may be, at least partially, acting as replacement therapy for the reduced endogenous cortisol production (HPA insufficiency).

4. Therapeutic use of glucocorticoids

GCs are widely used for several rheumatic diseases in various dosages. Often it is not clear what is meant with the semiquantitative terms used for dosages, such as ‘low’ or ‘high.’ Based on pathophysiologic and pharmacokinetic data, standardization has been recently proposed to minimize problems in interpretation of these generally used terms (Buttgereit et al., 2002a; Table 3).

4.1. Indications

An overview is given in Table 4, which only summarizes the general use and dosages of GCs in different rheumatic diseases. Without detailed description, some of the indications could be considered questionable at first glance. For instance, in systemic sclerosis, GCs, especially in high doses, are contraindicated because of the risk of scleroderma renal crisis (DeMarco et al., 2002), but they may be useful for myositis or interstitial lung disease. As can be seen, GCs are a basic part of the therapeutic strategy in myositis, PMR, and systemic vasculitis; for other diseases, GCs are adjunctive therapy or are not used at all. In osteoarthritis, for example, GCs are not given, except for intra-articular injection when there are signs of synovitis of the osteoarthritic joint (Gaffney et al., 1995). For generalized soft tissue disorders, GCs are not indicated, and for localized soft tissue disorders, they should only be used for intralesional injection.

Table 3
Terminology of dosages of glucocorticoids for use in rheumatology

Low dose	$\leq$7.5 mg prednisone or equivalent per day
Medium dose	$>$7.5 mg, but $\leq$30 mg prednisone or equivalent per day
High dose	$>$30 mg, but $\leq$100 mg prednisone or equivalent per day
Very high dose	$>$100 mg prednisone or equivalent per day
Pulse therapy	$\geq$250 mg prednisone or equivalent per day for one day or a few days

4.2. Glucocorticoid therapy in rheumatoid arthritis

4.2.1. Signs and symptoms

As can be seen in Table 4, RA is the only disease in which GC therapy is often started and maintained at a low dose as adjuntive therapy (Jacobs and

Table 4
Use of glucocorticoids in rheumatology in the general patient, excluding exceptional clinical situations

	Initial[a] oral dose				Intravenous/intra-articular Pulse injection
	Low[b]	Medium[b]	High[b]	Very high dose[b]	
Arthritides					
Gouty arthritis, acute	–	–	–	–	2
Juvenile idiopathic arthritis	–	1	1	–	1
Osteoarthritis	–	–	–	–	1
Pseudogout	–	–	–	–	2
Psoriatic arthritis	–	1	–	–	2
Reactive arthritis, Reiter's syndrome	–	–	–	–	1
Rheumatic fever	–	1	1	–	–
Rheumatoid arthritis	2	2	1	1	2
Collagen Disorders					
Dermatomyositis, polymyositis	–	–	3	1	–
Mixed connective tissue disease	–	1	–	1	1
Polymyalgia rheumatica	–	3	–	1	–
Sjögren's syndrome, primary	–	–	1	–	–
Systemic lupus erythematosus	–	2	1	1	–
Systemic sclerosis	–	1	–	–	–
Systemic Vasculitides					
In general	–	–	3	1	–

Symbols: –, rare use; 1, infrequent use or use for therapy-resistant disease, complications, severe flare, and major exacerbation; 2, frequently added to the basic therapeutic strategy; 3, basic part of the therapeutic strategy.

[a] Initial dose is the dose at the start of therapy and will often be decreased in time depending on disease activity.

[b] Dose in prednisone equivalents a day: low: $\leq$7.5 mg; medium: $>$7.5 but $\leq$30 mg; high: $>$30 but $\leq$100 mg; very high: $>$100 mg.

Bijlsma, 2005). GCs are highly effective for relieving symptoms in patients with active RA in doses of less than 10 mg/day; many patients become functionally dependent on this therapy and continue it long term (ACR Subcommittee on Rheumatoid Arthritis Guidelines, 2002). A review of 7 studies (253 patients) concluded that GCs, when administered for a period of approximately 6 months, are effective for the treatment of RA (Criswell et al., 2000). After 6 months of therapy, the beneficial effects of GCs seem to diminish. However, if this therapy then is tapered off and stopped, patients often—during some months—experience aggravation of symptoms. More patients are given GCs in the United States than in Europe (Bijlsma et al., 2003).

4.2.2. Radiologic joint damage

The disease-modifying properties of GCs in RA are particularly interesting. In 1995, joint-preserving effects of 7.5 mg of prednisolone given daily for 2 years in patients with RA of short and intermediate duration who were also treated with disease-modifying antirheumatic drugs (DMARDs) were described. The group of RA patients participating in this randomized, placebo-controlled trial was heterogeneous, not only in respect to disease duration but also to stages of the disease and the kind and dosages of DMARDs (Kirwan., 1995). In another trial published in 1997, patients with early RA were randomized to either step-down therapy with two DMARDs (sulphasalazine and methotrexate) and prednisolone (start 60 mg/day, tapered in six weekly steps to 7.5 mg/day and stopped at 26 weeks), or sulfasalazine alone. In the combined-drug-strategy group, a statistically significant and clinically relevant effect in retarding joint damage was shown, compared to the effect of sulfasalazine alone (Boers et al., 1997). In an extension of this study, long-term (4–5 year) benefits were also shown regarding radiologic damage following the combination strategy (Landewe et al., 2002).

In 2002, the results of the Utrecht study, a placebo-controlled trial on the effects of prednisolone in DMARD-naïve patients with early RA were published. Ten milligrams of prednisolone daily in these patients (who only got DMARD therapy as rescue) clearly inhibited the progression of radiologic joint damage (Van Everdingen et al., 2002). In this study, a 40% decreased need for intra-articular GC injections, 49% decreased need for acetaminophen use, and 55% decreased need for NSAID use was found in the prednisolone group, compared to the placebo group. In clinical trials evaluating the clinical effect of DMARDs or GCs, additional therapies should, thus, be taken into account. In an extension of this study, 3 years after the end of the study and 2 years after tapering off and stopping the prednisolone therapy, beneficial radiologic benefits of prednisolone were still present (Jacobs et al., 2006).

There are also negative studies on the effect of GCs on radiologic damage (Hansen et al., 1999; Paulus et al., 2000), but in early RA, evidence of joint-sparing properties of GCs seems convincing, classifying GCs as DMARDs. The jury is still out, however, on whether or not GCs can also inhibit progression of erosions in RA of longer duration. It could well be that there is a so-called window of opportunity in the treatment of RA (O'Dell, 2002). If this window exists, effective treatment of early RA with GCs, as well as DMARDs, may result in an effect that lasts for a long period of time, whereas if effective treatment starts later, this opportunity may be lost and erosive progression may continue.

However, as many questions yet remain to be answered, such as how the effect of GCs compares with that of high dosages of methotrexate or that of TNF blockers and for how long GCs should be prescribed and in what dosages, the final place of GC therapy in RA still has to be determined. Presently guidelines on how to use GCs as a DMARD and how to monitor GC therapy are being developed (Hoes et al., 2007).

5. Adverse events of glucocorticoids

The concept that GC therapy is associated with frequent and serious side effects is deeply rooted in most physicians' minds. However, recent systematic reviews of the literature have highlighted that there is scarce published evidence to support many of those fears, especially when low-dose therapy

(below 7.5 mg prednisone equivalent/day) is considered (Da Silva et al., 2006; Hoes et al., 2007).

Very few studies have ever focused on the toxicity of these agents and most of the evidence is derived from observational data or trials designed to assess efficacy. Collection and interpretation of the data is made extremely difficult by the enormous variety of doses, regimens, routes of administration (from low dose daily oral or inhaled, to intravenous pulses), duration of treatment, underlying diseases and comorbidities.

Confounding by indication is one of the major problems of the published literature. Many of the side effects commonly attributed to GC, can be manifestations of the disease they are used to treat (e.g., osteonecrosis, myopathy, atherosclerosis, and psychosis in connective tissue diseases). GCs, especially at higher doses, are usually selected for patients with especially aggressive diseases, exactly those-who are at higher risk of deleterious progression, comorbidity, and disease-related complications. This makes it difficult to clearly define the responsibility of GCs in many of these adverse events.

The toxicity profile may also differ between different molecules, as they are associated with varying degrees of potency, GC, and mineralocorticoid actions, as well as different degrees of receptor-mediated, genomic, and non-genomic effects. Clearly, the toxicity of GCs is highly dose-dependent. Some side effects, such as osteonecrosis, steroid myopathy, steroid psychosis, and pancreatitis are hardly ever seen with low-dose therapy. Table 5 presents the estimated incidence of side effects with low-dose GCs derived form a recent systematic review of the literature.

Table 5
Incidence of AEs in GC-treated patients with rheumatic diseases

Type of AE	Median: (25th–75th percentiles) (AEs per 100 patient years)
Cardiovascular (dyslipidemia, atherosclerosis, cardiovascular disease, water and electrolyte imbalance, oedema, renal and heart function, hypertension)	15 (3–28)
Infectious (viral, bacterial, skin infections)	15 (3–15)
Gastro-intestinal (peptic ulcer disease, pancreatitis)	10 (4–20)
Psychological and behavioral (minor mood disturbances, steroid psychosis)	9 (2–236)
Endocrine & metabolic (glucose intolerance and diabetes, fat redistribution, interference with hormone secretion)	7 (3–34)
Dermatological (Cutaneous atrophy, acne, hirsutism, alopecia)	5 (2–80)
Musculoskeletal (osteoporosis, osteonecrosis, myopathy)	4 (3–9)
Ophtalmological (Glaucoma, cataract)	4 (0–5)

This table shows the occurrence of adverse events of GCs in the rheumatic diseases studies of a general literature search ($n = 18$, total patients using GCs = 963) (Hoes et al., 2007).

5.1. Musculoskeletal

Osteoporosis is the most well-established side effect of GCs. Although this is strongly time- and dose-related, there is evidence to suggest that significant bone loss and increased fracture risk can be expected with doses as low as 2.5 mg/day (van Staa et al., 2002). Though the underlying disease itself may be associated with an increased incidence of fractures, the chronic use of GCs further enhances this increased risk by a factor of 2 (Da Silva et al., 2006). There is strong support for the concept that patients under GC tend to suffer fractures at a higher bone mineral density than controls, suggesting that these medications deteriorate bone resistance beyond densitometry-based expectations (Sambrook and Lane, 2001). GC-induced osteoporosis seems, on the other hand, reversible after stopping the medication and several drugs and other interventions have been shown to prevent GC-induced osteoporosis and associated fractures. Pathophysiology, epidemiology, and prevention of GC-induced osteoporosis have been the objective of many international reviews and recommendations (American College of Rheumatology ad hoc Committee on Glucocorticoid-Induced

Osteoporosis, 2001; The Royal College of Physicians et al., 2002; Geusens et al., 2004).

5.2. Cardiovascular and renal function

Observational studies in a variety of conditions (e.g., transplant, asthma, systemic lupus, RA) suggest that long-term treatment with moderate-to-high doses of GCs, are associated with deleterious changes in lipid profile and may increase the risk of coronary heart disease (Manzi et al., 1997). These effects seem to be dose-dependent. However, these results may be confounded by indication, as some of these conditions (e.g., SLE and RA) are, themselves associated with increased risk of atherosclerosis. The association between elevated C-reactive protein and accelerated coronary artery disease, opens the possibility that GCS may actually reduce atherosclerotic disease in the context of inflammatory diseases. Actually, data from RA cohorts show that disease activity is associated with an unfavorable blood lipid profile, which can be improved by effective treatment (including GC treatment) (Boers et al., 2003).

Detrimental effects on renal function are frequently feared. However, synthetic GCs have little mineralocorticoid effects, and their administration increases glomerular filtration rate and induces kaliuresis and natriuresis (Whitworth, 1987). Such effects are referred as the basis for positive effects observed with GCs in patients with heart failure (Liu et al., 2006). Nevertheless, hypertension is a well-demonstrated adverse effect of GCs, observed in about 20% of patients exposed to exogenous GCs (Manzi et al., 1997). This is dose related and is less likely with medium- or low-dose therapy.

5.3. Endocrine and metabolic adverse effects

GC-related hyperglycemia is dose-dependent. However, even doses as low as 0.25–2.5 prednisone equivalent per day have been associated with increased risk of starting antidiabetic medication (Gurwitz et al., 1994). Family history of diabetes mellitus, increasing age, obesity and previous gestational diabetes mellitus, have been pointed out as risk factors to develop new-onset hyperglycemia during GC therapy (Hirsch and Paauw, 1997). This is usually rapidly reversed upon GCs interruption, but some patients will go on to develop persistent diabetes (Hricik et al., 1991). Administration of GCs to diabetic patients requires careful control of glucose metabolism and adaptation of antidiabetic therapy. There are no preventive measures apart from the use of lower doses of GCs.

Redistribution of body fat (centripetal fat accumulation with sparing of the extremities) and weight gain are common side effects of even low-dose GCs. Centripetal fat accumulation with sparing of the extremities is a characteristic feature of patients exposed to long-term excessive GC. It is frequently seen even with low-dose GCs.

Chronic pharmacological doses of GCs inhibit linear growth in children (Allen, 1996). Prevention should include consideration of the dose, type, and regimen of steroid used. Chronic administration of human growth hormone can counteract the effects of GCs upon growth and body composition (Bechtold et al., 2001).

5.4. Cutaneous

Clinically relevant side effects on the skin, such as cutaneous atrophy, purpura, striae, easy bruisability, impaired wound healing, steroid acne, and hair effects have been reported to affect over 5% of those exposed to ≥ 5 mg prednisone equivalent for ≥ 1 year (Wolverton, 2002).

5.5. Ocular

Reports on the frequency of cataract with long-term low-dose systemic GC therapy are scarce. In a group of 25 RA patients treated with 5–15 mg/day of prednisone (mean 6.1 ± 3.1) for 6.2 ± 4.6 years, had a prevalence of cataracts of 15%, compared with 4.5% of matched RA controls not using prednison (Saag et al., 1994). There is no evidence that alternate-day therapy reduces the risk.

Elevation of intraocular pressure with GC administration is common but highly variable between individuals. In the general population, 18–36% of those exposed to GCs will experience an increase in intraocular pressure. Patients with diabetes mellitus, high myopia, and relatives of those with open-angle glaucoma are reported to be more vulnerable to GC-induced glaucoma (Tripathi et al., 1999).

5.6. Infections

The incidence of infectious complications of low-dosage GCs may be overestimated. In a meta-analysis of 71 trials involving over 2000 patients with different diseases and different dosages of GCs a twofold increase in the relative risk of infection was found. However, trials with low-dose therapy in RA patients showed no increased risk (Stuck et al., 1989). Some of the risk may be related to the underlying disease, as in systemic lupus.

5.7. Psychologic and behavioral disturbances

Psychological and behavioral disturbances are thought to affect 5–6% of all patients treated with GCs (Gourley et al., 1996). However, most cases are associated with high doses of GCs and the influence of the underlying disease, such as SLE, is frequently difficult to exclude.

GC treatment has been associated with a variety of low-grade disturbances, such as depressed or elated mood (euphoria), irritability or emotional lability, anxiety and insomnia, memory, and cognition impairments. Most studies relate these outcomes to doses of ≥ 80 mg of prednisone equivalent per day and they seem to be rare with doses of less than 20–25 mg prednisone equivalent per day (Reckart and Eisendrath, 1990).

5.8. Prevention

With the exception of osteoporosis and growth retardation, there is little sound evidence on which to support recommendations for the prevention of GC-associated side effects. An evidence-based approach to this topic may be found in recent recommendations produced by a working party of EULAR (Hoes et al., 2007).

6. Conclusions

GCs are still the most effective and most used immune moderators in systematic autoimmune rheumatic diseases. In this chapter an update on its pathophysiological role and use is given.

Key points

- Glucocorticoids influence every cell in the immune system.
- Glucocorticoids exert genomic as well as non-genomic actions.
- Glucocorticoids may exert their effect by transrepression mechanisms, associated with anti-inflammatory effects as well as by transactivation mechanisms, associated with unwanted adverse events.
- Glucocorticoids play an essential role in the sympathetic nerve system driven stress response.
- Changes in the hypothalamic–pituitary–adrenal axis influence the level of cortisol and vice versa.
- Some patients with rheumatic diseases, especially RA, have a reduced endogenous cortisol production (HPA-axis deficiency?).
- Therapeutic use of low-dose GCs in RA influences signs and symptoms in the short run, but have also a beneficial long-term effect on the progression of erosive changes on radiographs of hands and feet.
- Adverse effects of glucocorticoids are many, but many adverse effects of low doses are quite preventable and manageable.
- In early RA, the balance between advantages and disadvantages of low-dose GCs is beneficial.

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CHAPTER 21

Estrogen and Prolactin: Contributions to Autoimmunity in Murine Models of Systemic Lupus Erythematosus

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Hormones and the immune system interact through a number of pathways (Grossman, 1984; Besedovsky and Del Rey, 1996), and understanding the stimulating properties of individual hormones and their interactions in autoimmunity affords the opportunity to design relatively nontoxic treatments to change hormone concentrations and thereby treat autoimmune illnesses. This chapter will discuss the immunostimulating properties of estrogen and prolactin in murine models, as well as the interactions between estrogen and prolactin, in the context of treating the autoimmune illness, systemic lupus erythematosus (SLE).

1. Effects of estrogen in murine models of lupus

Sex hormones play an important role in regulating the severity of disease in the F_1 hybrid New Zealand Black (NZB) × New Zealand White (NZW) (NZB/NZW) mouse, a model of SLE that spontaneously develops antibodies to double-stranded DNA (anti-ds DNA) and dies early with immune complex glomerulonephritis. Disease in females starts early and progresses rapidly, and females die on the average 4 months earlier than males (Andrews et al., 1978). Of interest, serum estradiol and prolactin concentrations in the NZB/NZW model are comparable to mice that do not develop autoimmune disease (Brick et al., 1985; Walker et al., 1994) and females do not have abnormal estrogen metabolism that would alter 2-hydroxylated or 16-hydroxylated products (Baer and Green, 1990).

Treatment with estrogen accelerates disease in weanling NZB/NZW mice (Walker and Bole, 1973). Roubinian et al. (1978, 1979) and Steinberg et al. (1979) gave castrates pharmacologic doses of hormones in the form of crystalline implants containing 6–7 mg of estradiol-17β. Recipient mice of both sexes had stimulated autoantibody production and died prematurely (Melez et al., 1978; Roubinian et al., 1978, 1979; Steinberg et al., 1979; Siiteri et al., 1980). Unexpectedly, surgical oophorectomy did not reduce the severity of lupus in NZB/NZW females (Roubinian et al., 1979). Estrogen implants in these early experiments (Roubinian et al., 1978, 1979; Steinberg et al., 1979) released extremely high concentrations of circulating estradiol, resulting in fatal estrogen toxicity (Melez et al., 1978; Carlsten et al., 1989; Walker et al., 1992; Verheul et al., 1995). Newer dosing regimes (Brick et al., 1985; Carlsten and Tarkowski, 1993), however, mimicked naturally occurring levels of estrogen and these treatments did shorten longevity in NZB/NZW hybrids and first-generation offspring of NZB/NZW × NZB backcross mice.

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Gender, and possibly female hormones, affected severity of disease in the SLE models developed by Wakeland (Mohan et al., 1999; Morel et al., 1999), in which specific lupus susceptibility genes were introduced into C57BL/6 mice by congenic matings. Bicongenic B6.NZMc1/c7 females that expressed both the *Sle 1* gene (high titers of antinuclear antibodies) and the *Sle 3* gene (glomerulonephritis) developed severe disease and very high levels of anti-ds DNA. It therefore appeared that the effects of female gender promoted the phylogenetic expression of specific genes.

Experiments with BALB/c mice, which develop autoantibodies with increased age (Van Griensven et al., 1997), further emphasized the propensity of estrogen to stimulate the autoimmune response in a murine model predisposed to autoimmunity. Mice of both sexes were implanted with capsules containing 2–3 mg of estradiol-17β and immunized 3 months later with the 16/6 Id idiotype of human anti-ds DNA. The mice developed high titers of antibodies to ds-DNA (Blank et al., 1990), and treatment with either tamoxifen or anti-estradiol antibody decreased the severity of subsequent proteinuria and protected against immune complex deposits in renal glomeruli (Dayan et al., 1997).

2. Immunologic imprinting of the fetus

The autoimmune stimulating properties of estrogen extend to prenatal life. In C57BL/6 mice, which do not ordinarily develop autoimmune disease, treating the dam with estrogen at 14–16 days of gestation resulted in offspring that had sialoadenitis and increased plaque forming cell responses to bromelain-treated erythrocytes (Ansar Ahmed et al., 1989; Talal et al., 1992). Immunologic imprinting in utero also affected the subsequent course of autoimmune disease in NZB/NZW mice. In the NZB dam and her NZB/NZW fetuses, production of steroid hormones was found to be regulated differently compared with ''nonautoimmune'' mice. Male NZB/NZW fetuses, which are destined to develop into adults that will live longer than NZB/NZW females, developed in an intrauterine environment containing abnormally high levels of estradiol, but the concentration of testerone in the testicles and placentas of the male fetuses was unexpectedly low (Keisler et al., 1995). These findings suggested that estrogen concentrations in the prenatal environment could affect the course of autoimmunity after the fetuses develop into adults. Estrogen also exerted a paradoxical immunosuppressive effect in newborn female NZB/NZW mice. A single injection of high-dose estradiol, given within 2 days of birth, increased longevity so that lifespans in the estrogen-exposed females resembled lifespans in untreated NZB/NZW males (Yamaguchi et al., 2003).

3. Estrogen receptors in murine lupus

Estrogen has powerful effects on the immune system through mechanisms that are regulated differently through two types of estrogen receptors. Mice from nonautoimmune lineages that were knockout for the estrogen α-receptor gene had immune complex glomerulonephritis by 1 year of age with proteinuria, destructive infiltration of B-cells in the kidney, and circulating anti-DNA antibodies (Shim et al., 2004a). In contrast, estrogen β-receptor knockout mice spontaneously developed myeloproliferative disease that resembled chronic myeloid leukemia with lymphoid blast crisis (Shim et al., 2003). Therefore, the presence of the estrogen α-receptor was associated with suppression of estrogen-induced autoimmunity. This finding contrasted with data from NZB/NZW mice. Treatment of castrated females with propyl pyrazole, a selective estrogen α-receptor agonist, accelerated disease, and recipients had early albuminuria, elevated anti-DNA antibody levels, and early mortality. In contrast, the estrogen β-receptor activator, diarylpropionitrile, decreased IgG2b anti-DNA. It therefore appeared that the β-receptor had immunosuppressive properties in this animal model (Li and McMurray, 2007).

Immunostimulatory effects of estrogen are expressed in B-cells in BALB/c mice, which have a genetic background that predisposes to loss of

B-cell tolerance. Diamond and associates (Offen et al., 1992) generated transgenic mice that express the γ2b heavy chain of a nephritogenic anti-DNA antibody on a BALB/c background. These mice are normally able to maintain tolerance by deleting DNA-reactive B-cells that arise in the immature repertoire, but long-term treatment with a physiologic dose of estradiol caused the mice to develop circulating anti-DNA antibodies and glomerular immune complexes. Estradiol was thought to have stimulated autoimmunity by increasing the resistance of transitional B-cells to apoptosis through upregulation of the anti-apoptotic protein, Bcl-2, and inhibitory signaling molecules CD22 and SHP-1. In estradiol-treated transgenic mice, there was a tenfold increase in marginal zone cells. Marginal zone B-cells were activated to secrete high affinity and potentially pathogenic anti-DNA antibodies, and were not susceptible to regulation by T-cells (Bynoe et al., 2000; Grimaldi et al., 2001, 2005). Treatment of the transgenic mouse model with the estrogen receptor blocker, tamoxifen, prevented the appearance of lupus. When tamoxifen was given in conjunction with estradiol, apoptosis was not affected. Autoreactive B-cells expanded but were anergic, and the mice did not develop anti-DNA antibodies or glomerular IgG deposits (Grimaldi et al., 2005).

4. Diet therapy for murine lupus

The observation that estrogen can stimulate the immune system led to an attempt to control autoimmune disease with diet. Aromatase-knockout (ArKO) mice are unable to make estrogen. ArKO mice raised on a phytoestrogen-free diet, however, developed B-cell hyperplasia and destructive leukocyte infiltrates in the salivary glands that resembled Sjogren's syndrome. The lesions were completely absent if the mice were fed a diet with normal levels of phytoestrogen. These results suggested that endogenous estrogen paradoxically protects against autoimmune exocrinopathy, and the study showed that phytoestrogens in food can play a role in replacing endogenous estrogenic hormones (Shim et al., 2004b).

Indole-3-carbinol, in contrast, appeared to be protective in the setting of spontaneous autoimmune disease. Indole-3-carbinol is abundant in cruciferous vegetables and shifts estrogen metabolism away from mitogenic 16α-hydroxyestrone, which may fuel disease activity, and toward less estrogenic metabolites. When indole-3-carbinol was fed to weanling NZB/NZW females, 80% of the treated mice were alive at 1 year of age compared to 10% of controls. Mice that received indole-3-carbinol from the age of 5 months were all alive at 1 year. The ratio of urine 2α-hydroxyestrone to 16α-hydroxyestrone was increased, reflecting a shift in estrogen metabolism that favored production of metabolites with less estrogen activity (Auborn et al., 2003).

5. Hormone manipulation to treat murine SLE

The aromatase inhibitor 4-hydroxyandrostenedione, which inhibits estrogen biosynthesis, suppressed disease in female NZB/NZW mice (Greenstein et al., 1993). The estrogen blocker, tamoxifen, was highly effective in treating NZB/NZW females; percentages of B-cells were decreased; and renal immune complexes were suppressed (Wu et al., 2000; Peeva et al., 2005). Intensive treatment with high-dose tamoxifen, 800 µg twice a week, resulted in significant prolongation of life. Serum anti-DNA antibodies of the IgG3 class, a class that appears to be pathogenic in murine SLE, were reduced and glomerular deposits were composed primarily of IgG2a (Sthoeger et al., 2002).

6. Effects of prolactin on the immune system

Prolactin, a peptide hormone, has the potential to stimulate the immune system and has been implicated as a factor that can activate autoimmune diseases (Walker and Jacobson, 2000), and the relationships between prolactin, estrogen, and autoimmunity have been addressed in recent reviews (Hooghe et al., 2001; Vera-Lastra et al.,

2002; McMurray and May, 2003). Prolactin is produced in the anterior pituitary as well as extrapituitary sites such as the brain and lymphocytes (Ben-Jonathan et al., 1996). Prolactin is a cytokine, with comparable structural motifs and similar receptor structures and signal transduction pathways. Prolactin receptors are distributed throughout the immune system (Weigent, 1996) and are included in a novel receptor family that includes receptors for IL-2β, IL-3, IL-4, and IL-6 (Thoreau et al., 1991). Prolactin can influence the immune system through the thymus (Dardenne et al., 1989), inducing IL-2 receptors on lymphocytes (Mukherjee et al., 1990). Lymphocytes synthesize and release a biologically active form of prolactin (Montgomery et al., 1992), which the lymphocytes employ as an autocrine and paracrine growth factor. It is possible that treatment with corticosteroids affects production of prolactin. Dexamethasone reduces circulating prolactin concentrations and inhibits gene expression of both pituitary prolactin and lymphocyte prolactin (Weigent, 1996).

In rodents, prolactin influences the immune system at almost every level (Yu-Lee, 1997; Walker et al., 1998) and has a key role in maintaining normal immune function and sustaining life. Rats that were deprived completely of prolactin by hypophysectomy and injections of anti-prolactin antibody became anergic and anemic and died within 8 weeks. Replacement injections of either prolactin or growth hormone stimulated expression of the *c-myc* growth promoting gene and reversed involution of the spleen and thymus (Berczi et al., 1990).

High levels of circulating prolactin stimulate immune responses. In mice, hyperprolactinemia was created by either implanting syngeneic pituitary glands or injecting exogenous prolactin, and primary humoral antibody responses were increased (Cross et al., 1989). Low levels of prolactin in cysteamine-treated mice were associated with thymic atrophy and immune suppression (Bryant et al., 1989).

Th-1 cytokines are involved in initiating autoimmunity, and Th-2 cytokines contribute to production of antibodies by B-cells. The transcription factor gene, interferon regulatory factor-1 (IRF-1), which is exquisitely sensitive to prolactin, is an important regulator of T-cell and B-cell differentiation and maturation. IRF-1 is required for Th-1 immune responses. Prolactin, which stimulates IRF-1, can regulate expression of Th-1 cytokines such as IFN-γ and IL-15 (Tada et al., 1997). The potential of IRF-1 to promote autoimmunity was demonstrated when type II collagen-induced arthritis was induced in mice that were either IRF-1 deficient ($-/-$) or IRF-1 positive ($+/-$). Disease was reduced in the IRF-1 $-/-$ mice compared to the $+/-$ mice (Tada et al., 1997).

7. Estrogen–prolactin interactions

Estrogen is a potent stimulus for production of pituitary prolactin in rodents, and estrogen stimulates autoimmunity in the NZB/NZW lupus model. Female NZB/NZW mice treated with very high doses of ethinyl estradiol or estradiol-17-β, the same doses that were employed by early investigators (Melez et al., 1978; Carlsten et al., 1989; Verheul et al., 1995), developed pituitary adenomas and extremely high serum prolactin levels, up to 91 times greater than controls (Walker et al., 1992). The primary cause of death was estrogen toxicity, manifested as urinary tract obstruction and endometritis. The secondary elevation of prolactin could have contributed to the apparent stimulation of autoimmune disease.

Elbourne et al. (1998) found that mice with high circulating estrogen and high serum prolactin had accelerated albuminuria and premature appearance of antibodies to DNA (75% positive at 16 weeks of age). In contrast, autoimmune disease was retarded in females with high estrogen levels that were treated with bromocriptine. These mice had delayed appearance of albuminuria and anti-DNA (10% positive at 16 weeks of age).

The importance of prolactin as a stimulator of autoimmunity was demonstrated in studies of the transgenic R2A γ2b BALB/c mouse model (Bynoe et al., 2000). Lupus autoantibodies developed when these mice were oophorectomized to remove the major source of estrogen and treated with a prolactin dose that caused a twofold increase

in circulating prolactin. The T1/T2 ratio was inverted, Bcl-2 was increased, and all B-cells were increased. The autoimmune stimulating actions of prolactin were determined genetically and did not occur in control R4A γ2b C57BL/6 mice. A second group of lupus-susceptible transgenic BALB/c mice were treated with both estrogen and bromocriptine in order to determine if estrogen could stimulate lupus in the presence of extremely low concentrations of prolactin. Bromocriptine did not block the appearance of DNA-reactive B-cells, but the cells that did develop were functionally inactive. It therefore appeared that an adequate amount of circulating prolactin was required in order for estrogen to stimulate lupus in a mouse with a permissive genetic background (Peeva et al., 2003, 2004).

8. Prolactin in murine models of SLE

Hyperprolactinemia in NZB/NZW mice resulted in premature death from autoimmune renal disease. Female NZB/NZW mice that were made chronically hyperprolactinemic by grafts of two syngeneic pituitary glands developed premature glomerulonephritis and early mortality. In contrast, mice treated with the prolactin-lowering drug, bromocriptine, had delayed appearance of antibodies to ds DNA and significantly prolonged lifespans (McMurray et al., 1991). Neidhart (1997) treated mature NZB/NZW females from the age of 36 weeks with a dose of bromocriptine (5 mg/kg/day) that suppressed serum prolactin to undetectable levels. After 12 weeks of treatment, autoantibodies were not suppressed in mice that received bromocriptine, but proteinuria was delayed. No mice treated with bromocriptine had histological evidence of glomerulonephritis, but glomerulonephritis was found in 70% of NZB/NZW controls.

Very high levels of prolactin were studied in female NZB/NZW recipients of four transplanted pituitary glands. Twelve weeks after implantion, 80% of recipients had antibodies to ds DNA and hypergammaglobulinemia (Walker et al., 1993). Male NZB/NZW mice, which develop an indolent form of SLE, also responded to hyperprolactinemia with accelerated disease (McMurray et al., 1994). Naturally occurring hyperprolactinemia, which is expected during gestation, was detrimental in parous NZB/NZW dams that had whelped and suckled 2 litters. Females that had experienced prolonged pseudopregnancy, which is associated with persisting hyperprolactinemia, also had accelerated disease (McMurray et al., 1993).

In summary, estrogen and prolactin both play important roles in stimulating the autoimmune disease, SLE. In experimental models of lupus, either hormone is capable of augmenting autoimmunity. When the effects of very high doses of estrogen were tested in mice, experimental results were obscured by estrogen toxicity, which led to early death from causes unrelated to autoimmune disease. In turn, the pharmacologic doses of estrogen stimulated the formation of pituitary adenomas and the presence of extremely high amounts of circulating prolactin. In lupus-prone mice, the immune-enhancing properties of estrogen were impeded by very low concentrations of prolactin.

Key points

- Early studies of the effects of estrogen on lupus mice used very high doses of hormone, and mice are believed to have died with estrogen toxicity.
- The high doses of estrogen likely stimulated formation of prolactinomas, resulting in very high concentrations of circulating prolactin.
- It is now recognized that either estrogen or prolactin is capable of stimulating autoimmunity in genetically susceptible mice. However, prolactin is necessary in order for estrogen to exert its stimulatory activity.

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CHAPTER 22

Dehydroepiandrosterone

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1. Introduction

Dehydroepiandrosterone (DHEA) is produced in the adrenal glands, and its sulfate ester DHEAS is the most abundant steroid hormone in the circulation. Serum concentrations of DHEA and DHEAS are approximately 10^{-8} and 10^{-6} M, respectively. Plasma DHEAS levels in adult men and women are 100–500 times higher than those of testosterone and 1000–10,000 times higher than those of estradiol. DHEAS must be viewed as a large substrate reservoir for androgens and estrogens, which are downstream metabolites of DHEAS (Labrie, 1991). During adrenarche, at the age of 6–8 years, adrenal secretion of DHEA and DHEAS increases. Concentrations in serum reach a peak between 20 and 25 years and thereafter DHEAS levels decrease steadily. At the age of 70 years, the serum concentrations are only 20% of corresponding values in young adults (Labrie et al., 2005).

DHEAS is a biologically inactive hormone, and it is converted to the downstream biologically active DHEA. For DHEA, several studies demonstrated a G-protein-coupled surface receptor (Liu and Dillon, 2002; Charalampopoulos et al., 2006), which might be relevant for some DHEA effects. In addition, DHEA effects depend on conversion to downstream steroid hormones in target cells.

Because plasma levels of DHEA decline with age, diverse biological functions of DHEA have become an area of interest and research in humans. It was suggested that DHEA has protective effects in chronic inflammatory diseases, and on bone loss, atherosclerosis, and diabetes mellitus. In this paper, we summarize how DHEA is converted to downstream steroid hormones in health and disease, how DHEA influences inflammatory diseases (including DHEA therapy), and how DHEA influences bone homeostasis.

2. Conversion of dehydroepiandrosterone to downstream steroid hormones

Analysis of peripheral sex hormone metabolism must take into account that conversion of androgens and estrogens depends on availability of androgen precursors such as DHEAS or DHEA. The main pathways of steroid conversion of DHEAS into androgens and estrogens are summarized in Fig. 1.

The first conversion step from DHEAS to DHEA has been demonstrated in synovial tissue, and the responsible enzyme, the steroid sulfatase (step 1 in Fig. 1), is expressed in synovial fibroblasts and macrophages (Weidler et al., 2005). This important enzyme step is inhibited by tumor necrosis factor (TNF) (Hennebold and Daynes, 1994; Weidler et al., 2005). Starting from DHEA,

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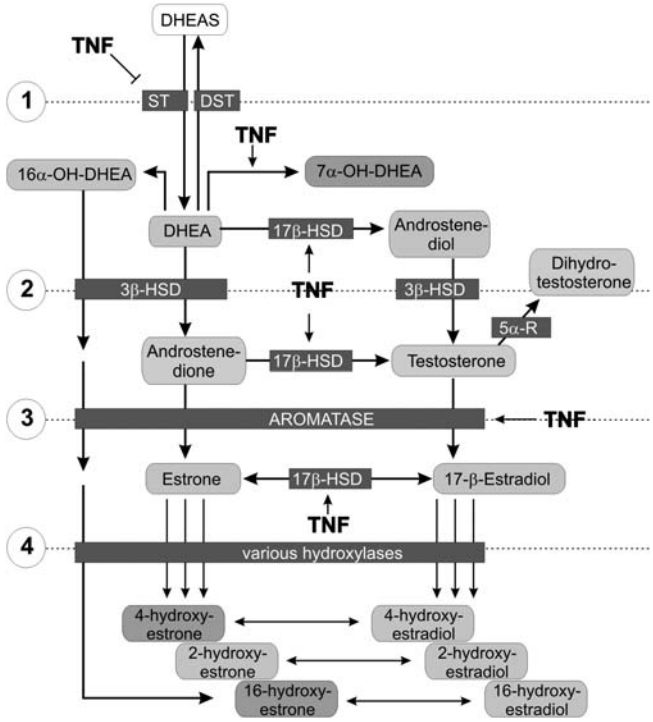

Figure 1. Steroid pathways in local inflammatory cells in patients with rheumatoid arthritis (RA). Starting from the precursor dehydroepiandrosterone sulfate (DHEAS), a total of four steps are needed for estrogen formation. Hormones in red (green) boxes indicate a proinflammatory (anti-inflammatory) influence. The role of hormones in gray boxes needs to be determined. The various hydroxylases relevant for estrogen metabolism need to be determined (step 4). Abbreviations: 3β-HSD, 3β-hydroxysteroid dehydrogenase; 5α-R, 5α-reductase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; DHEA, dehydroepiandrosterone; DST, DHEA sulfotransferase; OH, hydroxyl group; ST, sulfatase; TNF, tumor necrosis factor.

two enzymes are needed for the synthesis of testosterone: 3β-hydroxysteroid dehydrogenase (3β-HSD in Fig. 1) and a 17β-hydroxysteroid dehydrogenase acting as reductase (17β-HSD in Fig. 1). Only one further step is needed to convert androstenedione and testosterone to estrone and 17β-estradiol, respectively (step 3 in Fig. 1). This step is mediated by the aromatase complex (CYP19, step 3 in Fig. 1). In macrophages of healthy subjects, it has been demonstrated that DHEA can be converted to all mentioned downstream hormones (Schmidt et al., 2005). In recent years, these conversion steps have been studied in patients with rheumatoid arthritis (RA) using cells from the inflamed joint.

In patients with RA and osteoarthritis (OA), DHEAS can be converted to DHEA in synovial cells (step 1 in Fig. 1), and TNF inhibits this conversion step in RA but not in OA (Weidler et al., 2005). This indicates that in patients with a strong inflammation and high local TNF levels, the biologically active DHEA is most probably decreased in relation to the precursor DHEAS. In chronic inflammatory disease such as RA, all this happens in the presence of decreased systemic levels of DHEAS in the circulation (see below). Both factors contribute to an impoverishment of androgens in the inflamed tissue.

If DHEA is applied to mixed synovial cells (bypassing the above-mentioned step 1), DHEA can be converted to androstenediol, androstenedione, testosterone, and estrogens (steps 2–4 in Fig. 1) (Castagnetta et al., 2003; Schmidt et al., 2005). In addition, remarkable amounts of 16α-hydroxylated DHEA and 7α-hydroxylated DHEA appear, and these two conversion products might

serve a proinflammatory role (Dulos et al., 2005; Schmidt et al., 2005). Particularly, 7α-hydroxy-DHEA has been linked to proinflammatory effects as recently demonstrated in the collagen type 2 arthritis model in mice (Dulos et al., 2005). In synovial tissue, we expect that both pathways—to testosterone and to 7α-hydroxy-DHEA—compete for DHEA (Schmidt et al., 2005). In addition, 16α-hydroxylated DHEA might be the starting point of estrogen production (Fig. 1).

If androstenedione or testosterone are applied to mixed synovial cells (bypassing the above-mentioned two steps), remarkable amounts of the nonaromatizable pure androgens 5α-dihydro-androstenedione and 5α-dihydro-testosterone appear (Schmidt et al., 2005) (Fig. 1). Interestingly, the amount of these 5α-hydroxylated androgens is higher in RA as compared to OA (Schmidt et al., 2005). Since 5α-hydroxylated androgens cannot be converted by the aromatase complex, administration of androstenedione and testosterone might lead to strong anti-inflammatory effects. In addition, administration of androstenedione and testosterone did not lead to measurable amounts of estrogens, which stabilizes the pool of androgens (Schmidt et al., 2005). This might be the reason why testosterone therapy in patients with RA exerted beneficial effects (Cutolo et al., 1991; Booji et al., 1996).

We summarize that in synovial cells of patients with RA and OA, DHEAS can be converted to DHEA, DHEA can be converted to androstenedione, testosterone, and estrogens, and that androstenedione and testosterone inhibit production of estrogens. Expecting that low levels of estrogens in the picomolar range have proinflammatory effects, administration of androstenedione and testosterone most probably have an anti-inflammatory role. This might not be the same for administration of DHEA because this molecule can be converted to proinflammatory 7α-hydroxy-DHEA and 16-hydroxylated estrogens. The question remains what appears in synovial fluid of patients with RA.

In synovial fluid or superfusate of synovial tissue of patients with RA, two studies clearly delineated that estrogens are increased relative to androgens and relative to the normal situation (Castagnetta et al., 2003; Schmidt et al., 2005). This is indicative of an activated aromatase in the inflamed tissue, and it seems that estrogens in the picomolar range serve proinflammatory pathways (see Chapter 1, Capellino and Straub). At present, it is absolutely unclear whether estrogens can be further converted to 2-hydroxylated or 2-methoxylated estrogens, but there is evidence that estrogens can be converted to 4-hydroxylated, 4-methoxylated, and 16-hydroxylated downstream estrogens (Castagnetta et al., 2003) (see Chapter 1, Capellino and Straub), all of which might be proinflammatory at low concentrations.

In conclusion, the balance between anti-inflammatory androgens and proinflammatory estrogens depends on (1) the expression levels of converting enzymes, (2) the amounts of substrates available locally, (3) the cell type available within the tissue, and (4) presence of respective steroid hormone receptors (see Chapter 1, Capellino and Straub).

3. Dehydroepiandrosterone in inflammation

A clear decrease of serum DHEA has been linked to a number of inflammatory diseases such as RA (Sambrook et al., 1988), systemic lupus erythematosus (Straub et al., 1996), progressive systemic sclerosis (Straub et al., 1997), inflammatory bowel disease (Straub et al., 1998a), and pemphigus (de la Torre et al., 1995). In addition, low serum levels of DHEA were associated with diseases without a strong systemic inflammation such as coronary heart disease and atherosclerosis (reviewed in Alexandersen et al. (1996)), and Alzheimer's disease and dementia (Yanase et al., 1996). The reported loss of DHEA in chronic inflammatory diseases was thought to play a proinflammatory role due to the substantial decrease of androgen precursors in the tissue (see above paragraph). On that score, the question arises whether DHEA has a direct immunomodulatory role.

In animal models, DHEA has been shown to inhibit proinflammatory cytokines such as IL-6 (Daynes et al., 1993) or TNF (e.g., Kimura et al., 1998), but rodent models present the general problem that DHEA is not a physiological hormone, and most often high doses were applied. This leads to nonphysiological effects, which are

most probably not related to the human pathology. In human peripheral blood mononuclear cells, DHEA at 50 nmol/l decreased the production of IL-6, but DHEA had no effect at lower or higher concentrations (bimodal role of DHEA) (Straub et al., 1998b). DHEA also reduced the expression of IL-4, IL-6, and IFN-γ in human osteoblasts (Harding et al., 2006). In human epithelial cells, DHEA prevented the secretion of proinflammatory cytokines such as TNF (Gutierrez et al., 2007). In rabbit synovial cells and chondrocytes, DHEA decreased expression of IL-1β and matrix metalloproteinase-3, and DHEA increased tissue inhibitors of metalloproteinases (Wu et al., 2006). These examples demonstrate that DHEA might have some important beneficial effects, and it seems that these effects depend on the cell type and species investigated. The question remains whether DHEA has any beneficial effects in chronic human diseases.

One open study with DHEA was carried out in a small cohort of patients with RA, but DHEA had no effect on systemic inflammation or disease activity (Giltay et al., 1998). Under consideration of the above-mentioned conversion of DHEA in synovial cells, this result is not unexpected because DHEA might be converted to unwanted steroids in tissue of RA patients (7α-hydroxy-DHEA, 16α-hydroxy-DHEA, Fig. 1). We mentioned that conversion of DHEA happens in macrophages and fibroblasts. Since in the chronic phase of RA, macrophages and fibroblasts play a dominant role, DHEA might not be an adequate therapy to treat RA patients, because these cells convert DHEA to 7α-hydroxy-DHEA and 16α-hydroxy-DHEA (Fig. 1). This might be different in a chronic inflammatory disease where other cell types play a major role.

Three double-blind, placebo-controlled, multicenter trials with DHEA have been carried out in systemic lupus erythematosus (von Vollenhofen et al., 1999; Chang et al., 2002; Petri et al., 2004). It was shown that DHEA had a significant anti-inflammatory effect in patients with systemic lupus erythematosus. In addition, adjuvant DHEA treatment in systemic lupus erythematosus was able to maintain bone mineral density (BMD) (von Vollenhofen et al., 1999; Mease et al., 2005). Similarly, an open study in a small cohort of patients with ulcerative colitis revealed an impressive beneficial effect (Andus et al., 2003). It might well be that macrophages and fibroblasts do not play a similarly major role in systemic lupus erythematosus and ulcerative colitis as compared to RA. In earlier years, both diseases have been allocated to the T helper type 2 form of a chronic inflammatory disease, the pathophysiology of which might depend on quite different cell types compared to the T helper type 1 driven RA. Macrophages and fibroblast play a minor role in systemic lupus erythematosus, which might lead to separate downstream conversion products of DHEA in systemic lupus erythematosus compared to RA.

Further information comes from studies in the field of diabetology. It was demonstrated that DHEA possesses antioxidant effects (Nestler and McClanahan, 1992; Aragno et al., 2002; Yorek et al., 2002) and prevents tissue damage induced by acute and chronic hyperglycemia (Aragno et al., 2002). Treatment of diabetic rats with DHEA for 4–5 weeks blocks diabetes-induced increase in superoxide production (Nestler and McClanahan, 1992). Moreover, DHEA prevented the development of vascular and neural dysfunction in diabetic rats (Yorek et al., 2002). In rat hippocampus, DHEA treatment reduces activation of NF-κB, induced by chronic hyperglycemia. This reduces the severity of brain damage induced by diabetes (Aragno et al., 2002). Although most of these studies have been carried out in rodents, additional beneficial antioxidant effects might be attributed to DHEA. Since rodents do not produce high DHEA endogenously, the applied DHEA in higher doses is probably an unphysiologic therapy.

In conclusion, favorable effects of DHEA most probably depend on the most important proinflammatory pathways given in a certain chronic inflammatory disease. The proinflammatory pathways depend on different cell types involved. Since different cell types can convert DHEA to quite different downstream products, involved cell types control the role of DHEA. Sometimes, the character of a disease can change so that other cell types are involved. In such a situation, beneficial effects of DHEA might be present in one phase of the disease but not in another phase.

4. Role of dehydroepiandrosterone in bone homeostasis

Bone health and strength are dependent on coupling of bone resorption and bone formation. This process is mediated by the interaction of osteoclasts and osteoblast. Sex steroids such estrogen and testosterone bind to specific receptors in bone cells (Abu et al., 1997). Specific receptors for the weaker androgens such as DHEA have also been claimed to play a role but their exact nature is presently unknown (Notelovitz, 2002). Mechanism of androgen actions on bone is still a subject of debate. It might well be that estrogens play a major role since androgens are converted by the action of the aromatase complex (CYP19), and, thus, the effects of androgens on bone may be partially mediated via estrogen receptors (Riggs et al., 2002). Since DHEA is converted to downstream androstenedione, testosterone, and 17β-estradiol in osteoblasts and macrophages (Kuwano et al., 1997; Schmidt et al., 2000), DHEA might well exert bone-protective effects.

Indeed, treatment with DHEA for 1 year in 14 postmenopausal women significantly increased BMD of the femur, and DHEA also increased serum osteocalcin levels. In addition, the bone alkaline phosphatase (biochemical indicator of bone turnover) was decreased (Labrie et al., 1997). Another study demonstrated that in the hip (total, trochanter, and shaft regions), DHEA therapy tended to increase BMD. In the same study, a significant sex-specific response in these analyses has been found since the DHEA-mediated increase in lumbar spine BMD was larger in women than in men (Jankowski et al., 2006). The situation might be largely different in chronic inflammatory diseases since bone-destroying cytokines such as IL-1β and TNF play a dominant role and patients are treated with glucocorticoids.

For example, TNF and IL-1β play a central role in the pathogenesis of synovitis in RA. These cytokines are also found to be responsible for inducing osteoclast bone resorption (Strand and Kavanaugh, 2004). It has been shown that osteoclasts are stimulated through IL-6 and TNF. Since DHEA inhibits IL-6 and TNF secretion, an anti-resorptive influence of DHEA may be the consequence. Furthermore, positive interactions between DHEA and BMD have been described in healthy subjects and in patients with inflammatory diseases (Nordin et al., 1985; Sambrook et al., 1988; Mease et al., 2005). In the study by Mease et al. (2005), female prednisolone-treated patients with systemic lupus erythematosus profited from DHEA by an increase of BMD at the lumbar spine and hip.

Several studies pointed out that DHEA has anti-glucocorticoid activities with respect to immune function in mice after thermal injury (Araneo and Daynes, 1995), macrophage activity (Padgett and Loria, 1998), neurotoxic effects of dexamethasone (Kimonides et al., 1999), modulation of liver enzymes, lymphocyte proliferation, thymic involution, and glucocorticoid-induced hypertension (summarized in Kalami et al. (1994)). Since osteopenia is a frequent complication of long-term glucocorticoid therapy, the anti-glucocorticoid effects of DHEA would be most important. However, the biochemical and molecular mechanisms responsible have not been clarified yet. Cells from osteoblast lineage synthesize and secrete molecules that in turn initiate and control osteoclast differentiation during skeletal development and throughout life (Teitelbaum, 2000). Importantly, DHEA acts directly on osteoblasts enhancing the level of osteoprotegerin (Wang et al., 2006). This is another important indication how DHEA might exert bone-sparing effects.

In conclusion, the positive effects of DHEA on bone have been demonstrated in healthy controls and in patients with chronic inflammatory diseases. Although the direct effects of DHEA on inflammation might be relatively mild (and dependent on the disease), its bone-protecting effects are obvious.

5. Conclusions

In the 1990s, DHEA was categorized as a fountain of youth, which might be also relevant in chronic inflammatory diseases due to anti-inflammatory potential. Indeed, under certain circumstances DHEA has inhibitory effects on IL-6 and TNF secretion, two widely recognized proinflammatory

cytokines. DHEA may exert its effects via membrane and intracellular receptors but also through conversion to downstream steroid metabolites such as testosterone and 17β-estradiol. Conversion of DHEA to downstream metabolites depends on the cell type involved and microenvironmental conditions such as presence of cytokines. These conditions might vary in different chronic inflammatory diseases so that DHEA can exert beneficial or no effects. In RA and Sjögren syndrome (R. Derksen, Utrecht, personal communication), DHEA does not influence disease activity, but in systemic lupus erythematosus and ulcerative colitis, DHEA exerts favorable effects. In addition, the likelihood that DHEA has bone-sparing effects is gaining general acceptance.

In chronic inflammatory diseases, we and others pointed out that low-dose glucocorticoid therapy must be recognized as a cheap substitution therapy for functionally altered adrenal glands (see Chapter 1, Capellino and Straub). This concept can be similarly used for DHEA, the serum level of which is largely decreased in chronic inflammatory diseases. Since DHEA has mild anti-inflammatory activities and bone-sparing effects, therapy with this adrenal hormone should be considered as an additional therapeutic option to substitute for the adrenal dysfunction. We suggest that future studies address this important aspect of DHEA substitution therapy in chronic inflammatory diseases.

Key points

- In the serum of patients with rheumatic diseases DHEA sulfate is decreased.
- The adrenal androgen DHEA can be converted to androgens and estrogens in synovial cells of patients with rheumatoid arthritis.
- Administration of testosterone inhibits the aromatase and estrogen formation.
- DHEA is immunosuppressive in systemic lupus but probably not in rheumatoid arthritis.
- DHEA prevents bone loss in patients with rheumatic diseases.

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CHAPTER 23

Glucocorticoid Therapy for Polymyalgia Rheumatica

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1. Introduction

1.1. Definition and epidemiology

Polymyalgia rheumatica (PMR) is a clinical syndrome of unknown etiology characterized by inflammatory pain and stiffness of the shoulder and/or pelvic girdles accompanied with laboratory evidence of inflammation in a patient older than 50 years (Salvarani et al., 2004, Soubrier et al., 2006).

The clinical features of PMR have been widely reported during the last 20 years, but the incidence, duration, and outcome of this syndrome have been incompletely reported, probably due to the lack of universally accepted diagnostic and classification criteria.

PMR may be considered a common illness in certain population, with a reported prevalence of one case every 133 people over the age of 50 years (Salvarani et al., 1995a, b). The incidence of PMR increases with age, with a peak in people 70–80 years old, and is more frequent in females than in males. Even if the etiology is still unknown, the role of environmental (Elling et al., 1996; Cimmino, 1997) as well as of genetic factors (Salvarani et al., 2004) has been suggested.

The mortality rate in PMR is reported to be similar to that expected in general population, and the course of the disease seems to be more aggressive in women than in men (Cimmino et al., 2006).

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The association between PMR and giant cell arteritis (GCA) is well known, but the true relationship between PMR and GCA remains uncertain. Population-based studies have demonstrated the presence of biopsy-proven GCA in about 20% of patients (Salvarani et al., 1995a); on the other hand, symptoms of PMR have been observed in up to 60% of patients with GCA (Salvarani et al., 1995b). PMR may begin before, simultaneously with or develop after GCA; prompt recognition of GCA is extremely important because serious complications (e.g., blindness) may occur rapidly and may be prevented by immediate and appropriate treatment.

1.2. Clinical manifestations and diagnosis

Inflammatory pain with morning stiffness lasting more than 1 h in the shoulder and/or pelvic girdles is the typical presentation; the cervical spine may be affected as well. About one-third of patients have constitutional symptoms such as low-grade fever and weight loss. Peripheral musculoskeletal manifestations are also common, with up to 45% of PMR patients presenting nonerosive asymmetric polyarthritis (predominantly affecting knee and wrist), carpal tunnel syndrome, swelling of the hands with pitting edema, and tendonitis (Salvarani et al., 1998). Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are usually elevated at the beginning, even if cases without acute-phase reactants elevation have been described (up to 20% of cases). Imaging studies using ultrasonography and magnetic resonance demonstrated that bursitis and synovitis are very common in patients with

PMR, but the diagnostic value of these findings is still to be clarified (Cantini et al., 2001).

The diagnostic criteria for PMR are empirical, and based on clinical features. Three sets of criteria are the most widely used in clinical practice as well as in clinical trials (Bird et al., 1979; Chuang et al., 1982; Healey, 1984). Bird et al. (2005) compared the performance of different diagnostic criteria sets in a multicenter study and demonstrated that the Bird et al. (1979) and the Chuang et al. (1982) criteria performed best, with a sensitivity of 99.5 and 93.3%, respectively; authors suggested that these two sets of criteria should be used whenever possible (Table 1).

The differential diagnosis in a patient presenting with symptoms resembling PMR may be difficult; a wide variety of conditions, including other rheumatic diseases, may mimic the clinical picture of PMR (Gonzalez-Gay et al., 2000). Elderly-onset rheumatoid arthritis (EORA) may frequently present an abrupt onset with girdles involvement and it is frequently seronegative for classical rheumatoid factor (Van Schaardenburg and Breedveld, 1994). About two-third of patients with a PMR-like presentation may actually have, or will develop in a few months, an overt rheumatoid arthritis (Caporali et al., 2001); in many cases, only time can distinguish between PMR and EORA, even if specific laboratory tests may help in distinguish between the two forms (Brito et al., 1994; Ceccato et al., 2006). As a matter of fact, EORA with PMR-like onset show many similarities with true-PMR as for cytokine production and steroidal hormone patterns (Cutolo et al., 2006). Other rheumatic conditions that may present with a clinical picture similar to PMR are late-onset spondyloarthropathy, connective tissue diseases (systemic lupus erythematosus and polymyositis).

In addition to rheumatic diseases, malignancies (solid tumors and myeloma) and infections (bacterial endocarditis) are the conditions that more frequently mimic PMR. The lack of adequate response to corticosteroid therapy and the presence of atypical symptoms (high-spiking fever, absence of morning stiffness, and important weight loss) should suggest further investigations for an underlying infections or malignancy.

Table 1
Diagnostic criteria for polymyalgia rheumatica

A: Criteria developed by Bird et al. (1979): three out of seven features are required	
1	Bilateral shoulder pain and/or stiffness
2	Bilateral upper arm tenderness
3	Onset of illness within 2 weeks
4	ESR more than 40 mm/h
5	Morning stiffness more than 1 h
6	Age more than 65 years
7	Depression or weight loss or both
B: Criteria developed by Chuang et al. (1982): all criteria are required	
1	Pain for at least 1 month at two or more of the following sites: shoulders, pelvic girdle, and cervical spine
2	Morning stiffness more than 1 h
3	Age more than 50 years
4	ESR more than 40 mm/h
5	Exclusion of other diagnoses

2. Treatment

Nonsteroidal anti-inflammatory drugs have been suggested to treat mild forms of PMR; however, in the elderly this class of drugs may carry a high risk of complications (gastrointestinal, renal, and cardiovascular) (Gabriel et al., 1997).

In patients with PMR, the mainstay of the treatment still remains corticosteroids; a good therapeutic response to corticosteroids has been recognized as a feature of the condition, and constitutes part of the Healey's (1984) diagnostic criteria.

However, the exact starting dose, the duration of therapy, and the schedule of administration still remain a matter of debate.

2.1. Steroid regimens

2.1.1. Starting dose

The optimal corticosteroid starting dosage is not agreed, and varies widely across different studies (Li and Dasgupta, 2000). A starting dosage of 15–20 mg/day of prednisone is usually considered to be able to induce a dramatic clinical response

(symptoms resolution within 48–96 h) and a normalization of acute-phase reactants. However, lower dosage has been suggested, in particular for those patients presenting contraindications to corticosteroids (e.g., hypertension, glaucoma, diabetes, and severe osteoporosis). As a matter of fact, a starting dose at or below 10 mg/day is associated with fewer side effects, but many clinicians feel that it might be insufficient in most cases. Delecouillerie et al. (1988) compared high (15–30 mg/day) with low (7–12 mg/day) prednisone starting dose in two groups of PMR patients, and did not find significant difference in the relapse rate. Kyle and Hazleman (1989) observed that, to avoid relapse, PMR patients require prednisone 15–20 mg/day during the first 2 months, and that 65% of patients who receive an initial dosage of 10 mg/day experience relapse.

In a large series of PMR patients taking corticosteroids, the median starting dosage of prednisone was 20 mg/day, but with a very wide range of dosage (5–100 mg/day) (Chuang et al., 1982).

Thus, the issue of the ideal starting dosage is far from being settled and should be the matter of further studies (Luqmani, 2007).

2.1.2. Tapering and duration of treatment

The starting dose should be given for 3–6 weeks on average. No conclusive studies exist to determine the optimal tapering schedule of corticosteroid once achieved the complete remission of symptoms and acute-phase reactants normalization. To avoid relapses, the dosage is usually decreased by 10% every 10–15 days down to 10 mg/day; than from 10 mg/day to the end of steroid therapy, a slower rate of decrease (1 mg every month or every 2 months) is used. It should be underlined that although disease flares can occur more frequently when a rapid dose reduction is applied, spontaneous disease flares do occur independently of the dose (Salvarani et al., 1987). Kremers et al. (2005) showed that a higher starting dosage followed by a faster tapering rate was associated with a greater risk of relapses; however, a direct comparison in a prospective design between high starting dose with fast tapering and low starting dose with slow tapering is not available.

Different views exist also as for the definition of relapse and, accordingly, of remission. These topics are of outstanding importance in that the decision to taper or not steroid and to increase or re-start therapy is largely based on these concepts. In general, an isolated elevation of ESR does not warrant an increase in corticosteroid dosage; CRP is considered to be a better parameter to monitor the course of the disease (Soubrier et al., 2006) even if symptoms recurrence is mandatory to define a disease relapse. The same problem exists as for tapering or stopping therapy; disease remission should be defined as absence of symptoms and normal level of acute-phase reactants.

Relapses are most likely in the first 18 months of treatment, but they can occur after apparently successful treatment, when corticosteroids have been discontinued. In a retrospective evaluation of 256 PMR patients followed at a single center, 40% of the relapses were observed after 6 months from the treatment discontinuation (Caporali and Montecucco, unpublished data). At present, there is no way of predicting those patients most at risk.

Leeb and Bird (2004) recently developed an activity score for PMR (PMR-AS). The score is obtained by summing the CRP level (mg/dl), the patient-assessed disease activity (on a visual analog scale), the duration of morning stiffness, and the ability to lift the arms (0–3). The disease is considered inactive when the PMR-AS is lower than 7, moderately active between 7 and 17, and highly active when it is greater than 17. As recently published by Binard et al. (2007), PMR-AS seems a good indicator for disease activity and useful in the clinical setting to tailor the glucocorticoid dose to the individual needs of each patient.

Similarly to the starting dose and the tapering modalities, the optimal length of therapy as well as the rate of drug cessation remains unknown. The length of corticosteroid therapy varies from different studies; while some authors reported a mean duration of therapy of 11–17 months (Chuang et al., 1982), other reported a mean duration of therapy up to 31 months (Behn et al., 1983). The same variability may be encountered when assessing the rate of patients that are able to stop the therapy. Most European studies report that between 33 and 50% of the patients are able to

discontinue steroids after 2 years of treatment. Other studies from the United States reported a higher frequency of discontinuation (75% of patients discontinued therapy after 2 years). However, a large study from the Mayo Clinic confirmed the European view (Gabriel et al., 1997), being the median duration of therapy of 1.8 years and with a cumulative dose of prednisone between 4.5 and 5.4 g.

2.1.3. Steroid side effects

Side effects attributable to long-term corticosteroid therapy may be significant, between 20 and 50% of patients may experience serious side effects; moreover, in women over the age of 50, PMR and rheumatoid arthritis are the conditions showing the greatest corticosteroid dosage for the longest time as shown in a large population-based study (Chantler et al., 1993). PMR patients had a two to five times greater risk compared to age-matched controls of developing diabetes, vertebral, and hip fractures; a long-term follow-up study found that 65% of the patients undergoing treatment developed at least one adverse event (Gabriel et al., 1997). Increasing age at diagnosis, a higher cumulative dose, and female sex are independent predictors of the risk of adverse events (Salvarani et al., 2004).

Bisphosphonates, vitamin D, and calcium have been found to be effective for preventing bone loss in corticosteroid-treated patients. The American College of Rheumatology recommends calcium and vitamin D supplementation, lifestyle modification, regular weight-bearing exercise, and bisphonates therapy for prevention of glucocorticoid-induced osteoporosis, and suggests bone-density assessment for patients receiving long-term therapy (American College of Rheumatology ad hoc committee on glucocorticoid-induced osteoporosis, 2001). In order to avoid side effects due to long-term steroid therapy, various possibilities have been suggested (the use of apparently safer compounds, different treatment modalities, and the use of steroid-sparing drugs).

2.1.4. Deflazacort

This oxazoline derivative of prednisolone has been subject to interest as an alternative to prednisolone because it seems to have fewer side effects on bone and glucose metabolism (Gennari et al., 1984; Olgaard et al., 1992; O'Connell et al., 1993). Studies have been performed to explore the efficacy and safety of deflazacort in PMR. In the majority of them, deflazacort proved to be as effective as prednisone in treating PMR (Lund et al., 1987; Cimmino et al., 1994; Krogsgaard et al., 1995). However, it is still a matter of debate either the exact equipotency ratio between deflazacort and prednisolone and the effective more pronounced safety of deflazacort with respect to prednisolone in PMR. As for the first issue, the equipotent dose of 5 mg prednisolone calculated in different studies is >6 and <7.5 mg deflazacort (Cimmino et al., 1994; Krogsgaard et al., 1995; Saviola et al., 2007). As for safety, even if there are no data on long-term, controlled studies, preliminary data seem to confirm a more favorable profile on bone metabolism of deflazacort with respect to prednisolone, almost at low-intermediate dosage (Cimmino et al., 1994; Lippuner et al., 1998; Saviola et al., 2007).

2.1.5. Noncontinuous steroid regimens

Noncontinuous steroid treatment could be useful to reduce steroid-related side effects. In a multicenter prospective study involving 60 patients, it has been shown that intramuscular methylprednisolone can confer a similar remission rate to oral prednisolone (Dasgupta et al., 1991). Methylprednisolone was administered IM at a dosage of 120 mg every 3 weeks; with this regimen, the side-effect profile seems to be better, especially with respect to a lower fracture rate and a lower proportion of patients with weight gain (Dasgupta et al., 1998). This better safety profile may be due to a significantly reduced cumulative dose of steroids. The authors feel that this treatment may be suggested as initial treatment in PMR, although patients with more severe disease may require conversion to oral administration (Li and Dasgupta, 2000).

Similarly, it has been suggested that methylprednisolone acetate injections into the glenohumeral joints (40 mg, four times at 1-week interval) may be a valid alternative to systemic corticosteroid therapy (Salvarani et al., 2000).

Limited experience exists as for pulse intravenous steroid regimens for PMR. In a pilot study on four patients, Cimmino et al. (2004) failed to

demonstrate any steroid-sparing effect of three intravenous methylprednisolone boli (250 mg each on 3 consecutive days) used as first-line therapy.

2.2. Steroid-sparing agents

Patients who are unable to reduce the dosage of prednisone because of recurring symptoms or with serious steroid-related side effects may pose particular problems. In this setting, different immunosuppressive drugs have been suggested as steroid-sparing agents. Years ago a prospective study suggested that hydroxychloquine may be effective (David-Chausse et al., 1983); however, no large, randomized, and controlled studies exist to confirm this preliminary results.

De Silva and Hazleman (1986) suggested the possibility of reducing the overall amount of steroids with azathioprine; no other studies explored this issue.

In recent years, methotrexate is the drug more extensively studied as steroid-sparing agent in PMR. Two open-label and three placebo-controlled studies addressed this issue, giving conflicting results. The studies are difficult to compare, due to different methotrexate dosage, different steroid starting dose, and different strategies in reducing steroid dosage. However, both the placebo-controlled studies seem to demonstrate that methotrexate may be helpful. In the earliest study, involving 27 patients, the mean cumulative dose of steroids was significantly lower in methotrexate-treated patients with respect to placebo-treated, as well as the rate of patients in clinical remission after 1 year of treatment (Ferraccioli et al., 1996). The other study used a double-blind, randomized placebo-controlled design to evaluate 72 patients, followed up for 18 months (Caporali et al., 2004). At the end of the study, 28 of the 32 methotrexate-treated patients were no longer on prednisone, compared with only 16 of the 30 placebo-treated. Moreover, the number of relapses was lower in actively treated patients (27 versus 50) as well as the mean cumulative prednisone dose. These data, to be confirmed on larger groups of patients, may suggest a role of methotrexate as steroid-sparing agent.

Recently, a possible role of TNF-α inhibition in PMR treatment has been suggested. While a preliminary pilot study on refractory patients with PMR showed positive results (Salvarani et al., 2003), a recent randomized, double-blind, controlled trial provided evidence that adding infliximab (a chimeric anti-TNF-α monoclonal antibody) to prednisone for treating newly diagnosed PMR is of no benefit (Salvarani et al., 2007). It remains to be evaluated the role of TNF-α blocking agents in steroid-resistant patients.

Key points

- Corticosteroids still represent the mainstay of the treatment of polymyalgia rheumatica.
- The exact steroid starting dose, duration of therapy and schedule of administration are still a matter of debate.
- Methotrexate may be useful as steroid-sparing agent.

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CHAPTER 24

Danazol Therapy

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Danazol is a synthetic attenuated androgen that has been used to treat immune-mediated diseases (Tomino et al., 1987; Ahn, 1990; Lee et al., 1993; Cervera et al., 1995). This drug suppresses the pituitary–ovarian axis. This suppression probably results from a combination of depressed hypothalamic–pituitary response to lowered estrogen production, alteration of sex steroid metabolism, and interaction of danazol with sex hormone receptors. Danazol has weak androgenic activity. It depresses the output of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Due to these effects, this drug has been indicated for the management of conditions such as endometriosis (Jackson and Telner, 2006) and fibrocystic breast disease (Andrews, 1990). With regard to autoimmune diseases, danazol has been used to treat autoimmune diseases as shown in Table 1. The potential role of danazol in the management of these disorders is discussed in this chapter.

1. Danazol in the management of autoimmune diseases

1.1. Autoimmune thrombocytopenia

Effective treatment for immune thrombocytopenia should be based on a definitive understanding of mechanisms whereby a given therapy increases platelet counts. Classically, the major factor causing thrombocytopenia in immune-mediated diseases has been increased platelet destruction by platelet-bound antibodies and/or abnormal function of splenic macrophage Fc (IgG) receptors. These two mechanisms could shorten the survival of circulating platelets. However, in some cases the predominant cause of thrombocytopenia could be ineffective marrow platelet production rather than accelerated platelet removal. In this regard, Gernsheimer et al. (1989) established that in idiopathic thrombocytopenia purpura (ITP), corticosteroids increase production of platelets and splenectomy increases platelet survival by removing the major organ of peripheral destruction.

Danazol is useful in treating both refractory ITP and autoimmune thrombocytopenia associated with connective tissue diseases (Cervera et al., 1995; Blanco et al., 1997).

The mechanism of action of danazol in autoimmune thrombocytopenia is unknown. Studies of

Table 1
Danazol therapy in the treatment of autoimmune diseases

Autoimmune thrombocytopenia
Idiopathic
Associated with rheumatic diseases
Systemic lupus erythematosus
Primary antiphospholipid syndrome
Rheumatoid arthritis
Autoimmune hemolytic anemia
Autoimmune-mediated C1 inhibitor deficiency
Other immune-mediated diseases
Henoch–Schönlein purpura
IgA nephropathy

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the effect of danazol on antiplatelet antibody levels have yielded contradictory results. Some investigators found a reduction in the level of antiplatelet antibodies. However, others did not find such a decrease and it was postulated that danazol acted by inhibiting the mononuclear phagocyte system (West and Johnson, 1988; Ahn et al., 1989). The structure of danazol is similar to cholesterol and it is possible that danazol is incorporated into and affects the functions of cell membranes. In this context, danazol might modify the interaction of anticardiolipin antibodies with their antigens in platelet membranes (Kavanaugh, 1994). It has been suggested that an effect of the drug is to downregulate the number of Fc receptors on splenic macrophages, thereby prolonging the platelet survival.

1.1.1. Danazol in the management of idiopathic thrombocytopenic purpura

Primary ITP is a common hematologic disorder manifested by immune-mediated thrombocytopenia. The diagnosis remains one of exclusion after other thrombocytopenic disorders are excluded based on history, physical examination, and laboratory evaluation.

An understanding of the natural history of untreated ITP, which is different in children and adults, provides part of the rationale for deciding which patients should be treated. Many children receive no specific therapy for ITP, since 70–80% of those affected experience complete remission of the disease within 6 months. Initial treatment of children with corticosteroids, intravenous immunoglobulin, or anti-D therapy may cause more rapid increase of the platelet count compared to no therapy. In contrast, spontaneous remissions are unusual in adults, occurring in 9% in one series (Stasi et al., 1995).

ITP is caused by accelerated destruction of antibody-sensitized platelets. The antibody-coated or immune-complex-coated platelets are destroyed prematurely by the reticuloendothelial system, resulting in peripheral blood thrombocytopenia. In children, ITP is usually an acute disorder. In adults it is usually chronic, although there is considerable variation in the clinical course and most patients eventually attain safe platelet counts off treatment. Although 70–75% of adults with ITP respond to standard-dose corticosteroids and splenectomy, maintaining safe platelet counts without requiring further treatment, a subset of up to 20–30% of patients has severe disease that is refractory to all treatment modalities. This subgroup is subject to considerable morbidity and mortality, requiring further therapy due to bleeding or very low platelet counts $<10{,}000$–$30{,}000/mm^3$ (Stasi and Provan, 2004; Cines and McMillan, 2005).

As pointed out by Stasi and Provan (2004), current guidelines for the treatment of ITP are based on expert opinion rather than evidence because there is a lack of clinical trials and research into more effective therapies. Treatment of patients with ITP and severe bleeding and/or extremely low platelet counts who are refractory to corticosteroids and splenectomy requires careful evaluation of disease severity, patient characteristics related to risk of bleeding, and adverse effects associated with the specific therapies aimed at treating this disorder.

Danazol has been found to be useful in male and nonpregnant female adult patients with refractory ITP (Stasi and Provan, 2004). Ahn et al. (1989) reported the outcome of 22 patients treated with danazol at a dose of 200 mg two to four times daily for more than 2 months. Fifteen of them had undergone splenectomy without significant improvement. Around 60% showed elevation of the platelet count above $50{,}000/mm^3$, sustained for more than 2 months. Older patients and those who had undergone splenectomy were the best responders.

In line with the above, Andres et al. (2003) found that danazol treatment was more effective in treating older patients with ITP than younger patients.

In a review article that encompassed 25 publications on danazol in ITP, Ahn and Horstman (2002) described a favorable outcome of danazol therapy in 21 of the 25 reports. Danazol yielded a sustained platelet increase in 30% of patients. The platelet counts of an additional 10% increased to $50{,}000/mm^3$. However, Ahn and Horstman (2002) found that only a very few patients with severe ITP

responded to this drug. These authors emphasized the importance of maintaining this therapy for at least 6 months and preferentially for 1 year because clinical response in some patients was delayed for as long as 10 months. With respect to this, the majority of studies showed no improvement when danazol was used as a single agent and was discontinued after 2–4 months (Ahn and Horstman, 2002).

Maloisel et al. (2004) found that a high proportion of patients improved following danazol therapy. Their prospective study was conducted at a tertiary referral center and included 57 adult patients (range 21–91 years) with ITP diagnosed between December 1987 and January 1984. Among their subjects, 27 had refractory ITP and 30 had contraindications to splenectomy, refused this option, or had contraindication to corticosteroid therapy. As described previously (Ahn et al., 1989), danazol was used at a fixed dose of 600 mg/day and was started after other treatments were discontinued. In those patients who responded to danazol treatment, the drug was continued at the same dose for at least 6 months and was then decreased to 400 mg/day for the next 3 months. If remission continued, the dose was continued at a maintenance dose of 200 mg/day. Danazol was discontinued in patients with severe side effects or toxicity. However, in those with moderate side effects, the dose was reduced by 200 mg/day. Thirty-eight subjects (67%) experienced partial or complete responses. In this group of patients, the mean platelet count increased from 13,000 to 142,000/mm^3. Also, 12 of the 19 nonresponders had a decrease in bleeding despite any change in platelet counts. Response to danazol was unrelated to gender, severity, and duration of thrombocytopenia and prior splenectomy. Younger patients tended to respond less frequently (Maloisel et al., 2004).

In accord with Ahn and Horstman (2002), Maloisel et al. (2004) recommended at least 6 months of full-dose treatment before this therapy would be considered ineffective. In addition, Maloisel et al. (2004) recommended more than 12 months of treatment in the responder group, progressively decreasing the dose and monitoring carefully for side effects, in particular abnormal liver function tests. These authors also suggested the use of danazol as a therapeutic alternative to splenectomy in older patients with ITP.

According to pharmacokinetic studies, danazol concentrations are variable in plasma and blood cell membranes (Horstman et al., 1995). This fact may explain why patients who failed to respond to a standard danazol dose of 400–800 mg/day may respond to a very low dose of 50 mg/day. This apparent contradiction suggests that in some cases, an excessively high-blood concentration may have adverse effects on platelets (Ahn et al., 1987).

Remissions following long-term danazol therapy in ITP may last for years, even after discontinuation of this drug (Ahn and Horstman, 2002). In the series by Maloisel et al. (2004), the mean time to response for the 38 responders was 3 months, the mean duration of danazol therapy was 3 years, and the mean duration of remission was 10 years.

1.1.2. Danazol therapy in autoimmune thrombocytopenia associated with rheumatic diseases

Thrombocytopenia is an important complication of several autoimmune diseases (Harris et al., 1986). It occurs in 20% of patients with systemic lupus erythematosus (SLE) and is severe ($<50{,}000$ platelets/mm^3) in 5% (Fernández et al., 2007). It has also long been recognized as one of the main manifestations of the antiphospholipid syndrome (Alarcón-Segovia et al., 1992). In this regard, thrombocytopenia has been associated with the presence of antiphospholipid antibodies. In a series of 1000 patients with antiphospholipid syndrome, thrombocytopenia was found in up to 22% of cases, in particular in those associated with SLE (Cervera et al., 2002).

Danazol improved platelet counts in patients with thrombocytopenia associated with SLE (West and Johnson, 1988; Cervera et al., 1995; Blanco et al., 1997) or the antiphospholipid syndrome (Kavanaugh, 1994). It also improved thrombocytopenia in patients with rheumatoid arthritis (RA) (Dasgupta and Grahame, 1989; Blanco et al., 1997).

Cervera et al. (1995) prospectively assessed the efficacy of danazol in treating 16 adult patients

with SLE associated with either autoimmune thrombocytopenic purpura or Evans's syndrome (autoimmune thrombocytopenic purpura and autoimmune hemolytic anemia (AIHA)) that was refractory to corticosteroid therapy. In five instances, danazol was given after splenectomy. Seven of the eleven SLE patients with autoimmune thrombocytopenic purpura and two of five with Evans's syndrome had severe thrombocytopenia, defined as platelet counts $<30{,}000/mm^3$. In all cases, the initial danazol dose was 200 mg/day. Subsequently, the dose of danazol was increased by 200 mg every 4 weeks, according to the clinical response, to a maximum of dose of 1200 mg/day. In this series, danazol was added to the medications the patient was already taking. When the thrombocytopenia or hemolysis had been resolved for at least 1 month, the corticosteroid dose was tapered and danazol was continued. In patients who experienced sustained remission with danazol or who suffered side effects, the dose of danazol was reduced gradually to 200–400 mg/day. In all five patients who had undergone splenectomy, thrombocytopenia resolved within 6–8 weeks of starting danazol therapy. Four of the five patients received daily doses between 600 and 900 mg, and only one required 1200 mg/day. The mean platelet count of these five patients was $>150{,}000/mm^3$ during the mean follow-up period of 22 months. The mean dose of danazol that was required to maintain remission was 380 mg/day. Clinical remission was also observed in the remaining 11 patients on danazol therapy who had not undergone splenectomy. In six, there was a good response, manifested by platelet counts $<30{,}000/mm^3$ before danazol therapy and platelet counts $>50{,}000/mm^3$ following danazol treatment. In the other six patients, there was an excellent response to danazol with platelet counts $>50{,}000/mm^3$ (Cervera et al., 1995). All 11 patients were able to taper prednisone therapy from a mean dose of 20–2.5 mg/day. In 10 patients, thrombocytopenia resolved within 8 weeks of starting danazol. The mean dose required to maintain remission was 372.7 mg/day. Danazol was well tolerated in most patients. These authors supported the use of danazol in thrombocytopenia associated with SLE and suggested starting treatment with a dose of 200 mg/day, regardless the severity of thrombocytopenia, and increasing the dose to about 600 mg/day at week 8 of treatment. They also recommend maintained danazol therapy for at least 1 year, because relapses were more common if danazol was withdrawn during this period. Using this protocol, the authors were able to reduce the daily dose of corticosteroids (Cervera et al., 1995).

Blanco et al. (1997) assessed the efficacy of danazol in treating refractory autoimmune thrombocytopenia associated with various autoimmune diseases. Their study included four patients with SLE, two with RA, and one with primary antiphospholipid syndrome. Platelet counts were below $40{,}000/mm^3$ and bone marrow biopsy showed megakaryocytes in normal or increased numbers with morphology. Follow-up was at least 12 months. In addition to corticosteroids, all patients had received an additional therapy including methotrexate, azathioprine, or intravenous immunoglobulin. Danazol was started at an initial dose of 100 mg every 6 h, and the dose was increased progressively to a maximum of 200 mg every 6 h. The dose of prednisone was kept at a constant amount for at least 1 month. Then it was tapered progressively based on the platelet counts. All cases achieved acceptable platelet counts within the first 4 weeks of danazol therapy, permitting doses of prednisone to be tapered. After 4 weeks of danazol therapy, the platelet counts were between $90{,}000$ and $213{,}000/mm^3$ (median $130{,}000/mm^3$). No important side effects related to danazol therapy were observed (Blanco et al., 1997).

Based on the reports by Cervera et al. (1995) and Blanco et al. (1997), danazol is useful in treating thrombocytopenia in the setting of SLE and other rheumatic diseases. According to their observations, patients with connective tissue diseases and autoimmune thrombocytopenia may be treated initially with corticosteroids (1 mg/kg/day or greater) for at least 1 month. If no response is observed or patients require continued high-dose corticosteroid (prednisone doses >20 mg/day) to maintain normal platelet counts, danazol may be added. Blanco et al. (1997) suggested starting danazol, 100 mg q.i.d. for 1 month, and then, according to response, the dose should be progressively increased to a maximum of 200 mg

q.i.d., so that clinicians can set an optimal dose without overtreatment. When optimal response is reached, this dose should be maintained for another month and then corticosteroids should be progressively reduced to the lowest required dose. Thereafter, when corticosteroids are tapered to a low dose (prednisone 10 mg/day), if the platelet counts remain in an acceptable range ($>100{,}000/mm^3$) for at least another month, an attempt should be made to reduce danazol by 100 mg/day every month.

Based on these observations (Cervera et al., 1995; Blanco et al., 1997), danazol also seems to be a well-tolerated treatment for refractory immune thrombocytopenia associated with different rheumatic diseases. In this regard, danazol had the highest probability of being continued in a study that examined long-term termination rates of several drugs used to treat hematological manifestations of SLE, mainly thrombocytopenia (Avina-Zubieta et al., 2003).

1.2. Danazol in the management of autoimmune hemolytic anemia

Initial therapy for warm antibody-mediated AIHA should be corticosteroids, such as prednisone at conventional doses of 1–1.5 mg/kg/day orally. Approximately 20–30% of patients achieve a lasting remission with corticosteroid therapy, yet 50% continue to require low-dose maintenance prednisone for months, and another 10–20% either do not respond to corticosteroids or require unacceptably high doses of prednisone (Petz, 2001). In these cases, splenectomy has the advantage over therapeutic options in that it has the potential for complete and long-term remission.

Danazol has been used in combination with corticosteroids to improve the response rate and allow complete withdrawal of corticosteroids in some patients with AIHA. Ahn (1990) reported excellent or good response in a series of 28 AIHA patients. This author described improvement in 77% of patients with idiopathic AIHA and 60% of patients with secondary AIHA. In this series, danazol was discontinued after 1 year or more of therapy. Interestingly, up to 5 years of remission was achieved in several cases (Ahn, 1990).

Pignon et al. (1993) assessed the efficacy of danazol in 17 adults with either idiopathic or secondary AIHA. Ten were initially treated with prednisone (1 mg/kg/day) and danazol (600–800 mg/day) as first line therapy. The remaining seven patients, who either had refractory AIHA or had relapsed after an initial favorable response to prednisone, were also treated with danazol (600–800 mg/day) plus prednisone. In this series, the mean duration of danazol therapy was 28 months. Eight of the ten patients treated with prednisone and danazol as first line therapy had an excellent response. However, only three of the seven patients from the second group had excellent responses. Danazol therapy has also been found useful in the treatment of AIHA associated with SLE (Chang and Sack, 1991; Cervera et al., 1995).

1.3. Danazol in autoimmune C1-inhibitor deficiency

C1 inhibitor (C1-INH) protein is the only in vivo inhibitor of the first component of human complement, and it is an important element in controlling the contact activation and kinin system by the inhibition of factor 12 and kallikrein (Davis, 1988). The genetic defect of C1-INH produces hereditary angioedema (HAE), a disease characterized by recurrent swelling of subcutaneous and mucous (gastrointestinal and laryngeal) tissues (Agostoni and Cicardi, 1992). Also, an acquired form of C1-INH deficiency with identical symptoms, called acquired angioedema, has been reported mainly in association with lymphoproliferative disorders, and is occasionally associated with autoimmune, neoplastic, or infectious diseases (Gelfand et al., 1979).

Prophylaxis of angioedema symptoms in C1-INH deficiency states is based on the administration of an attenuated androgen such as danazol (Cicardi et al., 1993). Danazol increases C1-INH plasma levels and effectively cures HAE, reversing biochemical abnormalities (Gelfand et al., 1976; Cicardi et al., 1991). In this regard, Gelfand et al. (1976)

assessed the role of danazol in preventing attacks of HAE in a double-blind study with nine patients. Of 47 placebo courses, 44 ended with attacks, but during 46 danazol courses only one attack occurred. C1-INH levels increased three to four times, and levels of the fourth component of complement (C4) increased by a factor of 15. These changes began during the first day of therapy and were maximal by 1–2 weeks. However, when danazol therapy was stopped, C1-INH and C4 levels decreased rapidly. Similarly, Cicardi et al. (1991) assessed the effects of two attenuated androgens, stanozolol and danazol, in 56 patients affected with HAE who had one or more severe attacks per month. The minimal effective doses usually did not exceed 2 mg/day of stanozolol or 200 mg/day of danazol. In two patients, these doses were not sufficient to achieve the complete disappearance of symptoms. Plasma levels of C1-C1-INH complexes at different doses of stanozolol were measured in four patients with HAE. These complexes were elevated before treatment, but reverted rapidly to normal values during androgen therapy and remained normal with reduction of dose as long as the patient remained free of symptoms. In addition, attenuated androgens have been shown to prevent symptoms and increase C1-INH plasma levels in non-autoantibody-mediated acquired angioedema (Sheffer et al., 1985).

1.4. Danazol in other autoimmune diseases

Danazol has been useful in treating other immune-mediated diseases such as Henoch–Schönlein purpura (Lee et al., 1993). Tomino et al. (1987) showed that 200 or 300 mg of danazol was effective in reducing the amount of proteinuria and increasing serum concentrations of complement components in patients with IgA nephropathy.

2. Side effects of danazol therapy

In most cases, danazol is tolerated well. However, weight gain, increased transaminases, and peliosis hepatitis has been described. Therefore, careful monitoring with adjustment of dose is recommended (Nesher et al., 1985). Other side effects are generalized as skin rash, lethargy, myalgia, itching, hair loss, mild virilizing side effects (voice change and hair growth), vertigo (Ahn et al., 1989), and arterial thrombosis have been reported (Alvarado et al., 2001).

Key points

- Danazol can be used in individuals with idiopathic thrombocytopenic purpura refractory to corticosteroids.
- Danazol therapy can be considered in patients with thrombocytopenia in the setting of SLE or other autoimmune rheumatic diseases when response to corticosteroids is not achieved.
- Danazol is useful in HAE.

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PART IV:

Novel Therapies

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CHAPTER 25

Islet Transplantation for the Treatment of Type I Diabetes

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1. Historical perspectives on islet transplantation

The first clinical attempt of islet transplantation occurred in 1893 in Bristol, UK, 28 years before the discovery of insulin (Williams, 1894). Watson-Williams and his surgical colleague, Harsant, transplanted three pieces of freshly slaughtered sheep's pancreas, 'each the size of a Brazil nut', into the subcutaneous tissues of a 15-year-old boy dying from uncontrolled ketoacidosis. The operation, performed under chloroform anesthesia, was completed 'within twenty minutes of the death of the sheep'. Although there was a temporary improvement in glucose control before the boy's death 3 days later, this xenograft was destined to fail without immunosuppression. The idea was not new, Oscar Minkowski had already carried out a similar procedure in a pancreatectomized dog in 1892 and had described a temporary reduction in glycosuria (Minkowski, 1892).

In 1920, Frederick Banting discovered that a ligation of the pancreatic duct in dogs led to acinar degeneration and an enhanced recovery of the 'internal secretions' for the treatment of diabetes (Banting, 1920). The effect was dramatic, and studies by Banting, Best, Collip and Macleod rapidly led to the introduction of exogenous insulin into clinical practice in 1922. By the following year, Eli Lilly was producing insulin in virtually unlimited quantities (Bliss, 1982). Diabetes was transformed from being rapidly fatal after the onset of ketoacidosis to a chronic incurable illness, most patients with diabetes developing one or more end-stage secondary complications during their lifetime.

The historical threads of islet transplantation were picked up again by the pioneering work of Paul Lacy in the 1960s and the successful isolation of rat islets at Washington University in St. Louis (Lacy and Kostianovsky, 1967). Transplantation of these islets showed to reverse diabetes in animal models (Ballinger and Lacy, 1972). Despite these initial successes, clinical application has been difficult, mainly because of the technical challenge to isolate large-scale human islets. The first report of insulin independence after islet transplantation in a subject with type 1 diabetes and previous kidney transplant was reported in 1989 (Scharp et al., 1990).

Improved outcomes in clinical islet transplantation were reported not long afterwards. Insulin-independence rates of more than 50% at one year was achieved in 10 patients receiving combined liver and islet allotransplants following upper abdominal exenteration (including pancreatectomy) for cancer (Ricordi et al., 1989, 1992). However, insulin independence, in this series of islet allotransplants, was not achieved in any patient with pre-existing autoimmune diabetes. Interestingly immunosuppression did not include steroids, in contrast to the regimen employed in diabetic subjects.

Successful islet transplantation, predominantly in association with kidney transplantation, in type

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1 diabetes was achieved by several groups including Giessen, Germany and the GRAGIL consortium (based in Geneva, Switzerland, and France) (Bretzel et al., 1999; Benhamou et al., 2001). This improved success was attributed to advances both in peritransplant management and in immunosuppressive therapy. However, insulin-independence rates at 1 year were only around 20%, mainly because the protocols included steroid-based immunosuppression.

Till the late 1990s, most islet allotransplants were combined with another solid organ transplant, usually a kidney, which required and justified immunosuppression. Despite earlier optimism, serious complications and relatively disappointing insulin-independence rates suggested that the benefits of islet-alone transplantation were outweighed by the risks. The future of islet-alone transplants seemed in doubt, but the report in 2000 of insulin independence in all of seven type 1 diabetic subjects receiving islet-alone transplants at the University of Alberta in Edmonton (Shapiro et al., 2000) was followed by much interest in the medical and scientific communities as well as among the general public. Subsequently, there has been a huge expansion in clinical islet transplantation as well as basic and clinical research.

2. Current procedures for islet isolation and transplantation

2.1. Pancreas donor selection

The increasing interest and demand for islet transplantation are challenging the current islet isolation facilities. The scarce availability of donors and the costs of islet isolations require a selection of donors in order to avoid the exclusion of eventually appropriate donors and to reduce the number of isolation procedure with insufficient islet yields. In an attempt to select the best donors only, several islet isolations centers established selection criteria (Benhamou et al., 1994; Brandhorst et al., 1994; Zeng et al., 1994; Lakey et al., 1996; Toso et al., 2002; O'Gorman et al., 2005).

Donors should have no history of diabetes, pancreatitis, or alcohol abuse. The age has an impact on islet yields. Lower isolation results are achieved with pancreata from donors younger than 20 years. This may be explained by a softer parenchyma with less connective tissue around the islets. A debate remains regarding the inclusion of pancreas from old donors. However, 60 years appear as a reasonable limit. A high body mass index (BMI) is positively correlated with the isolation yields. The total islet mass may be increased in over-weighted patients, reflecting the higher metabolic demand in obese individuals. In addition, a higher islet yield in obese donors may be also explained by a higher content of fat cells in the parenchyma and reduced amount of collagen, what has been demonstrated to increase the yield (Mahler et al., 1999). The occurrence of hemodynamic instability with sustained hypotension in the donor is associated with a trend toward poorer isolation, especially when associated with biochemical abnormalities ($>50\%$ increase in creatinine or a doubling in liver function tests).

After donor selection, tight coordination is essential between organ retrieval, transportation and isolation teams. Secondary warm ischemia and cold ischemia times are relevant factors for the outcome of islet isolation (Ketchum et al., 1994; Lakey et al., 1996; Toso et al., 2002). Every effort should be made to shorten both. The pancreas should be dissected before aortic cross-clamp, to facilitate placement of ice behind and in front of the pancreas in the lesser sac, while taking care to preserve capsule integrity (Lakey et al., 2002). It must be harvested before the kidneys and either before or at the same time as the liver. Finally, procuring hospitals should be within a distance to the islet core facility to allow the initiation of digestion within 8 h of aortic clamping.

Such selection criteria have been applied within the GRAGIL collaborative network between the isolation center in Geneva (Switzerland) and several French universities. Over a 2-year observation period, 260 pancreas were offered, but only 104 (40%) were finally accepted for isolation. Reasons for refusal include the absence of suitable recipient ($n = 10$), medical ($n = 105$), and logistic ($n = 41$) issues (Kempf et al., 2005).

2.2. Islet isolation

Current islet isolation techniques are derived from the original work of Camillo Ricordi, describing an automated technique to achieve large-scale production of islets (Ricordi et al., 1989). Strong international collaboration between centers led to increased efficiency of the process and had a major impact on enhancing the consistency and quality of highly purified islet preparations for safe transplantation into patients.

Pancreas glands are first distended with collagenase (Lakey et al., 1998a). Subsequently, the pancreas is cut into small pieces and placed in the Ricordi chamber, a stainless steel container, along with stainless steel marbles. The chamber is perfuse with a blend of enzymes at 37°C and agitated. With this combination of chemical and mechanical dissociation, islets are freed from exocrine tissue. Digestion of the pancreas is generally complete within less than 30 min (typical digestion times are 11–18 min).

A major advance in islet isolation occurred with the development of refined collagenase, Liberase-HI and subsequently Liberase-CI by the Boehringer-Mannheim Corporation (now Roche Biochemicals, Boehringer Mannheim, Inc., Indianapolis, IN, USA). These are purified enzyme blends, low in endotoxin (Linetsky et al., 1997; Lakey et al., 1998b). Liberase has been found to be superior to crude collagenase preparations in yielding larger number of islets without compromising functional viability (Lakey et al., 1998b). With time, some lot-to-lot variability appeared, leading to inconsistency in pancreatic digestion. An alternative company, Serva, developed a collagenase blended with a neutral protease to be added at the time of use. Preliminary experience suggested promise, but further demonstration of reproducibility is needed (Bucher et al., 2005). It should be emphasized that despite recent advances in collagenase science, this enzyme blend remains the key determining factor in the success of islet isolation, but the enzymatic constituents accounting for this success have not yet been fully characterized.

After digestion, cells are washed from collagenase and islets are separated from exocrine tissue over a series of density gradients using a COBE cell separator. The separation is based on the observation that islets are less dense than exocrine cells. Density gradient involved Ficoll, the high molecular weight (400 kDa) polymer of sucrose (Robertson et al., 1993). In 1991, Euro-Collins, a cold-storage preservation solution, was used as the vehicle for dissolving the Ficoll powder (Olack et al., 1991). Hypertonic-density solutions such as EuroFicoll prevent edema of the exocrine tissue at low temperatures and result in an improved separation of the islets from the exocrine tissue (Lakey et al., 1996). The large-scale purification of islets is performed; thanks to the Cobe 2991 blood cell processor. This system, originally designed to separate blood components via centrifugation, offers decreased operating time and the ability to process an entire preparation of human pancreatic tissue digest in a self-contained sterile disposable tubing system.

In the original Edmonton protocol, islets were transplanted directly after isolation (Shapiro et al., 2000). While long-term culture of islets is associated with a loss of endocrine cells (Schmied et al., 2000), it is now clear that islets can be successfully cultured for several days prior to transplantation without any significant loss of potency (Benhamou et al., 2001; Hering et al., 2005). Indeed, the ability to culture islets prior to transplantation has a number of advantages. Safety is improved since transplants can be scheduled when the whole transplant team can be present. Time is also available to administer conditioning or other immunosuppressive therapy, avoiding exposure of islets to the cytokine release associated with many cell-depleting induction therapies. Logistically, patients no longer need to live close to the transplant center for indeterminate periods of time. Islets can be safely cultured within 24–72 h. It is not entirely clear at this time whether the islet engraftment and long-term function is improved by the use of fresh or cultured islets.

2.3. Islet transplantation networks

While islets can be cultured for 2–3 days prior to transplant, collaboration have been developed

between centers. The benefits of concentrating expertise in the challenging task of islet isolation lead to enhanced efficiency with higher rates of isolation success, and have been associated with reduced costs linked to islet-processing laboratories. In the GRAGIL network, the average cost of an islet transplant reaches approximately $80,000 (islet isolation and 1-year follow-up), with the main costs being linked to islet preparation (30%), adverse events (14%), drugs (14%), and hospitalization (13%) (Guignard et al., 2004).

Islets can be successfully shipped from the isolation facility to distant transplant centers (Goss et al., 2004). Collaboration appears feasible between centers up to 10 h away (Kessler et al., 2004). This opens up the potential for a small number of core islet isolation facilities, which provide considerable economy of scale, since islet isolation must be performed in a cGMP facility. Preliminary data from a multi-center trial of islet transplantation indicates superior outcomes from experienced centers and confirms the steep learning curve associated with islet isolation (Shapiro et al., 2003, 2005).

Shipment between centers is performed using bags. At the arrival in the recipient site, the bag can simply be hooked to a line connected to the portal vein catheter (Baidal et al., 2003). This simple procedure reduces manipulations and minimizes risks of errors. Prior to islet infusion in the recipient center, however, a series of quality control, product-release criteria assessment, and islet quantification recounting is mandatory.

2.4. Selection of islet preparation and recipients

Prior to transplant, islet preparations should fulfil several quality criteria including $>30\%$ purity, $>70\%$ viability, <10 ml packed cell volume, <5 EU/kg endotoxin content (based on recipient weight) and negative Gram staining of the islet culture medium. Transplantation of pure islets reduces the risk of intra-portal thrombosis.

The tissue matching of donor islets with recipients requires mandatory compatibility for ABO-type matching. Where a potential recipient has become sensitized to HLA antigens through with previous pregnancy, blood transfusion, or previous transplant, further matching is required, including prospective cytotoxic cross-match being negative for both T and B cells. Furthermore, the number of islets needed per infusion is generally selected at a minimum of greater than 5000 islet equivalents per kilogram, based on recipient weight.

2.5. Islet transplantation

Purified islets are implanted into the liver by way of the portal vein using two accepted approaches.

The most common one is the radiological approach. It is relatively simple and avoids general anesthesia. There is, however, a potential risk for uncontrolled bleeding when a percutaneous transhepatic portal access catheter transgresses the hepatic parenchyma. We encountered a high rate of bleeding from the liver surface when the track was partially plugged with Gelfoam pledgets. Recently we switched to the routine use of Avitene paste (microfibrillary collagen powder made up in radiological contrast media). The advantage of the Avitene paste is that it may be visualized directly on fluoroscopy, so one can be sure it has adequately and completely ablated the entire catheter tract within the liver, without risk of central embolization. We have experienced no further episodes of bleeding with these track-ablative approaches in the past 50 consecutive cases.

The second method involves surgical laparotomy and canulation of a mesenteric venous tributary of the portal system. The advantage of this approach is that it is carried out with complete surgical control. This approach is generally recommended for patients on anti-coagulation, if there is a hemangioma on the right side of the liver that may be at risk for puncture and bleeding, or in case of combined kidney/islet transplantation. Additionally, the surgical approach is used where

there is only limited local interventional radiological expertise. The disadvantage of this approach is that a surgical incision is required, and there is risk for wound infection and wound herniation, which may be exacerbated when the drug sirolimus is used post-transplant, as this drug interferes with wound healing. Adhesion formation is also a potential risk of the surgical approach.

At the University of Geneva, Switzerland, 2 partial and reversible portal vein thrombosis and 7 bleedings were observed among 62 percutaneous transhepatic injections, but no complication was related among 16 open surgical injections (Bucher et al., 2004).

Alternative routes of portal access, including transjugular intrahepatic techniques (adapted from the TIPPS procedure), may diminish the risk of bleeding but are more challenging and prolong the duration of the procedure.

3. Islets and pancreas transplantation

Either islet or whole pancreas transplantation can provide effective beta-cell replacement. As such, their primary utility is in patients with type I diabetes, where the disease is linked to a depletion of insulin-producing cells. In contrast, due to increased peripheral insulin resistance, type II diabetes is, in general, not considered as an indication for transplantation. There has been relatively limited experience of whole pancreas transplantation in lean subjects with type II diabetes and positive outcome, but this approach has yet to be explored further in islet transplantation.

Potential transplant recipients may be considered in two groups—those with type I diabetes with or without end-stage renal disease. Each of these subjects may be potential candidates for either islet or whole pancreas transplantation.

3.1. Recipient selection for islet and whole pancreas transplantation

The main difference between the two techniques of beta-cell replacement is that a lower mass of insulin-producing cells is transplanted with islet compared to whole organ transplantation. Islet transplantation is usually biased toward selection of patients with lower insulin requirements. Females should weight less than 70 kg and males less than 75 kg and BMI should be under 26 kg/m^2. They should further require less than 50 U of insulin a day, or less than 0.7 U/kg of body weight/day. Patients respecting all these criteria have the best chances to achieve insulin independence and long-term function. Islet transplant is generally restricted to patients under 65 years old.

While pancreas transplant is a more challenging surgical procedure, it is addressed to younger patients, with a generally accepted age limit of 50 years. These patients should further have no major cardiac or respiratory disease. Results tend to decrease in obese patients, but there is no absolute limit regarding weight, BMI, and insulin requirement.

3.2. Non-uremic patients with type I diabetes

Currently, islet transplantation has been studied mostly in subjects receiving islet transplant alone (ITA) (Shapiro et al., 2000; Markmann et al., 2003; Goss et al., 2004; Hering et al., 2005; Ryan et al., 2005a, b).

Clinical islet transplantation has progressed in the past 6 years from being considered as medical curiosity to an established therapy available only at selected number of centers for the effective treatment of unstable forms of type 1 diabetes. The initial report by the Edmonton Group of 100% success in achieving insulin independence in the first seven treated subjects served to galvanize the field and generate enthusiasm and activity worldwide (Shapiro et al., 2000). This led to a huge burst in clinical islet transplant activity worldwide, with more than 600 islet transplants performed at more than 40 international centers since the year 2000 (Fig. 1). More recently, however, it has become clear that while short-term insulin independence is achievable in approximately 60–80% of treated

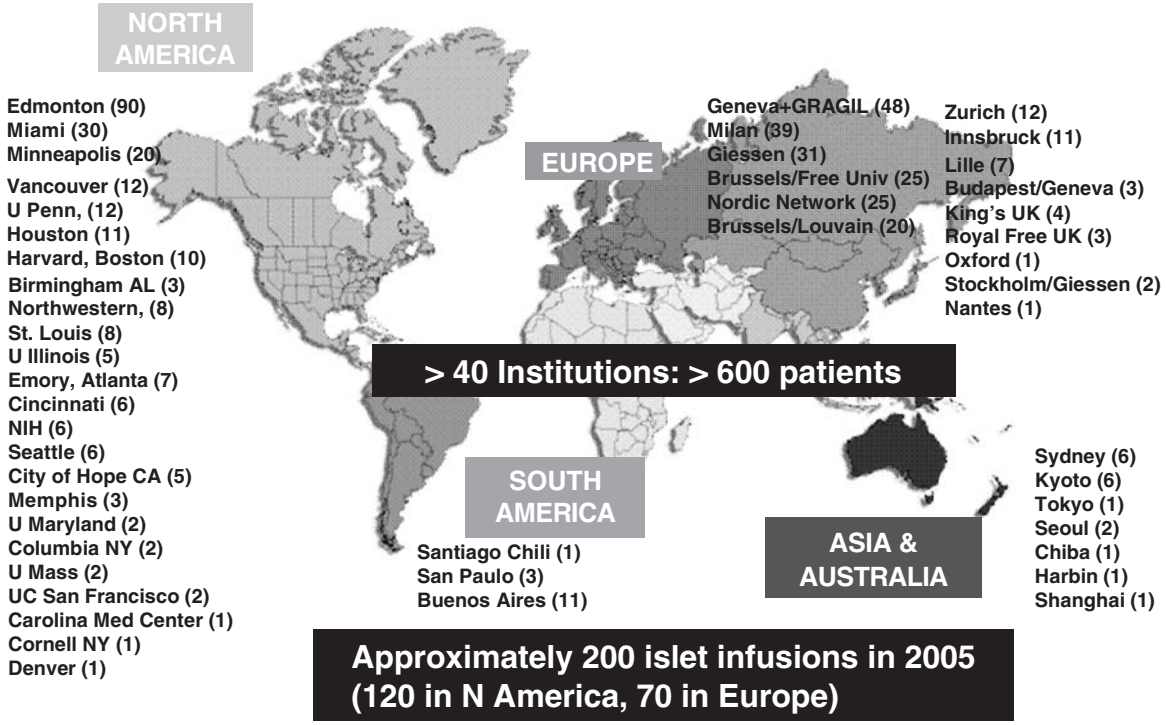

Figure 1. Islet transplant activity (1999–2006).

subjects (given two or more islet infusions), more long-term durability has been far harder to maintain, with only 40–50% of subjects remaining insulin-free at 3 years, and only 11% remaining insulin-free at 5 years post-transplant (Ryan et al., 2005a, b). It should be emphasized that while insulin independence may not be sustained beyond 5 years in most patients, persistent C-peptide secretion continues in approximately 80% of subjects. This renewed endogenous insulin secretion is usually sufficient to effectively counteract episodes of severe hypoglycemia and glycemic lability, and from both the patient's and endocrinologist's perspective, partial restoration of endogenous regulated insulin secretion is generally regarded as a highly effective intervention.

Despite recent progress in islet transplantation, the majority of subjects with type 1 diabetes will still be more safely managed with chronic insulin injections rather than facing the potential risks and significant side effects of current anti-rejection therapies. While type I diabetes without uremia is a common disease, ITA must be restricted to a small number of highly selected patients with very unstable blood-glucose regulation. These patients experience repeated hypoglycemias, which restrict their daily social life and expose them to potential traumas. This happens despite intensive and professional endocrinological management.

Subjects receiving ITA could also potentially be treated with a pancreas transplant alone (PTA). There has been much recent discussion relating to potentially detrimental outcomes of PTA on mortality, but with recent re-analysis of data, the benefits of PTA are clear (Venstrom et al., 2003; Gruessner et al., 2004). Our own approach in Edmonton has been to support ITA and not PTA for our subjects with unstable type I diabetes without uremia, but we recognize that our bias reflects ready access to high-grade human islets. ITA is generally linked to less morbidity compared

with the surgical implantation of a pancreas graft. Long-term stabilization of blood glucose may be readily achieved with either islet or pancreas transplantation. Therefore, we would advocate that either islet or whole pancreas transplantation be considered as potential therapeutic options, and where a subject is unsuitable for one approach, the alternative approach should at least be considered.

3.3. Patients with type I diabetes and end-stage renal disease

The current policy is to offer simultaneous pancreas/kidney transplantation (SPK) to patients with type 1 diabetes reaching renal failure, with the aim to improve patient survival and quality of life (Gruessner et al., 2004). Ideally, this procedure should be performed prior to onset of dialysis-dependent end-stage renal disease. Simultaneous islet/kidney transplantation (SIK) has been offered to older patients—usually over 50—with more co-morbidities and to those unwilling to take the risk of a SPK procedure. With such a policy, there should be a relatively small number of subjects with type 1 diabetes receiving a kidney-alone, unless they are receiving first a living donor kidney with a plan to proceed with a subsequent islet/pancreas graft. Data from the Swiss consortium clearly indicates that where preferential use of pancreas organs is given to whole pancreas transplantation, this does not lead to a significant deficit of valuable organs for islet isolation (Ris et al., 2004). Pancreas-after-kidney transplantation (PAK) is becoming an increasingly attractive option for patients with type 1 diabetes who either never were offered a pancreas transplant, lost their pancreas transplant for technical reasons, or have a live kidney donor available. The procedure is thought to prolong patient survival and delay or prevent the occurrence of secondary diabetic complications thanks to the recovery of glycemic control (Hariharan et al., 2002). Along this line, offering islet-after-kidney (IAK) transplantation to patients who are not candidates for a pancreas transplant seems a logical option to achieve a similar long-term goal. This rationale is supported by the association of successful IAK transplantation with long-term improvement of diabetic macro- and microangiopathy (Fiorina et al., 2001).

In the context of an IAK transplant, the subject is already facing the pre-existing risks associated with immunosuppression, and therefore it may be regarded as less of an issue in case of islet transplant in presence of a kidney graft. We can thus anticipate that the number of such transplants will increase in the coming years and will reach a similar number of ITA transplants.

A preliminary series of the University of Geneva explored the applicability of the steroid-free sirolimus/low-dose tacrolimus Edmonton immunosuppression protocol in the IAK setting (Toso et al., 2006a). This study showed that islet transplantation can be done in the IAK setting with equal efficacy and safety as in the ITA setting. All eight recipients achieved insulin independence, with five having reached 1-year follow-up without exogenous insulin.

The issues pertaining to the IAK and ITA populations are completely different. In the ITA setting, patients with type 1 diabetes mellitus must have a highly unstable disease on a metabolic standpoint, to warrant the introduction of immunosuppression with its well-known, sometimes poorly tolerated, side effects and risks of infection and malignancy. In the IAK setting, the risks of immunosuppression become less of an issue because these patients are already immunosuppressed.

On the other hand, patients run the risk of destabilizing the function of their kidney grafts at the time of switching immunosuppression and/or weaning of steroids at the time of islet transplant. This was the case in one patient in the Geneva's series, who lost his kidney graft several months after IAK transplantation. This complication urges for strict selection of recipients. The described patient had a declining creatinine clearance while on the waiting list and was under 50 ml/min at the time of transplantation. It is questionable whether this patient should have been transplanted under this protocol. Cut-off values cannot be determined from this study, but they can be adapted from policies applied for transplantation of pancreas after kidney (Hariharan et al., 2002).

Creatinine clearance >50 ml/min and proteinuria <0.5 g/day appear as reasonable minimal recipient selection criteria in the IAK setting, and are in accordance with previously published data (Kaufman et al., 2002). However, it seems to us that the absolute value of creatinine clearance is probably of less importance than the stability of kidney graft function. Practically, stability of serum creatinine values over the previous year should be ascertained before listing a patient for IAK transplantation. Although there are only few data about baseline biopsies, they could also help assessing a pre-existing nephropathy.

3.4. How many islet preparations should be transplanted?

At the present time and whatever the setting (ITA, IAK, SIK), most islet recipients receive two to three islet preparations. These repeated procedures increase the risk of injection-related complications. Patients exposed to more donor antigens also have a higher risk of immunological sensitization. As a consequence, the identification of subsequent suitable compatible islet preparations is more challenging. Moreover, if these patients receive a kidney transplant, such a sensitization can cause significant delays in identifying suitable donors and they would have to remain longer on dialysis. Repeated islet injections further exacerbate the critical lack of pancreas organs, as the indications for islet transplantation expand earlier into the course of type 1 diabetes.

On the other hand, the primary indication for islet transplant is blood-glucose instability and the occurrence of severe and repeated hypoglycemia. These events are already effectively controlled after a first islet infusion (Ryan et al., 2004, 2005a, b). The subsequent islet infusions usually lead to insulin independence, but only marginally further improve blood-glucose stability. Furthermore, the improvement of quality of life after islet transplant is mainly related to the absence of hypoglycemia and not to insulin independence (Barshes et al., 2005; Poggioli et al., 2006). It is thus questionable whether islet transplant should be viewed solely as a way of avoiding exogenous insulin injections or as a way of stabilizing blood glucose only, and therefore whether just one or several islet infusions should be performed. It remains unclear whether supplemental islet infusions (or indeed continued low-dose insulin administration) will help maintain the existing islet mass and avoid undue metabolic stress. This controversy is clearly inadequately addressed in the available literature, and further studies are needed to answer this question.

4. Immunosuppression

4.1. Steroids

Evolutions in immunosuppressive strategies over the past 5 years have led to much less chronic exposure to corticosteroids. Corticosteroid use is particularly troublesome in islet transplantation, as high-dose steroid exposure leads to insulin resistance and islet exhaustion, as well as numerous systemic side effects. When glucocorticoids were combined with calcineurin inhibitors (cyclosporin or tacrolimus), potent synergistic toxicity developed, with an irreversible decline in islet autograft function coupled with an increase in peripheral insulin resistance (Morel et al., 1992; Rilo et al., 1994; Shapiro et al., 1998). Clinical evidence showed that up to 20% of previously non-diabetic organ recipients developed new-onset non-autoimmune diabetes as a direct result of calcineurin/glucocorticoid therapy.

Damage caused by diabetogenic immunosuppression was particularly apparent in islet transplantation in which the islet engraftment reserve is sub-physiological. As a consequence, only marginal numbers of islet recipients were remaining insulin-free with the steroid-containing immunosuppression used till 2000.

4.2. Sirolimus

More potent, more specific, and less toxic immunosuppressive agents have recently become available

for clinical application. It is now possible to provide greater immunological protection while avoiding diabetogenic side effects by using an agent such as sirolimus.

Sirolimus is a macrolide antibiotic, structurally related to tacrolimus. The name was derived from Rapa Nui, a region of Easter Island, where rapamycin was first isolated from soil samples returned by the Canadian geological expedition by Suren Sehgal and his team. It was originally reported as a fungicidal antibiotic (Vezina et al., 1975).

Sirolimus acts by forming an active complex with a cytosolic immunophyllin—the FK binding protein 12—but unlike the CNI, this has little effect on inhibiting the signal 1 MHC/T-cell receptor pathway that leads to nuclear factor of activated T-cells (NFAT) initiation. Rather the sirolimus/FKBP12 complex negatively regulates kinases referred to as mammalian targets-of-rapamycin (m-TOR) that leads to blockade of the B7-1/B7-2 to CD28 co-stimulatory signal 2 pathway interfering with NF-κB induced secretion of IL-2 and other cytokines (Lai and Tan, 1994); and abrogation of the signal 3 pathway signal transduction pathway from cytokine/growth factor receptors to the nucleus to initiate cell cycling and proliferation (Yakupoglu and Kahan, 2003).

The US Food and Drug Administration approved sirolimus in 1999 for its use in kidney recipients in combination with cyclosporin and corticosteroids. In multi-center solid organ trials, the combination of sirolimus with CNI and steroids was reportedly as good as or better than classical CNI and steroids regimens in preventing rejection in solid organ transplantation (Wiesner et al., 2002a, b). In non-randomized single center trials, sirolimus combinations with reduced tacrolimus dosing reported frequency of acute cellular rejection at least equal to historical control groups (McAlister et al., 2000; Trotter et al., 2001; Dunkelberg et al., 2003).

In the islet transplant setting, survival was improved with sirolimus combined with subtherapeutic doses of cyclosporin, whereas either drug given alone did not benefit survival (Yakimets et al., 1993). Kneteman et al. (1996) found a significant improvement in glucose tolerance in canine islet autografts as a result of increased insulin secretion and reduced insulin clearance.

Sirolimus is currently the backbone of immunosuppression for islets. It is given at a loading dose of 0.2 mg/kg orally immediately pre-transplantation, with maintenance initially at 0.1 mg/kg per day adjusted to 24 h target serum trough levels of 12–15 ng/ml for 3 months, being reduced to 7–10 ng/ml thereafter (measured by high-performance liquid chromatography). Low-dose tacrolimus is begun at 2 mg orally given twice daily but adjusted to target 12 h trough levels of 3–6 ng/ml, representing about a quarter of the usual standard dose for other transplants.

4.3. Anti-IL2 receptor antibodies

In the Edmonton Protocol, glucocorticoids were completely avoided because of the previously described concerns. Immunosuppressive efficacy was maintained with an induction course of an anti-interleukin (IL) 2 receptor monoclonal antibody (anti-CD25), Zenapax, 1 mg/kg intravenously immediately pre-transplantation, and four doses given bi-weekly post-transplantation.

Zenapax is a genetically engineered mouse antibody that has been modified to have 90% human component. As a result, the incidence of acute hypersensitivity reactions is negligible. The risk of anti-idiotypic antibodies is minimal, allowing repeated injections, without risk of decreased efficiency (Koch et al., 2002). In patients in whom a second islet graft was given beyond the 10 week induction window, the induction course of daclizumab was repeated.

Daclizumab was approved in 1997 by the FDA to be used in conjunction with a standard course of immunosuppressive therapy in kidney recipients. It was the first monoclonal antibody to achieve a treatment label for prevention of rejection as OKT3 was originally approved for the treatment of acute rejection rather than prophylaxis.

Studies in solid organ transplant recipients showed that daclizumab prophylaxis reduced the rate of acute cellular rejection. A non-randomized study at the University of Pennsylvania using daclizumab

induction reported less acute rejection episodes compared to no induction in patients maintained on calcineurin inhibitors (CNI)/mycophenolate mofetil (MMF)/steroids (Eckhoff et al., 2000; Sellers et al., 2004). These findings were confirmed by a second study using a similar protocol, but with a single dose of daclizumab at 2 mg/kg (Yan et al., 2003). In comparison to a historical control group receiving OKT3 prophylaxis, patients treated with daclizumab demonstrated similar rates of acute rejection (Emre et al., 2001).

4.4. Future immunosuppression perspectives

More recently in Edmonton and at other centers, it has become increasingly clear that the high doses of sirolimus (levels of 12–15 ng/ml) used early post islet transplantation have led a relatively high rates of chronic side effects. New emerging drugs would probably allow designing novel protocols, which could ideally be steroid, calcineurin inhibitor-, and mTOR inhibitor-free, in order to minimize side effects, and most of all nephrotoxicity. Alternative strategies include induction with anti-T cell therapies including anti-CD3 (hOKT3-ala-ala) in Minnesota, alemtuzumab (anti-CD52) in Edmonton and Miami, thymoglobulin in a number of islet centers, and future strategies involve clinical evaluation of co-stimulatory blockade with Belatacept (LEA29Y), sphingosine-1-receptor blocking drugs (e.g., FTY720), Janus Kinase inhibitors (e.g., JAK3 inhibitors such CP690,550), and other newer anti-rejection approaches. These are predicted to be more 'islet-friendly' in terms of avoidance of diabetogenic side effects, while simultaneously improving islet engraftment and neovascularization.

5. Risks and side effects

5.1. Injection-related complications

The principle, injection-related complications include bleeding and thrombosis.

Bleeding is manifest at the surface of the liver after percutaneous puncture. In the previous years, bleedings were more frequent because of the use of larger catheter. In Edmonton, an unexpectedly high rate of bleeding after percutaneous islet implantation was recorded in 2003. At this time we had completed 85 islet infusions, and encountered bleeding in 19 cases (22% risk of bleeding). We defined post-procedural bleeding as an acute 20% fall in Hb after transplant, associated with the presence of free fluid on ultrasound, need for blood transfusion, or surgical intervention for control of bleeding. They have been observed more often after second and third transplants (Villiger et al., 2005).

Bleeding is now a rare event, thanks to the exclusion of patients with coagulopathy, the use of small catheter ($\leq$4.5 French), and effective embolization of the liver tract with the microfibrillary collagen Avitene paste after transplant. The radiologist should further be experienced to prevent repeated punctures of the liver capsule.

Post-transplant bleeding was a significant problem not only because of the complication itself but also because it was restricting the use of anti-coagulation after transplant. A more aggressive anti-coagulation appears as an important step to prevent early activation of the coagulation cascade after transplant and the instant blood-mediated inflammatory reaction (IBMIR) (Johansson et al., 2006). Such strategies might in the future further improve islet transplant results.

Thrombosis of the main portal vein is an extremely rare event (Shapiro et al., 1995). Thrombosis of a right or left branch, or peripheral segmental veins, has been encountered in 3–5% of the procedures (Bucher et al., 2004; Villiger et al., 2005). Of note, most of these thromboses were discovered on routine ultrasound, while patients were asymptomatic. This risk could be better controlled by limiting the islet packed cell volume to a maximum of 10 ml, by diligent monitoring of portal pressures throughout the islet infusion (Casey et al., 2002), and by injecting intra-portal heparin (35 U/kg mixed with the islets), followed by low molecular weight heparin (Lovenox 30 mg). Liver function tests should also be monitored after infusion. Transaminases should classically not

exceed 200 U/L in the first week after transplant (Rafael et al., 2003).

In Edmonton, two patients (out of 85) with small arteriovenous fistulae were identified on routine follow-up ultrasound. This undoubtedly relates to hepatic parenchymal injury occurring during percutaneous islet implantation. Both of these fistulae have been small (less than 1 cm areas in the liver) and not associated with significant shunting of arterial-portal blood, and have been clinically completely asymptomatic. They have been managed conservatively, with Doppler monitoring. If these become more progressive, we anticipate that they could be easily resolved with minimal morbidity by localized arterial embolization if indicated.

5.2. Infections

Like in any transplantation, immunosuppression increases the risk of infection. After islet transplant, infections involve various organs, but are mainly located in the upper respiratory airways and the lungs (Hafiz et al., 2005; Toso et al., 2006a). A rare complication of sirolimus toxicity is interstitial pneumonitis (Haydar et al., 2004).

Cytomegalovirus (CMV) transmission was also previously a challenging problem in solid organ transplantation but has been encountered only on extremely rare occasions in the islet setting, provided valganciclovir (900 mg/day) prophylaxis is given to CMV-negative recipients of CMV-positive donor islets (Hafiz et al., 2004).

5.3. Impaired renal function after islet transplant

The risk of renal dysfunction resulting from calcineurin inhibition has been reduced using low-dose tacrolimus-based regimens (Fig. 2). Nonetheless, the drug sirolimus has been associated with additional renal toxicity, especially in patients with significant underlying diabetic nephropathy, which may experience increased or new onset proteinuria (Andres et al., 2005; Letavernier et al., 2005). Some rare patients even finally reach end-stage kidney failure after transplant. Ongoing surveillance for proteinuria in sirolimus-treated transplant patients and evaluation of long-term impact on renal function appears mandatory. Patients with pre-transplant borderline renal function should further be excluded from transplant.

There remains a need to develop non-nephrotoxic alternatives to the calcineurin inhibitors and the mTOR inhibitors if islet transplantation is to be more broadly applied in the diabetes population. Newer therapies such as newer sphingocine-1-phosphate receptor antagonists (FTY720 and alternatives in development), and the new class of costimulation inhibitor antibodies (e.g., belatacept (LEA29Y)) offer considerable hope on the horizon.

5.4. Hematological, metabolic disorders, and others

Leucopenia and anemia have been observed in 90–100% of islet recipients (Hafiz et al., 2005). They are known side effects of sirolimus (Ciancio et al., 2004; Mendez et al., 2005; Larson et al., 2006). Although frequent, they can most of the time be well controlled with growth factors, G-CSF and EPO.

Metabolic disorders, mainly involve dislipidemia, with about 80% of patients involved (Hafiz et al., 2005). They usually need introduction or increase of lipid-lowering drugs.

Mouth ulcers have been observed in virtually all islet recipients treated with sirolimus (Hafiz et al., 2005; Ryan et al., 2005a, b; Toso et al., 2006a). While they usually can be controlled with local means, without stopping sirolimus, they have major implications upon patient quality of life. Ulcers have recently also been described in the small bowel and required to discontinue sirolimus (Molinari et al., 2005). Of note ulcers are frequent in the islet transplant setting, but are rare in solid organ recipients. This remains unexplained, but may possibly in part be related to a more liberal use of steroids in case of solid organ transplantation.

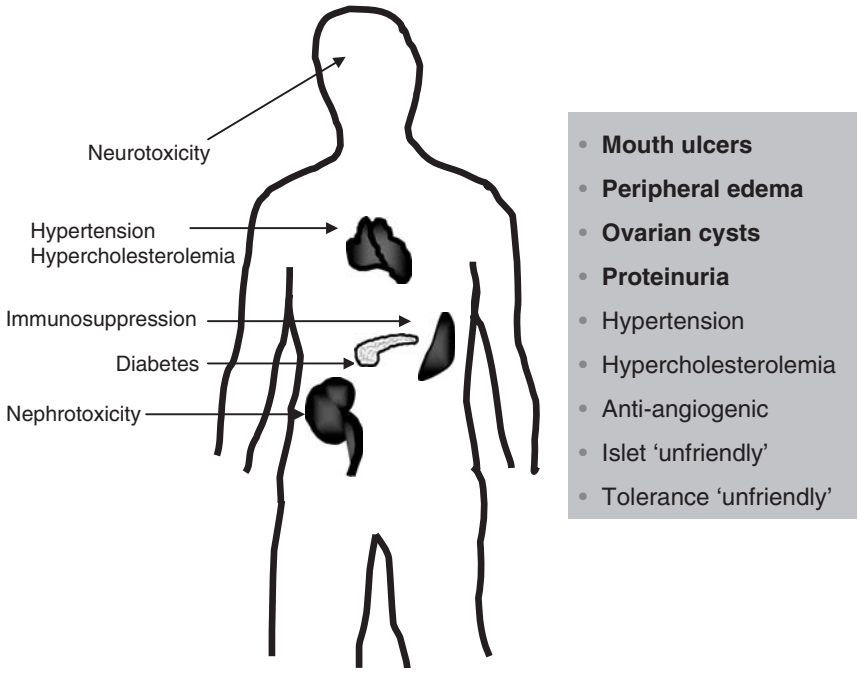

Figure 2. Edmonton protocol drug side effects.

Several other side effects have been observed after islet transplantation (Hafiz et al., 2005). They include various disorders: neurological (insomnia, headache, fatigue), gastrointestinal (diarrhea, vomiting, nausea), dermatologic (acneiform rash, leg edema), gynecologic (uterine bleeding, vaginal infection, ovarian cyst). The risk of all types of malignancy are also increased in chronically immunosuppressed individuals, but squamous epithelial cancers are the most common and most readily treatable. The lifetime risk of lymphoma is estimated to be 1–2% in transplant recipients. This risk may be an overestimate for islet recipients, in whom glucocorticoids and OKT3 are avoided.

6. Islet graft monitoring

In the recent years, islet transplantation results have improved significantly with an insulin-independence rate of about 80% after 1 year (Ryan et al., 2001, 2005a, b). However, patients, who underwent a transplantation have beta-cell function of only 20% of that of healthy individuals, even though they had received islets from more than one donor (Ryan et al., 2001). This low rate of islet engraftment is the consequence of a loss of endocrine cells, which results from multiple accrued injuries. Damage of islets during isolation or at the time of injection, non-specific inflammatory reactions and activation of the coagulation cascade play an early role after transplantation, while allo- and auto-immunity have a more delayed action.

Current clinical monitoring includes the level of serum c-peptide and glucose and the monitoring of exogenous insulin requirements and of the number of hypoglycemic episodes. Monitoring can further be improved by measuring the amount of insulin produced by the islets during a glucose challenge (Shapiro et al., 2001), but all these techniques only reflect late stages in the loss of islets and no method is currently available to accurately monitor islet grafts. Such an assessment could target islet mass or rejection monitoring. Current studies are taking two novel approaches to address this problem: imaging of islets after transplantation and measurement of

islet rejection markers in serum. Islets monitoring has major implications as various drugs could potentially prevent their loss.

6.1. Islet graft imaging

In April 2006, the third workshop on 'Imaging of pancreatic beta cell in health and disease' has emphasized the need for transplanted islet imaging (Paty et al., 2004). Two main modalities demonstrated possible clinical applicability in the near future: magnetic resonance imaging (MRI) and positron-emission tomography (PET).

MRI studies have been conducted by several groups. Pancreatic islets can be labeled prior to transplant with nano-particles of iron. These iron-containing agents are specifically designed for MRI and are broadly used in the clinical setting for liver imaging. After intra-portal transplantation, labeled islets can be identified within the liver of rats and appear as hypointense spots on T_2^*-weighted MR images. The signal remains stable within the liver and can allow imaging of islets for several months after syngeneic transplantation (Jirak et al., 2004; Evgenov et al., 2006). In case of allo-transplantation and in absence of immunosuppression, no more MR signal can be detected 3 weeks after transplantation (Kriz et al., 2005).

PET imaging have been limited by the short half-life of most tracers and by their lack of beta-cell specificity (Sweet et al., 2004). Another limitation is the fact that the liver, usual site of implantation of islets, has a high uptake for most tracers. The signal-to-noise ratio must thus be very intense to allow detecting islets (Toso et al., 2006b). Preliminary studies have labeled rat islets with 2-[^{18}F]fluoro-2deoxy-d-glucose (FDG) and transplanted them into the portal vein. Islets could be followed for the first 6 h after transplant only (Toso et al., 2005). Longer follow-up would require a tracer with a longer half-life, than ^{18}F (110 min). Most β^+-emitting radionucleotides, such as ^{11}C, ^{13}N, and ^{15}O, have similar half lives, or have high rates of cellular outflow as it is the case for ^{64}Cu. Only a radionucleotide with both a longer half-life and a high intracellular retention rate would improve results for ex-vivo labeling of islets.

Another approach is to improve the specificity of the tracer for beta cells, in order to image islet grafts after implantation. [^{11}C]Dihydrotetrabenazine (DTBZ) is a radio-ligand currently used in clinical imaging of the brain. It binds specifically to VMAT2, a transporter found specifically in the brain and on beta cells. Longitudinal PET imaging of the pancreas of diabetic BB rats could demonstrate a decline of signal, reflecting the decrease of beta-cell mass (Souza et al., 2006). This technique appears promising, but still needs to be replicated in the islet transplant setting with the general high uptake of the liver.

6.2. Serum markers for monitoring islet graft

In a preliminary study, the group of Geneva University demonstrated that circulating mRNA for insulin can be detected by RT-PCR immediately after islet transplantation (Ritz-Laser et al., 2002). This reflects early islet damage in the engraftment period, with release of beta cells in the peripheral blood. Circulating mRNA was observed during a longer period in patients treated with steroid-containing immunosuppression. In a recent report, the quantitative dosage of insulin mRNA could predict the occurrence of subsequent event signaling islet damage (increase in the amount of injected exogenous insulin, decrease in c-peptide levels) (Berney et al., 2006). This assay is indicative of beta-cell shedding in general and is not specific of allorejection. Islets damage could also be observed in case of autoimmune destruction, non-specific inflammatory mechanisms or loss of function by progressive exhaustion. In this regard, detection of insulin mRNA could be coupled with the one of circulating mRNA for the cytotoxic lymphocyte genes of granzyme B, perforin, and Fas-ligand. This test has been tested in non-human primate and in human recipients of islet transplants (Han et al., 2002, 2004). The combination of these assays still requires a better

clinical validation and the determination of its sensitivity and specificity to predict islet rejection.

7. Conclusion

Outcomes after islet transplantation have improved step by step over the years. The first one was the development of techniques allowing large-scale isolation of islets by Camillo Ricordi et al. The next one was probably the introduction of the Edmonton protocol with insulin independence achieved in most patients.

While most patients can currently stop insulin, most of them have to return to smaller amounts of exogenous insulin treatment over time, although a degree of islet function is still maintained on long-term which is often sufficient to protect against hypoglycaemias. This progressive exhaustion of the islets should be better understood in order to be able to impact on it. As such, a better monitoring of the islet graft appears as an absolute requirement. We need to more fully understand the mechanisms occurring at the time of islet engraftment. This would possibly allow a larger number of infused islets to implant viable at the transplant site. Finally, new immunosuppression strategies should be introduced to minimize side effects.

While several issues could still improve results, islet transplantation has already entered clinical reality and should clearly be counted as a possible key in the management of selected patients with type I diabetes.

Key points

- Islet transplantation is a valid therapeutic option in selected patients with type 1 diabetes mellitus.
- It can be performed in non-uremic patients with labile glucose control, and after or in combination with kidney transplantation.
- Future developments require more potent immunosuppession with less side effects, and a better long-term monitoring.

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CHAPTER 26

The Immunoendocrine Role of Vitamin D in Autoimmunity

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Vitamin D has a well-recognized effect on calcium metabolism but it is less acknowledged that this hormone also takes part in the regulation and differentiation of the several arms of the immune system both directly and indirectly.

The 25(OH)D_3-1-α-hydroxylase that converts 25(OH)D to 1,25$(OH)_2$D in the kidney is also expressed in activated macrophages and dendritic cells (DC) (Monkawa et al., 2000; Fritsche et al., 2003). However, in contrast to renal cells, in antigen-presenting cells this enzyme is not suppressed by the action of PTH or 1,25$(OH)_2$D, whereas cytokines such as IFN-γ enhance its production (Hewison et al., 2003).

In this chapter, we will review the various functions of vitamin D in the immune system components, and the data that links vitamin D to different autoimmune diseases.

1. The effect of vitamin D on immune system components

1.1. Lymphocytes

Vitamin D has a direct effect on T- and B-cells and contributes to the molding of their responses to antigenic stimulation.

Quiescent $CD4^+$ T-cells express vitamin D receptors (VDRs) at low concentrations, which increases fivefold after activation (Mahon et al., 2003). The main impact 1,25$(OH)_2$D has on the acquired, antigen-specific immune response is inhibiting T-lymphocyte proliferation (Bhalla et al., 1984; Lemire, 1992), particularly of the Th1 arm (Mattner et al., 2000).

Th1-cell activation is essential for strong cell-mediated immune responses, including host responses to tumors and intracellular pathogens. In autoimmune diseases, Th1 cells are misdirected against self proteins, resulting in pathologic conditions such as multiple sclerosis (MS), type 1 diabetes mellitus (DM), and inflammatory bowel disease (IBD).

Addition of 1,25$(OH)_2$D to CD4 T-cells inhibits Th1 cell proliferation and Th1-mediated cytokine production (Boonstra et al., 2001). IL-2 and IFN-γ secretion decreases while IL-5 and IL-10 production increases, which consequently tilts the T-cell response toward Th2 dominance (van and Mathieu, 2005).

Th2 cells primarily play a role in antibody-mediated immunity and are seminal to the response against extracellular pathogens and to the generation of allergic reactions and graft rejection. Vitamin D has a complex interplay with Th2-mediated cytokines. The Th2-associated cytokine IL-4 production has been shown to be upregulated in vivo by 1,25$(OH)_2$D3 treatment yet other studies demonstrated its inhibition (Cantorna et al., 1998; Staeva-Vieira and Freedman, 2002).

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In B-cells vitamin D has been shown to inhibit antibody secretion and autoantibody production (Linker-Israeli et al., 2001).

2. Antigen-presenting cells

2.1. Dendritic cells

In contrast to the antiproliferative effects of $1,25(OH)_2D$ on some cell types (Jones et al., 1998b), generation of DCs from bone marrow is not impaired by $1,25(OH)_2D$ although an attenuated progression of maturation occurs (Griffin et al., 2001). $1,25(OH)_2D$ affects autoimmune CD4 T-cell responses by regulating DC function.

In vitro, $1,25(OH)_2D3$ inhibits the differentiation of monocytes into DCs and impedes the stimulatory activity that T-cells exert on them (Berer et al., 2000; Penna and Adorini, 2000; Griffin et al., 2001). It has been demonstrated that $1,25(OH)_2D_3$ is one of the most powerful blockers of DC differentiation and of IL-12 secretion. In vitro $1,25(OH)_2D_3$ also stimulates phagocytosis and killing of bacteria by macrophages and also suppresses the antigen-presenting capacity of these cells and of DCs (Griffin et al., 2000).

In vitro DCs treated with VDR agonists markedly decrease IL-12 and INF-γ production, whereas IL-10 and TGF-β synthesis are enhanced (Adorini, 2005; Gauzzi et al., 2005; Lyakh et al., 2005).

2.2. Macrophages

Vitamin D induces monocytic differentiation into mature macrophages but prevents their release of inflammatory cytokines and chemokines (Helming et al., 2005). Vitamin D deficiency impairs macrophage ability to mature, to produce macrophage-specific surface antigens, to produce the lysosomal enzyme acid phosphatase, and to secrete H_2O_2, a function integral to their antimicrobial function (bu-Amer and Bar-Shavit, 1994).

Prostaglandin E2, a suppressive cytokine, is stimulated by $1,25(OH)_2D3$, while the granulocyte macrophage-colony-stimulating factor (GM-CSF) is suppressed. Moreover, $1,25(OH)_2D_3$ can decrease the antigen-presenting activity of macrophages to lymphocytes by reducing the expression of MHC-II molecules on the cell surface (Lemire, 1992; Zittermann, 2003; Cantorna and Mahon, 2004).

Some immune cells, in particular activated macrophages and DCs, contain the enzyme 1α-hydroxylase, which is necessary for the final activating step of the conversion of vitamin D_3 to the metabolically active molecule. These cells therefore hold the capacity to synthesize and secrete $1,25(OH)_2D$. The 1α-hydroxylase present in immune cells is identical to the renal enzyme, but regulation of its expression and activity is different. In contrast to the renal enzyme that is principally under the control of calcemic and bone signals, the macrophage enzyme is primarily regulated by immune signals such as IFN-γ (Overbergh et al., 2000).

3. Evidence of vitamin D receptor role in autoimmunity

After activation by the active vitamin D metabolite, the VDR, this receptor not only plays a role in the regulation of calcium homeostasis, but also exerts immunomodulatory effects as well.

The VDR is present in multiple cells of the immune system. VDR can be detected in over 30 different tissues, including circulating monocytes, DCs, and activated T-cells (Jones et al., 1998a). VDR is found in significant levels of the T lymphocyte and macrophage populations. However, its highest concentration is detected in immature immune cells within the thymus and in mature CD8 T lymphocytes regardless to activation status (Stio et al., 2006). VDR is required for $1,25(OH)_2D$ to induce the differentiation of bone marrow progenitors into monocytes/macrophages, but monocyte/macrophage differentiation can occur in the absence of VDR. Expression of VDR was shown to be important for the generation of a Th1-type immune response by spleen cells. Fewer Th1 cells are generated in the absence of VDR in response to antibody stimulation (O'Kelly et al., 2002).

4. Vitamin D deficiency and autoimmune diseases

4.1. Inflammatory bowel disease

IBD is more prevalent in areas with decreased sunlight exposure. The disease is more frequent in Northern climates such as North America and Northern Europe (Podolsky, 1991; Sonnenberg and Wasserman, 1991; Cantorna, 2006). Serum concentrations of 25(OH)D levels are low in patients with IBD (Jahnsen et al., 2002). It is unclear why vitamin D deficiency occurs more frequently in IBD. It is probably an outcome of combined effects such as low-vitamin D intake, malabsorption of many nutrients including vitamin D, and decreased outdoor activities and sun light exposure. Experimental IBD has also been shown to be accelerated by vitamin D deficiency and suppressed by 1,25(OH)2D3 treatment (Froicu et al., 2003; Kamen et al., 2006).

IL-10 knockout mice develop spontaneous IBD; however, supplementing these mice with sufficient vitamin D improved the intestinal status (Cantorna, 2000). Experimental treatment with a low-calcemic vitamin D analog has been shown to display a prophylactic as well as therapeutic profile in Th1-like experimental colitis in mice (Daniel et al., 2006).

4.2. Multiple sclerosis

MS is a demyelinating disease of the central nervous system that can progress into a debilita ting and sometimes fatal course (Hayes et al., 1997).

MS prevalence shows a striking geographic variance; it rises in parallel to the increasing latitude in both hemispheres, from a low of 1–2 cases per 100,000 people near the equator to a high of above 200 cases per 100,000 people at latitudes higher than 50°. This peculiar distribution suggests that one disease-determining environmental risk factor is somehow linked to latitude (Acheson et al., 1960). A study set up to investigate bone metabolism in MS patients revealed a prevalence of insufficient serum 25(OH)D levels (<50 nmol/l) in 77% of the patients (Nieves et al., 1994).

Munger et al. (2006) recently studied the association between circulating vitamin D levels of more than 7 million active-duty US military personnel and the incidence of MS. The results show that among Caucasians, the risk of MS is significantly decreased with increasing levels of 25-hydroxyvitamin D. Another study checked at the vitamin D intake in more than 187,000 women from two separate cohorts; the Nurses' Health Study (NHS; 92,253 women followed from 1980 to 2000) and Nurses' Health Study II (NHS II; 95,310 women followed from 1991 to 2001). A 40% risk reduction of MS was reported in women who used supplemental vitamin D (Munger et al., 2004). Another interventional study in MS patients demonstrated that daily supplementation with 16 mg Ca/kg body weight, 10 mg Mg/kg body weight, and 125 mg vitamin D/day for 1–2 years decreased the rate of flares of MS (Goldberg et al., 1986).

More evidence that vitamin D is a natural inhibitor of MS comes from experiments done using the experimental autoimmune encephalitis (EAE) system as a model of MS. Immunizing mice with a spinal cord homogenate containing myelin basic protein induced a progressively paralytic autoimmune disease that resembles MS. When vitamin D was provided shortly before induction of disease, the neurological manifestations were prevented. In addition, when vitamin D was provided post-induction, when disease symptoms were established, their severity decreased. Finally, if vitamin D supplementation was discontinued, disease symptoms reappear (Mark and Carson, 2006).

4.3. Systemic lupus erythematosus (SLE)

In the United States, African Americans have a threefold increased incidence of this disease, developing it at an earlier age, and sustain increased morbidity and mortality compared to Caucasians (Alarcon et al., 1999).

Kamen et al. (2006) observed significant lower serum 25-hydroxyvitamin D levels in recently

diagnosed SLE patients compared to controls, and a higher overall prevalence of vitamin D deficiency. Similar results were also obtained with lupus patients who had a longer disease course (O'Regan et al., 1979; Muller et al., 1995). These results, however, are debatable, Huisman et al. (2001) failed to repeat these results; in a cross-sectional study of 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, and PTH levels in 25 Caucasian SLE and 25 fibromyalgia female patients, this team did not disclose any significant differences between the two groups, with half of all patients were found to be vitamin D deficient.

Recently, VDR gene *Bsm*I polymorphisms have been used as genetic markers to determine their association with SLE. A Japanese study of 58 patients with SLE found that the BB genotype might trigger the development of SLE and that the BB genotype was associated with lupus nephritis. A Taiwanese study of 47 Chinese patients with SLE also found an increased distribution of the VDR BB genotype in SLE but indicated no association between the frequency of VDR allelic variations and clinical manifestations or laboratory profiles (Ozaki et al., 2000; Huang et al., 2002).

Oral administration of vitamin D3 to MRL/lpr spontaneous developing lupus mouse model significantly improved longevity and reduced proteinuria. These findings were concordant to less severe histopathological findings in the kidneys and joints of the mice (Abe et al., 1990).

4.4. Diabetes mellitus type 1

$1,25(OH)_2D$ has been successfully used to prevent autoimmune insulitis and to reduce diabetes from developing in the non-obese diabetic (NOD) mouse model of type 1 diabetes (Mathieu et al., 1992, 1994).

Investigators in several epidemiologic studies have reported that dietary vitamin D supplementation during infancy and childhood reduces the risk of type 1 diabetes. An important paper in this regard examines the risk ratio for developing DM type 1 with respect to vitamin D supplements in infancy in Finland. In a more recent Finnish investigation, regular vitamin D supplementation of 50 mg/day during infancy in the 1960s was associated with a marked reduction in the risk of type 1 diabetes 30 years later in comparison with unsupplemented infants (RR 0.12). Children suspected of having rickets during the first year of life had a threefold increased prevalence of type 1 diabetes in comparison with those without such a suspicion (The EURODIAB Substudy 2 Study Group, 1999; Hypponen et al., 2001).

In the Diabetes Autoimmunity Study in the Young, Fronczak et al. (2003) reported that the presence of islet autoantibodies in offspring was inversely correlated with maternal dietary vitamin D intake during pregnancy.

4.5. Rheumatoid arthritis (RA)

Vitamin D may play a role in the course of arthritis. Low $1,25(OH)_2D$ was associated with higher RA disease activity in cross-sectional studies. Epidemiological data indicate that more than 60% of rheumatoid patients have 25(OH)D levels below 50 nmol/l (Aguado et al., 2000) and that 16% have levels in the range of vitamin D deficiency (<12.5 nmol/l). However, the finding of a positive correlation between $1,25(OH)_2D$ and alkaline phosphatase indicates this may in part reflect that people with higher disease activity have increased bone resorption (Oelzner et al., 1999).

Interventional trials with a dosage of 1 mcg 1α-vitamin D were not associated with an improved outcome (Hein and Oelzner, 2000). However, administration of higher amounts of 1α-D or other vitamin D forms was associated with decreased pain sensation and a significant reduction in C-reactive protein, a marker of inflammatory disease activity (Andjelkovic et al., 1999).

5. Thyroiditis

Experiments in animal models and in humans emphasize the critical role of $1,25(OH)_2D3$ in the prevention of autoimmune thyroiditis. In a study recently published (Lin et al., 2006), a significant

difference between Hashimoto thyroiditis patients and normal controls was detected in the prevalence of VDR SNP, similarly a statistical correlation between VDR-FokI polymorphisms and HT formation was also shown. Other studies have demonstrated an association between VDR polymorphism and Graves disease in Japanese, German, and Polish populations (Ramos-Lopez et al., 2005).

5.1. Addison's disease

Autoimmune Addison's disease is a rare disorder causing primary adrenal failure. One study has been published investigating the role of VDR polymorphisms in Addison's disease (Pani et al., 2002). The genotype distribution of *Bsm*I differed significantly between patients and controls, but neither the 'b' allele, nor the 'bb' genotype nor the *Apa*I polymorphism showed any association with Addison's disease development.

6. Conclusions

The common denominator that rises from these studies is that vitamin D affects the immune response in numerous levels and by multiple immunomodulatory mechanisms. Vitamin D affects different immune cells, takes part in the genetic regulation and secretion of cytokine production expression by VDRs, and enhances or hinders important biological processes by which these cells interact. Vitamin D by and large confers an immunosuppressive effect. These preliminary results should encourage further clinical trials in order to evaluate the potential role vitamin D may have in practice.

Key points

- Vitamin D exerts multiple immunosuppressive effects particularly by inhibiting Th1 cell cytokine secretion, autoantibody secretion, and macrophage proliferation and differentiation.
- Vitamin D supplementation confers a beneficial clinical effect in several autoimmune animal models. Preliminary studies indicate that this effect exists also in patients with autoimmune conditions such as multiple sclerosis, prevention of diabetes type 1, rheumatoid arthritis, and other conditions.

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CHAPTER 27

Modulation of Hormone Axes by Anti-TNF Therapy

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1. Introduction

Tumor necrosis factor (TNF) blockers were first licensed for clinical use in 1998. Three TNF inhibitors have been approved for treatment of rheumatoid arthritis (RA): infliximab, etanercept, and adalimumab (Feldmann and Maini, 2001; Atzeni et al., 2005). TNF blockers have been evaluated in a series of randomized, controlled trials enrolling nearly 60,000 patients with RA (Scott and Kingsley, 2006). Although TNF inhibitors induce adverse events such as reactions at the injection site, hypersensitivity reactions, upper respiratory tract infections, and malignancies (Desai and Furst, 2006). TNF inhibitors are associated with clear improvement with respect to symptoms and signs of RA and, more important, with a reduced risk of joint destruction and deformity. Anti-TNF therapy has strong direct anti-inflammatory effects. However, such therapy may also support other important anti-inflammatory pathways such as hormonal axes because these axes are disturbed by chronically elevated TNF (Straub et al., 2006b) (Fig. 1).

2. The role of tumor necrosis factor in arthritis

TNF, a soluble 17-kDa protein consisting of three subunits, is a potent pro-inflammatory cytokine produced by multiple cell types (including monocytes, macrophages, B- and T-cells). TNF plays a pivotal role in the pathogenesis of RA, Crohn's disease, psoriatic arthritis, and ankylosing spondylitis (Feldmann and Maini, 2001). In the pathogenesis of RA, pro-inflammatory cytokines such as TNF and interleukin (IL)-1β play a key role. The synovial membrane of RA patients shows hyperplasia, increased vascularity, and infiltration by inflammatory cells. CD4+ T lymphocytes play an important role, which, when activated by an antigen, stimulate monocytes, macrophages, and synovial fibroblasts to produce IL-1, IL-6, and TNF, and to secrete matrix metalloproteinases (MMPs) as a result of cell-surface signaling by means of CD69 and CD118 (Atzeni et al., 2004), and through the release of soluble mediators such as interferon (IFN)-γ, IL-1, IL-6, IL-17, and TNF (Choy and Panayi, 2001). Activated CD4+ T-cells also stimulate B-cells by means of cell–cell contacts and the binding of α sub 1 and β sub 2 integrin, CD40 ligand and CD28 to produce immunoglobulin (Ig), including rheumatoid factor (RF). The precise role of RF is unknown, but it is most probably involved in activating complement as a

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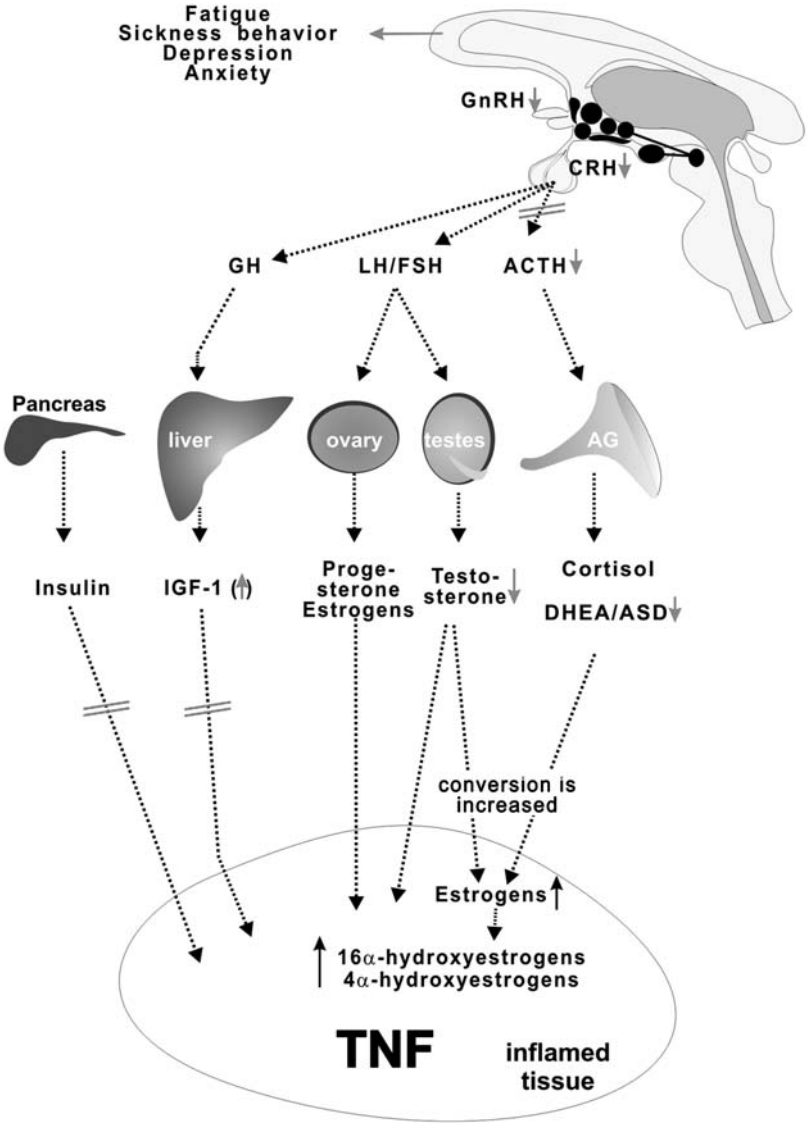

Figure 1. Summary of neuroendocrine alterations in patients with rheumatoid arthritis. The red downward arrows indicate the deleterious effects of TNF on several levels of endocrine and neuronal supersystems. Double red bars delineate a reduction of the respective pathway. The pathways of the parasympathetic system are not demonstrated. Abbreviations: ACTH, adrenocorticotropic hormone; AG, adrenal gland; ASD, androstenedione; CRH, corticotropin-releasing hormone; DHEA, dehydroepiandrosterone; FSH, follicle stimulating hormone; GH, growth hormone; GnRH, gonadotropin releasing hormone; IGF-1, insulin-like growth-factor-1; LH, luteotropic hormone; TNF, tumor necrosis factor. Modified according to Straub et al. (2006b).

result of the formation of immune complexes. Activated CD4+ T-cells express RANK that stimulates osteoclastogenesis via RANK ligand (Choy and Panayi, 2001). Activated macrophages, lymphocytes, and fibroblasts can also stimulate angiogenesis, which is responsible for the synovial hypervascularity found in RA patients (Choy and Panayi, 2001). Synovial endothelial cells are activated and express adhesion molecules that promote recruitment of inflammatory cells to the joint. Finally, TNF induces production of several cytokines, and stimulates fibroblasts to express adhesion

molecules that interact with the ligands on the surface of leukocytes, thus increasing the number of inflammatory cells in the joint. TNF exhibits an immunostimulatory role that can alter the balance of T-regulatory cells (Scott and Kingsley, 2006).

Moreover, TNF interferes with the neuroendocrine axes (Bijlsma et al., 2005). In a chronic inflammatory disease such as RA, the HPA axis is altered: (1) the spontaneous and stimulated secretion of cortisol is inadequate in relation to inflammation, (2) the secretion of adrenocorticotropic hormone (ACTH) is inadequate in relation to inflammation, and (3) adrenal androgens are decreased (Straub et al., 2002c; Capellino and Straub, Ch. 1, this volume).

3. Hypothalamic–pituitary–adrenal axis

In patients with RA with high levels of TNF, serum levels of ACTH and cortisol in relation to inflammation are inadequately low (in the normal range). It seems that continuous stimulation of the hypothalamus and the pituitary gland with TNF and IL-6 induces a hypothalamic–pituitary adaptation that makes these organs unresponsive (Straub et al., 2002c). In patients with RA, cytokine levels remain elevated, whereas the levels of hormones become normal or lower than normal (Straub et al., 2002c). The lower levels of adrenal hormones are probably attributable to a direct inhibitory effect of TNF on the expression of the steroidogenic acute regulatory protein and on ACTH-stimulated expression of steroidogenic enzymes such as P450ssc, P450c21, and P450c11 in adrenocortical cells (Jäättelä et al., 1991).

Recently, a rapid increase in ACTH levels was demonstrated in prednisolone-naïve patients with RA after injections of anti-TNF antibody (Straub et al., 2003). In addition, cortisol levels in relation to serum IL-6 continuously increase during 16 weeks of anti-TNF treatment, which indicates a normalization of the immune and neuroendocrine systems (Fig. 2). Furthermore, the ratio of serum cortisol to serum ACTH decreased during anti-TNF treatment, suggesting a sensitization of the pituitary gland. Moreover, cortisol-binding globulin (CBG) produced by liver cells is normal in RA and no significant change is observed during anti-TNF treatment (Straub et al., 2005b). Thus, the variations in cortisol concentrations induced by anti-TNF agents cannot be attributed to changes in serum levels of CBG. In conclusion, long-term therapy with anti-TNF sensitizes the pituitary gland and improves adrenal androgen secretion in prednisolone-naïve patients. These changes are indicative of a normalization of the HPA axis and must be considered as evidence of an additional anti-inflammatory influence of anti-TNF treatment in patients with RA.

In a recent study, we investigated the predictive role of HPA-axis hormones for immediate clinical improvement during anti-TNF antibody therapy (Straub et al., 2006a). We demonstrated that rapid clinical improvement in treatment responders may be attributed to an increase in serum cortisol as TNF inhibits adrenal conversion of 17-hydroxyprogesterone into cortisol (leading to low serum cortisol) (Straub et al., 2006a). These findings indicate that some patients rapidly benefit from anti-TNF agents probably by restoring steroidogenic enzymes such as P450c21 and P450c11 in the adrenal glands and by restoring the pituitary–adrenal axis (Straub et al., 2006a). Furthermore, in prednisolone-naïve patients with psoriatic arthritis, an increase of serum cortisol relative to serum 17-hydroxyprogesterone or androstenedione was related to clinical improvement (Atzeni et al., unpublished observation).

4. Hypothalamic–pituitary–gonadal axis

Androgens such as dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), androstenedione, and testosterone are markedly reduced in patients with RA and systemic lupus erythematosus (SLE) (reviewed in Cutolo et al., 2004). Data from animals with chronic inflammation and patients with RA suggest that androgens exert anti-inflammatory effects (reviewed in Cutolo et al., 2004). The serum levels of estrogens compared to androgens are not significantly changed in RA patients (van den Brink et al., 1993). High concentrations of estrogens have been found in

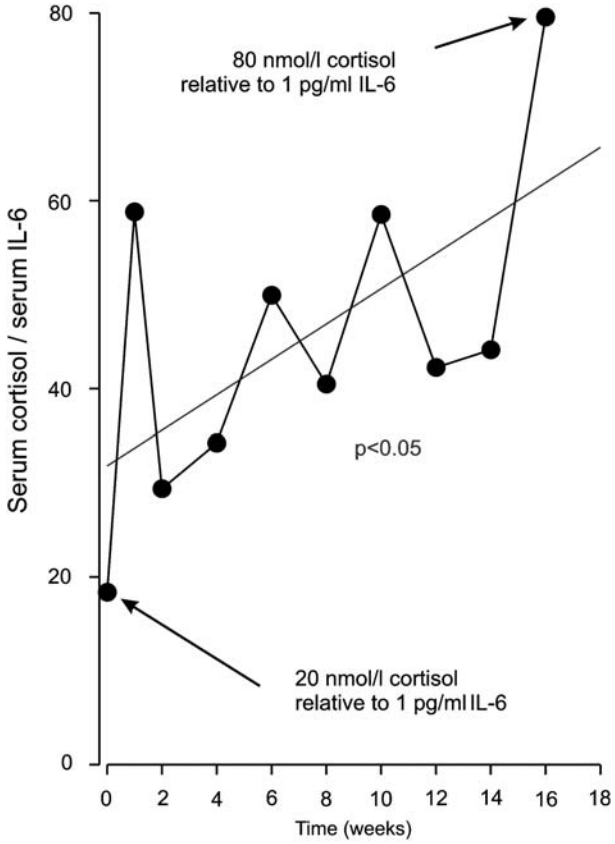

Figure 2. Increase of cortisol relative to IL-6 during anti-TNF therapy. The *p*-value indicates the positive correlation between the two variables. Modified according to Straub et al. (2003).

synovial fluid of RA patients (Castagnetta et al., 2003). These findings suggest an accelerated peripheral metabolic conversion of androgens to 17-β estradiol (Castagnetta et al., 2003). Pro-inflammatory cytokines such as TNF and IL-6 stimulate the aromatase enzyme in nongonadal cells to produce estrogens. In patients with RA, it was demonstrated that local levels of pro-inflammatory estrogens related to androgens are elevated in patients compared to trauma controls, which is probably related to TNF-induced aromatase activity (Castagnetta et al., 2003). In addition, it was reported that in synovial fluid of RA patients, an increased estrogen concentration is observed in both sexes and that it is characterized by appearance of 16α-hydroxyestrone, which is a mitogenic endogenous hormone (Castagnetta et al., 2003). It can be speculated that TNF blockers can inhibit androgen to estrogen conversion, which needs to be demonstrated in RA synovial cells. In such a situation, the beneficial effects of restoring synovial androgens might be clinically more evident in male RA patients since they suffer more from the lack of local androgens (Cutolo et al., 2006).

We demonstrated that therapy with anti-TNF antibodies for 12 weeks did not change serum levels of typical sex hormones in patients with RA, although baseline values were different from controls (Straub et al., 2005a). In patients with long-standing RA, this indicates that alterations of serum sex hormones and altered activity of respective converting enzymes are imprinted for a long-lasting period over at least 12 weeks. Furthermore, we found no changes in the serum levels of typical sex hormones in patients with

psoriatic arthritis during 12 weeks of etanercept treatment (Atzeni et al., unpublished observations).

5. Hypothalamic–pituitary–liver–muscle axis

Patients with RA suffer from decreased muscle function and loss of body cell mass (Munro and Capell, 1997). Insulin-like growth-factor-1 (IGF-1) is an important determinant of muscle mass because it promotes growth and suppresses protein degradation (Heszele and Price, 2004). TNF inhibits synthesis of IGF-1 from liver cells, and it also inhibits IGF-1-mediated anabolic effects on peripheral tissue (Straub et al., 2006b). In parallel, glucocorticoids induce IGF-1 resistance and add to muscle degradation (Straub et al., 2006b). IGF-1 resistance is indicated by an increase of IGF-1 in the presence of corticosteroid treatment. We showed that anti-TNF antibody therapy over 12 weeks in patients with RA improved corticosteroid-induced IGF-1 resistance without influencing IGF-1 binding protein 1 (IGFBP-1) and IGFBP-3. We did not test muscle strength during the study, but the improvement of the score of the Health Assessment Questionnaire (HAQ) suggests an improvement of muscular function (Sarzi-Puttini et al., 2006).

6. Adipose tissue and hormones of the HPA axis

Leptin and adiponectin are hormones of the pluripotent white adipose tissue, which does not only store energy (Fantuzzi, 2005). Adipose tissue is involved in the conversion of sex hormones and glucocorticoids. For example, biologically active cortisol can be converted into biologically inactive cortisone via the 11β-hydroxysteroid dehydrogenase type 2 and vice versa by 11β-hydroxysteroid dehydrogenase type 1. Moreover, the adipose tissue is capable of converting androgens into estrogens by aromatization. Furthermore, adipose tissue produces cytokines such as TNF, IL-1β, IL-6, IL-8, IL-10, etc. (Tilg and Moschen, 2006).

Leptin is a 16 kDa adipokine that links nutritional status with neuroendocrine and immune functions (Härle and Straub, 2006b). This hormone regulates body weight by inhibiting food intake and stimulating energy expenditure, and it inhibits the HPA-axis function and steroid production in adrenal cells (Härle and Straub, 2006b). The question remains as to how leptin can influence the HPA axis. Leptin acts by indirect mechanisms: (a) leptin inhibits the HPA-axis during stress, e.g., hypoglycemia, which has been shown in explanted and superfused rat hypothalamus (Park et al., 2005); (b) it suppresses the increase of corticotropin-releasing hormone under hypoglycemic conditions in a concentration-dependent way (Nowak et al., 2002); (c) moreover, the diurnal rhythm of leptin runs anti-cyclic to the diurnal rhythm of corticosterone in mice (Härle and Straub, 2006b); (d) leptin inhibits the expression of multiple enzymes of steroidogenesis. For example, in the white adipose tissue, leptin stimulates the expression of 11β-hydroxysteroid dehydrogenase type 1 that converts the inactive cortisone into cortisol. Cortisol upregulates the expression of aromatase in fat tissue leading to enhanced synthesis of estrogens.

Recent studies reported that androgens conferring anti-inflammatory effects have a negative correlation to serum leptin concentrations (Sarraf et al., 1997). This has been confirmed in patients with RA (Härle et al., 2004). The acute stimulation with pro-inflammatory cytokines leads to an increase in serum leptin levels. In contrast, chronic IL-6, IL-1, or TNF stimulation leads to a reduction of leptin concentrations. These results have been shown in adipose tissue cultures (Bruun et al., 2002) and in clinical studies in patients with RA, where inflammatory indices (IL-6 and C-reactive protein) negatively correlated with serum leptin concentrations (Popa et al., 2005). However, in patients with RA, we did not find any correlation between serum levels of leptin or adiponectin and the number of swollen and tender joints, or serum levels of IL-6 or CRP (Härle et al., 2006a). Furthermore, in prednisolone-naïve patients with RA after 12 weeks of treatment with adalimumab, we did not find a decrease or increase of serum levels of leptin or adiponectin (Härle et al., 2006a).

7. The nervous system

7.1. The sympathetic nervous system (SNS)

In a recent study in patients with Crohn's disease and ulcerative colitis, we have observed a preponderance of the tone of the SNS over the HPA axis: serum levels of neuropeptide Y (NPY) (an excellent indicator of sympathetic activity) were increased, whereas serum cortisol levels were normal or decreased (Straub et al., 2002b). We called this phenomenon uncoupling of the SNS and the HPA axis in order to emphasize the loss of cooperative anti-inflammatory activities of these two endogenous response systems. Another study demonstrated an anti-inflammatory cooperation between norepinephrine and cortisol that led a strong reduction of TNF, IL-6, and IL-8 secretion in cultured mixed synovial cells of patients with RA (Straub et al., 2002a).

Using NPY, we demonstrated an increased SNS outflow in relation to the HPA-axis tone in patients with SLE and RA. This supports uncoupling of the two main response axes in patients with SLE and RA (Härle et al., 2006c). Uncoupling is enhanced in patients treated with steroids because prednisolone stimulates the SNS and inhibits the HPA axis even in healthy controls (Härle et al., 2006c). After 12 weeks of anti-TNF antibody treatment in patients with RA, we showed a slight decrease in NPY levels and SNS dominance (Härle et al., 2006c). The relatively little effect of anti-TNF therapy may be due to the fact that TNF is not the sole and the main factor responsible for this phenomenon. In addition, uncoupling of these two response systems may be imprinted for a long time. All the studies indicate an increased sympathetic tone in patients with RA (and also other chronic inflammatory diseases). However, since sympathetic nerve fibers are lost in inflamed tissue, the increased sympathetic tone does not lead to higher local levels of anti-inflammatory sympathetic neurotransmitters in the synovium of patients with RA (Miller et al., 2000).

In conclusion, the relatively low serum levels of cortisol, the absolute loss of androgens, and the loss of sympathetic nerve fibers are pro-inflammatory signals. At present, it is unclear whether anti-TNF therapy can restore sympathetic nerve fibers in the tissue.

7.2. Central nervous system

Patients with RA or psoriatic arthritis suffer from chronic fatigue and depression (Wolf and Michaud, 2004). Recent studies demonstrated that elevated cytokine levels deteriorate sleep and declarative memory. TNF is an important mediator of these changes during the course of inflammatory disease (Straub et al., 2006b). Patients with RA treated with anti-TNF antibody demonstrated a marked reduction in fatigue scores and an improvement of well-being and joint pain (Wolf and Michaud, 2004). In conclusion, elevated levels of circulating TNF have an important impact on brain function.

8. Conclusions

In a chronic inflammatory disease such as RA, neuroendocrine axes are altered. TNF is an important mediator of these alterations. Long-term therapy with anti-TNF restores hormonal pathways leading to normalization of the *milieu interne*. Thus, anti-TNF therapy realizes an alternative mode of anti-inflammatory action.

Key points

- TNF disrupts several neuroendocrine pathways in patients with rheumatoid arthritis.
- Neutralization of TNF restores defect neuroendocrine pathways.
- Anti-TNF therapy normalizes the hypothalamic–pituitary–adrenal axis.
- Anti-TNF therapy restores the hypothalamic–pituitary–liver–muscle axis.
- However, anti-TNF therapy does not restore the hypothalamic–pituitary–gonadal axis and the increased sympathetic tone.

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Index

A

1a or immune mediated 211
A/G49 SNP 65
abdominal pain 96, 150
abortion 159
ABO-type 278
ACA/21-OHAbs 141–142
acne 96, 225
acquired sensitization 161
acrocentric chromosome 116
ACTH 5, 96–97, 142, 232, 303
  cortisol 232
  secretion 176
activator protein-1 (AP-1) 230
activators of transcription 30
active 96
  SLE 193
activin 193
activin A 191
activity score for PMR (PMR-AS) 259
adalimumab 301, 305
adaptive immune response 188
Addison's disease (AD) 33, 65, 85, 87, 99, 135, 139, 141, 144, 149, 179, 175, 216
adenohypophysial 87
adenosine 7
adhesion molecules 186, 302
16 kDa adipokine 305
adiponectin 305
adolescents 87
adrenal 149
  androgens 98, 303
  cells 150
  cortex extracts 135
  glands 150
  hemorrhage 96
  infarction 96
  involvement 96–98
adrenocortical antibodies (ACA) 34, 87
adult-onset Still's disease 192
African Americans 295
agglutination test 161
agonists 225
AIRE gene 128, 205
airway 205
alanine/threonine polymorphism 65
alcohol abuse 276
aldosterone 175
  response to stimulation 177
alemtuzumab (anti-CD52) 284
alkaline phosphatase 296
allele 66
allograft 32
allotransplants 276
alopecia 143
  areata 123, 139, 159
  universalis 106
alpha chain 217
alpha enolase 85
altered
  circadian rhythms 13–14
  mental status 150
Alzheimer's disease 115, 117, 125, 251
amenorrhea 164, 223
amniocentesis 114
amnion cells 187
amniotic fluid 187
ANCA-positive 201
androgens (A) 13–14, 21, 120, 173, 181, 185, 223, 225, 249, 265, 303
  blocker 190
  deficiency 181
  estrogen ratios 13–14, 24
  levels 123
  receptor gene 124
androstenediol 250
androstenedione 4–5, 96, 250, 253, 303
anemia 159, 285
anergy in T cells 120
aneuploidy 126
angiogenesis 302
angiogenic/ static factors 160, 187
ankylosing spondylitis (AS) 164, 192, 301
anterior pituitary 152
anti-β_2GPI 162, 192
anti-andrenal 216
anti-annexin 158
anti-beta 2GPI antibodies 192

antibodies 48, 122, 124–125, 157, 159, 174, 178, 192, 206
  to melanocytes 139
antibody 162
  binding self-structures 199
  sensitized platelets 266
anticarbonic anhydrase 107
anti-cardiolipin antibodies (aCL) 34, 96, 106, 124, 150–151, 162, 192, 224
anti-CD3 (hOKT3-ala-ala) 55, 284
anti-coagulant activity 96, 107, 165, 278
anti-complementary effects 193
anti-D therapy 266
anti-DNA antibodies 15, 23–25, 160, 180, 190
anti-DNA B cells 15
anti-dsDNA 17, 34, 107, 241
antiendometrial antibodies 106, 160
antiendomysial 122
antiendothelial cells 107, 160
anti-estradiol antibody 242
antiextractable nuclear antibodies 124
antigen presenting cells (APCs) 31, 47, 64
antigen 165
  antibody reaction 160
  4 gene on cytotoxic T lymphocytes (CTLA-4) 143
antigliadin 122
anti-glucocorticoid 253
anti-granulosa antibodies 159
antihistone 106
anti-idiotypic antibodies 165
anti-inflammatory androgens 251
anti-insulin 178
anti-interleukin (IL) 2 receptor monoclonal antibody (anti-CD25), Zenapax 283
anti-La 201
anti-laminin antibodies 160
antimicrobial function 294
anti-neuronal antibodies 181
antinuclear antibodies (ANA) 34, 106, 158, 160, 200, 203
anti-ooplasm antibodies 159
anti-ovarian 159, 160
antioxidant effects 252
anti-parietal cell/anti-intrinsic factor (PCA/IFAbs) 216
anti-peroxidase/anti-tyroglobuline (aTPO/TG) 216–217
anti-phosphatidylcholine 152
anti-phosphatidylethanolamine 163
anti-phosphatidylinositol 152
anti-phosphatidylserine antibodies 163
antiphospholipid 125
  antibodies (aPL) 157–158, 160, 162, 178, 192, 206
  syndrome (APS) 95–96, 115, 124, 149, 150–152, 157, 181, 206, 267
anti-pituitary antibodies (APA) 37–38, 85–86
anti-platelet antibody 266
anti-PRL antibodies 31, 35, 190, 244
anti-prothrombin 158
anti-rejection therapies 280
anti-Ro (SS-A)/anti-La 34, 106, 152, 201
anti-Ro/La-positive euthyroid mothers 201
anti-Ro/La-positive hypothyroid mothers 201
anti-Sm 107, 152
anti-smooth muscle 107
anti-sodium-iodide 77
anti-sperm antibodies (ASAs) 157, 161
anti-T cell therapies 284
anti-Tg autoantibodies 67
anti-Tg T-cell responses 67
anti-thyroglobulin antibodies 77, 161
anti-thyroid 157, 200
  antibodies 34, 38, 78, 174
  autoantibodies 160
  drug therapy 206
  peroxidase (TPO) antibodies 77, 161, 174
anti-tissue transglutaminase antibodies 122
anti-tissue transglutaminase/anti-endomysial (tTG/EMAbs) 216
anti-TNF therapy 7, 301
anti-zonal antibodies 159
anxiety 237
aortic cross-clamp 276
ApaI polymorphism 297
APECED locus 128
APGA 37
apolipoprotein H or β_2GP1 162
apoliprotein Eε4 alleles 117
apoptosis 30, 61, 77, 161, 164
  apoptotic endometrial cells 107
APP gene 117
APS type 1 3, 87
Arg74 polymorphism 67
arginine 50, 66–67, 121
  at position 63, 74
  vasopressin (AVP) 232
aromatase enzyme 304
aromatase-knockout (ArKO) 243
arterial thrombosis 224, 270
arterio-ventricular canal defects 115
arthralgia 105
arthritis 32, 158, 179
ASD 98
aspartic acid (D727E) 67
aspirin 96, 164, 166, 193–194
asplenia 141
assisted conception 114

asthenia 139
asthma 64, 105–106
aTG 217
atherosclerosis 235–236, 249, 251
(AT)n micro-satellite 66
atopic diseases 105–106
atrophic 63, 116
autoantibodies 22, 33
  to calcium-sensing receptors 139
  to tryptophan 141
autoimmune 32, 83, 87, 124, 159
  Addison's disease 297
  adrenal disease 159
  attack 50, 54
  destruction 45
  diabetes 45
  diseases 29, 83, 104, 122, 164
  epithelialitis 201
  gastritis 139
  gastropathy 216
  hemolytic anemia (AIHA) 268–269
  hepatitis 139
  liver disease 123
  ovarian injury 223
  polyendocrine syndrome (APS) 87, 135
  polyendocrine syndrome-I (APS-1) 47
  polyendocrinopathy–candidiasis–ectodermal–dystrophy (APECED) 138
  polyglandular syndrome type 1 (APECED) 128, 138
  polyglandular syndrome type II 141, 143, 216
  regulatory (AIRE) gene 47, 138
  thrombocytopenia 265
  thyroid disease 38, 61, 77, 122, 139, 150, 173, 199, 203, 205, 215
  thyroiditis 296
autoimmunity 77, 119
autologous endometrium 107
autoreactive B cells 22, 120
autoreactive T lymphocyte 214
Avitene paste 278
azathioprine (AZP) 90, 164, 261, 268
azoospermia 116, 164

B

1b or non-immune mediated 211
B cell 1 30–31, 47, 61, 64, 76, 120, 160–161, 177, 223, 244, 278, 294, 301
B lymphocytes 179–180
B/W mice 190, 241
B6.NZMc1/c7 242
B6.yaa mice 25
B7 in β-cells 214
B7-1 38
B7-1/B7-2 283
bacterial endocarditis 258
BALB/c background 243
BALB/c mice 162–163, 242, 245
baseline prolactin level 24
BB genotype 296
B-cell
  compartment 22
  hyperplasia 243
  maturation 22
  response 212
  tolerance 25
Bcl-2 32, 243
Belatacept (LEA29Y) 284
17 beta-estradiol (E2) 188
beta1 160
beta human chorionic gonadotropin (βhCG) 192–194
beta-cell replacement 279
bilateral adrenal hemorrhage 150
biliary 159
bioactive 190
biological clock 191
biopsy-proven GCA 257
bisphosphonates 260
blastocyst 164, 186
  stage 186
blocking estrogen synthesis 22
blood cells 158
blood transfusion 278
blood–brain barrier cerebral endothelium 84
body fat 236
body mass index (BMI) 276
bone alkaline phosphatase 253
bone loss 249
bone marrow hematopoietic cells 75
bone mineral density (BMD) 252–253
brain 117
  IL-6 85
breast development 114
breast feeding 190
bromocriptine (BRC) 21, 23, 35, 38, 180, 190–191, 244–245
  induced PRL reduction 33
  therapy 37
BsmI 297
bursitis 257
BXSB male mice 21
BXSB mice 24

C

+1858C/T 215
^{11}C 287
β-cells 55
β-cell-specific 50
β-cell 177, 211, 214
[^{11}C]Dihydrotetrabena-zine (DTBZ) 287
C/T polymorphism 64
C/T single nucleotide polymorphism 121
C1 inhibitor 188
  C1-INH protein 269
C1-C1-INH complexes 270
C1-INH 269
C1-inhibitor deficiency 269
C1r 188
C1s 188
C2 188
C3 106, 188
C3a 188
C4 106, 188
C4a 188
C5a 188
C57BL/6 fetuses 190
C57BL/6 mice 25, 242
cabergoline therapy 39
CAG trinucleotide 124
calcific-uremic arteriolopathy 153
calcineurin/glucocorticoid therapy 282
calcitonin gene-related peptide (CGRP) 7
calcium 139, 260
cAMP 84
cancer 224
Candida albicans 141
CAPS 150
carpal tunnel syndrome 257
carp-like mouth 114
cataract 236
Caucasians 295
CC genotype 64
CD22 243
CD28 283, 301
CD118 301
CD4$^+$ cells 88
CD4+ T lymphocytes 301
CD4+ T-cells 159, 205, 293, 301
CD4$^+$/CD8$^+$ ratio 123
CD4+/CD8+ 30
CD4$^+$ CD25− 64
CD40 31–32, 63–64, 121
CD40-CD40L 23–25
CD40L 24
CD40 ligand (CD154) 64, 301
CD40 SNP 64
CD45+/CD11b+ phenotype 75
CD4-related cytokines 24
CD59 188
CD69 301
CD8 T lymphocytes 294
CD8$^+$ cells 120
CD8+ 159
celecoxib 164
celiac 33
  disease (CD) 37, 114–115, 122, 125, 141, 144, 159, 179
cell 30, 47–48
  adhesion 162
  anti-25-OHase (AAA/25-OHAbs) 216
  cycle progression 30
  growth and apoptosis 15
  membrane antigens 158
  mediated autoimmunity 77
  mediated immunity 32
cellular immune responses 104
central
  dopaminergic tone 37
  embolization 278
  nervous system (CNS) 84, 158, 181
  tolerance 47, 119
centripetal fat 236
cereals 51
cGCR 229
cGMP 278
CGRP-positive nerve fibers 8
chemokines 187
chemotherapy 76
childhood 45
children 87, 266
cholecystokinin deficiency 141
chondrocytes 252
chorionic 188
  gonadotropin 186
  villi 187
  villus sampling 114
chromosomal abnormalities 126
chromosomal basis 116
chromosomal rearrangements 163
chromosome 217
  5p13 30
chromosome 1 121
  insulin gene variable number landem repeats (INS VNTR) 121
chromosome 14 121
chromosome 2 121, 143

chromosome 21 113, 139
chromosome 21q22.3 128
chromosome 5 at q14.3-15 201
chromosome 6 30, 120
chromosome 8 121
chromosome fluorescence in-situ hybridization (FISH) 116
chronic
  atrophic gastritis 87, 143
  fatigue syndrome 95, 104
  gastritis 144
  hepatitis C 127
  hypoparathyroidism (CHP) 139
  infections 231
  mucocutaneous candidiasis (CMC) 139
  stress 13–14
  thyroiditis 135, 143–144
ciliary neurotrophic factor (CNTF) 84
circadian PRL rhythm 35
circadian rhythmicity 232
circulating anticoagulant 149
circumventricular organs 84
cirrhosis 159
class 2 31
class II HLA genes 143
class III 143
classic atherosclerosis risk factors 178
classification 202
clonal anergy 120
clonal deletion 120
c-myc growth promoting gene 244
COBE cell separator 277
cod-liver oil 52
codon 620 (R620W) 66, 121
coeliac disease (CD) 215
coelomic metaplasia 103
co-factor 162
cognition impairments 237
cold ischemia 276
cold-storage preservation solution 277
collagen type 2 arthritis 251
collagenase 277
combined American-European 202
complement 3 106, 160, 301
  hemolytic activity (CH50) 188
  mediated immune attack 188
  proteins 188
  regulatory proteins 193
complex 190
computerized tomography scanning 139
concanavanine A 31
congenital
  cardiac defects 114
  heart defects 115
  lymphedema 114
  malformations 159
  rubella syndrome 52
  rubella 51
Conn's syndrome 177
convulsions 181
Copenhagen 202
coronary artery disease 152
coronary heart disease 236, 251
corpora lutea 159
corpus luteum 194
corticoliberin gene 98
corticosteroids 13–14, 160, 258, 266, 269, 282
corticotroph 86
corticotrophin 176
  releasing hormone (CRH) 84, 95, 97, 173, 176, 186, 191
  releasing hormone test 232
cortisol (C) 4–5, 96–97, 175, 187, 189, 303, 306
  binding globulin (CBG) 303
  levels 3
cosyntropin stimulation test 97
cotransmitter 7
cow's milk 51
  protein 54
CP690, 550 284
C-peptide 55, 214, 280
C-reactive protein (CRP) 98, 236, 257, 259, 296
creatine kinase 206
CRH receptor type 1 (CRHR1) 186
CRH-mRNA 95
Crohn's disease 123, 159, 301, 306
cross-reactive antigens 120
  molecular mimicry 120
Crry 188
cryptic 120
CSK 50
CT60 SNP 66
CTLA-4 216, 218
CTLA-4 +49A/G 215
CTLA-4 expression 64
CTLA-4 gene 65
CTLA-4 polymorphism 65
CTLA4+49G 217
^{64}Cu 287
Cushing's syndrome 194
cutaneous atrophy 236

cyclophosphamide 181, 158, 164, 223, 225
cyclosporin A 138, 283
CYP19 253
CYP-2A6 139
CYP-IA2 139
cysteamine-treated mice 244
cystic fibrosis 141
cytokine 22, 47, 84, 106–107, 165, 185–187, 258
  chemokine 187
  growth factor receptors 283
  production 180
  receptors 84
Cytomegalovirus (CMV) 285
cytoplasmic antigens 158
cytosolic GC receptor (cGCR) 229
cytosolic immunophyllin-the FK binding protein 12, 283
49-kDa cytosolic pituitary protein 85
cytotoxic cross-match 278
cytotoxic T lymphocyte-associated factor 4 (CTLA-4) gene 63–64, 121
cytotrophoblast 187
  cells 162
  proliferation 192

D

D2 receptors 36
Daclizumab 283
Danazol 265
DA-receptors 35–36
DBA/1 mice 191
DC/Th1 cells 32
decay accelerating factor (DAF) 188
decidua 194
decidual vessels 162
decidualization 186
decidualized endometrial stroma 186
declarative memory 306
defective placentation 192
Deflazacort 260
dehydroepiandrosterone (DHEA) 4, 13–14, 24, 96–98, 225, 232, 249, 303
dehydroepiandrosterone sulfate (DHEAS) 96–98, 189, 232, 303
dementia 117, 251
dendritic cells (DC) 30–31, 293–294
depression 7, 306
dermatomyositis 205
destruction of β-cells 46
dexamethasone test 188, 229, 232, 244, 253
D-group acrocentrics 116
DHEA in women with SLE 96
diabetes 45, 50–51, 53, 114, 157, 260, 275
  Autoimmunity Study in the Young 296
  insipidus 87
  mellitus (DM) 114–115, 152, 159, 237, 249
  mellitus type 1 (DM1) 177, 179
diabetic ketoacidosis 46
diabetic muscle infarction 178
diabetic rats 252
dialysis-dependent end-stage renal disease 281
diarrhea 286
diarylpropionitrile 242
diastolic blood pressure 224
diclofenac 164
dietary factors 51
dietary iodine intake 77
dihydrotestosterone (DHT) 14
1,25-dihydroxyvitamin D 187, 296
dioxin 104
disease 32–33, 175, 205
  modifying antirheumatic drugs (DMARDs) 234
dislipidemia 285
disomic homozygosity 128
dizygotic (DZ) twins 48, 62, 215
DNA synthesis 164
DNA-binding sites 229
DNA-reactive B cells 15, 243
docosahexanoic acid (DHA) 52, 54
dopamine 35
  agonist 38, 39
  delivery 38
  receptors 38
double non-disjunction 116
double stranded DNA 152, 199
double-blind 35
Down's syndrome 113, 115–117, 124
DPT-1 53–54
DQ8 214
DQA 0301allele 121
DQA1*0501 142
DQB1*02 143
DQB1*02/0302 212
DQB1*0201 142
DQB1*03 143
DRβ-Arg-74 63
DR1B1*01 143
DR1B1*013 143
DR1B1*05 143
DR2 and DR3 120
DR3 and DQA 120
DR3 64
DR3/4 49

DR3/4-DQ8 49–50
DR4 142
DRB1*03 143
DRB1*03,04 143
DRB1*0301 142
DRB1*04 143
DRB1*0401 216
drug-induced lupus 105
drugs 200
duplication of specific genes 24

E

early acute stress response 5
early RA 234
ectodermal dystrophy 141
ectopic endometrial growth 160
ectopic endometrial tissue 106
eczema 106
edema 88
EES-like 76
elated mood (euphoria) 237
elderly-onset rheumatoid arthritis (EORA) 258
electron microscopy 88
elevated muscle enzymes 205
ELISA 212
embryo 106, 225
embryonic 118
  development 163
  growth 162
  miscarriage 192
β^+-emitting radionucleotides 287
emotional lability 237
endocrine autoimmunity 135
endogenous opioids 7
endometrial
  antigens 160
  differentiation 194
  implants 104
  PRL gene 193
  stromal cell 108
endometriomas 104
endometriosis (EM) 103, 157, 160, 265
endometritis 244
endometrium 186
endothelial cells 158, 187, 229
end-stage renal disease 158, 178, 281
enolase isoforms 86
enteroviral infections 52
environmental factors 51, 119
eosinophils 88
epicanthic folds 114–115
epilepsy 115
epiphyseal fusion 116
epithelial cells 252
epitheliod histiocytes 88
EPO 285
erythrocyte sedimentation rate (ESR) 98, 257, 259
estradiol 159–160, 223
estradiol-17β 6, 14, 16, 17, 241, 244, 253, 304
estradiol-treated transgenic mice 15, 243
estrogen 5, 13–14, 21–22, 32, 119, 123, 160, 181, 185, 188, 223, 241, 244–245, 249–250, 253, 304
  activity 22
  deficiency 117
  implants 241
  induced lupus 24
  induced murine lupus 22
  levels 17
  mediated suppression 32
  metabolism 9
  α-receptor agonist 242
  α-receptor gene 242
  β-receptor activator 242
  β-receptor knockout mice 242
  receptors 242
  stimulated PRL secretion 32
  suppression 32
  toxicity 244
estrone 6
etanercept 204, 301, 305
ethinyl estradiol 244
Euro-Collins 277
Euthyroid Sick Syndrome (ESS) 76
exon 2–3, 6, 9, 33, 67, 141
experimental allergic encephalomyelitis (EAE) 33
experimental autoimmune encephalitis (EAE) 295
experimental autoimmune thyroiditis (EAT) 66
extra chromosome 21 116

F

2-[^{18}F]fluoro-2deoxy-d-glucose (FDG) 287
F_1 hybrid New Zealand Black (NZB) New Zealand White (NZW) (NZB/NZW) mouse 190, 241
Fab receptors 165
factor 12 38, 269
factor B 188
factor H 188
factor-binding 192
false-positive test for syphilis 149
familial aggregation 214
family history of DM 179

Fas ligand 187
fatigue 7, 96, 150, 158, 286, 306
Fc receptors 165, 266
female 32
  fat distribution 116
  Lewis (LEW/N) rats 95
  sex hormones 22
feminization 116
fertility defects 115, 186, 225
fertilization 161
fetal
  alloantigens 186
  deaths 118
  growth 163
  heart 162
  heart rate 193
  loss 192
  maternal microchimerism 15
  maternal interface 188
α1-fetoprotein 193
fever 96, 150, 158
fibroblast-like cells 35
fibroblasts 8, 35, 160, 229, 252, 302
fibrocystic breast disease 265
fibromyalgia 95, 104, 106, 296
fibrosis 77, 88, 162, 205
Ficoll 277
first trimester 162
first phase insulin response (FPIR) 53
first-degree relatives 104
flat occiput 115
flattened bridge 115
flutamide 190
folate 194
folic acid antagonist 164
follicle-stimulating hormone (FSH) 265
follicular
  B cells 23
  cells 15
  growth 160
  phenotype 22
folliculogenesis 161
fourth component of complement (C4) 270
Fox criteria 202
FOXp3 47
fracture 235
free androgens 10
free estrogens 10
free PRL 180
FSH 117
FSH levels 160
fungicidal antibiotic 283

G

$3G5^{+}$(age related) T cells 128
GAD Ab 34, 37
GAD65 48
gallstones 141
gamete fusion 161
gamma1 160
gamma/delta T cells 187
GC receptor abnormalities 232
GC/GCR 229
GC-induced glucoma 237
GC-induced osteoporosis 235
GCR 230
  mediated non-genomic effects 230
GCs 232, 234
G-CSF 285
gelatin agglutination test 161
Gelfoam pledgets 278
gender bias 21
gender-biased autoimmune disorders 22
gene 30, 50, 121, 186
  specific DNA demethylation 24
genetic 215, 231
  factors 21, 50
  predisposition 119
  risk 48
genome scanning 62
genomic distances 62
genotype/phenotype correlation 117, 119
gestation 164
GG 66
G-group acrocentrics 116
giant cell arteritis (GCA) 95, 98, 257
Giardia lamblia 141
glomerulonephritis 33, 159
glucocorticoids (GCs) 30, 35, 90, 95, 163, 188, 229, 232
  induced hypertension 253
gluconeogenesis 211
glucotoxicity 54
glutamic acid decarboxylase (GAD65) 212
glutamic acid decarboxylase autoantibodies (GADA) 212
glutamine 67
glutamine at position 63, 74
gluten 51
glycolytic enzyme 85
glycosylated hemoglobin (HbA1C) 159
GM-CSF 31
goiter 77, 126
goitrous 63
gonadal dysgenesis 122

gonadal hormones 13
gonadotrophins 159–160
gonadotropin-releasing hormone (GnRH) 105, 193, 225
β_2GP1 162
gp130 cytokines 84
gp130 receptor 85
G-protein-coupled surface receptor 249
GRAGIL 276
granulocyte 294
granulomas 88
granulosa cells 161
Graves' disease (GD) 33, 61–62, 65–66, 77, 87, 122, 127, 135, 151, 173, 179, 199, 204–206, 297
growth 192
  factors, 106–107, 186
  failure 114
  hormone 236, 244
  hormone receptor 30
  hormone therapy 123
  spurt 116
  stimulating antibodies 77
gynecomastia 116, 181

H

H_2O_2 294
hair effects 236
hair growth 270
hair loss 270
haplotype 142
HapMap project 62
haptoglobin 108
Hashimoto's disease 173
Hashimoto's thyroiditis (HT) 33, 38, 61, 77, 87, 122–123, 151, 159, 173, 179, 199, 297
αhCG 192
hCG-treated rabbits 164
headache 87, 286
Health Assessment Questionnaire (HAQ) 305
hearing defects 115
γ_2b heavy chain 243
hemangioma 278
hematopoietic stem cell transplantation 76
hemoglobin A1c 54
hemolytic 159
hemorrhagic 96
  infarction 150
Henoch–Schönlein purpura 270
heparin 158, 192
hepatic dysfunction 224
hepatic parenchyma 278
Hepatitis B infection 125
hereditary angioedema (HAE) 269
high estrogen/high prolactin 24
high estrogen/low prolactin 24
high myopia 237
hip 253
  fractures 260
hirsutism 96
histidine 67
  decarboxylase 141
histones 159, 160
HIV infection 153
HLA 47, 49, 186, 212
  A2 214
  antigens 278
  class I 143, 186
  DQ2, HLA DQ8 177
  DR4-DQ8 47
  genotype 50
  type B8, DR3 174
HLA-C 186
HLA-DQ2/DQ8 217
HLA-DQA1*0501 63, 143
HLA-DRβ1 63
HLA-DR 63, 67
HLA-DR3 63–64, 142, 214, 217
HLA-DR3 (DRB1*0301) 215
HLA-DR3-DQ2/DR4-DQ8 215
HLA-DR4 64, 142, 214, 216
HLA-DR5 63
HLA-DRB1*03 63
HLA-DRBi 34
HLA-E 186
HLA-G 186–187
HOMA-R 52
homozygous state 119
hormonal axes 301
hormone 173
  mediated breakdown of B-cell tolerance 25
  replacement therapy (HRT) 224
HPA axis 3, 36, 95, 98, 303
HPA dysfunction 97
HT 62–66
human chorionic gonadotrophin (hCG) 159
human fetal pituitary tissue 86
human leukocyte antigen(HLA) class II genes 47, 63, 120
human polyclonal ACL 192
humoral immune responses 104
humoral immunity 32
1α-hydroxylase 294
11β-hydroxysteroid dehydrogenase 163
11β-hydroxysteroid dehydrogenase type 1 305
11β-hydroxysteroid dehydrogenase type 2 305

3β-hydroxysteroid dehydrogenase 250
2-hydroxylated 241, 251
16-hydroxylated 251
5α-hydroxylated androgens 251
16α hydroxylated DHEA 250
7α-hydroxylated DHEA 250z
2-hydroxylated estrogens 17
16α-hydroxylated estrogens 6
16-hydroxylated products 241
16α-hydroxyestrone 16, 243, 304
17-hydroxy-progesterone 303
17α-hydroxylase (17α-OHAbs) 139
17β-hydroxysteroid dehydrogenase 250
25-hydroxyvitamin D 295–296
4-hydroxyandrostendione 23, 243
4-hydroxyestradiol 10
4-hydroxylated 251
21-hydroxylase (ACA) 139
hyalinized seminiferous tubules 116
hydrocortisone 176
hydroxychloroquine 23, 35, 165, 180, 261
hydroxylase (TPHAbs) 141
hypercalcemia 175
hypercortisolism 191
hypergammaglobulinemia 152, 190
hyperglycemia 54, 211, 236
hyperglycemic activity 38
hypergonadotrophic 181
hyperkalemia 96, 177, 224
hyperparathyroidism 175, 153
hyperpigmentation 139
hyperprolactinemia (HPRL) 23, 33, 36–38, 87, 152, 179, 190–191, 244–245
hyperresponsive B cells 152
hypertension 114, 177, 189, 236
hyperthyroidism 125, 199–200, 205
hypertonic-density solutions 277
hypertriglyceridemia 181
hypoglycemia 96, 139
hypogonadism 114, 124, 139, 144, 181
hypogonadotrophic hypogondism 124
hyponatremia 96, 181
hypoparathyroidism 87, 185, 175
hypophysectomy 244
hypophysitis 33, 83
hypopituitarism 88, 152
hyporeninemic hypoaldosteronism state 176
hypotension 96, 139, 150
hypothalamic 36, 95
  dopaminergic inhibition 32
  dopaminergic neurons 36
  dysfunction 152
  LIF 85
  pituitary axis 29, 121
  pituitary–adrenal 3
  pituitary–adrenocortical axis (HPA) 13, 84, 95, 191, 229, 231, 303
  pituitary–gonadal axis (HPG) 13
  pituitary–thyroid axis 75, 77
hypothalamus 84
  autonomic nervous system axis 8
hypothyroidism 38, 61, 76–77, 105, 122, 124, 151, 174, 199–200, 203, 205–206

I

IA-2 (ICA512) 48
ICAM-1 38
idiopathic
  diabetes 45
  inflammatory myositis 206
  thrombocytopenia purpura (ITP) 265
IgA nephropathy 270
IgE 64
IGF-1 binding protein 1 (IGFBP-1) 192, 305
IGFBP-3 305
IgG 34, 106, 160, 162, 166, 190
  anti-dsDNA 17
  class 206
  β2GP1 162
  IgA autoantibodies 160
  IgM antibodies 206
  23 kDa 190
  production 32
IgG2a 243
IgG2b anti-DNA 242
IgG3 22
IgG3 class 243
IgM 166
IgM aCL titers 152
IGT 54
IL-4 33, 107–108, 205, 244, 252, 293
IL-5 33, 107, 293
IL-6 5, 10, 17, 24, 33, 35, 84, 106–108, 187–188, 191, 232, 244, 251–253, 301
IL-8 10, 106–107
IL-10 knockout mice 295
IL-11 84
IL-12 secretion 294
IL-15 187, 244
IL-17 301
IL-18 187
IL-1Ra 192
IL-2 receptor 217, 244

IL-2β 244
immature B cell apoptosis 32
immature B cells 32
immature DC 31
immune
  activity 29
  cell–derived PRL 30
  complexes 23, 178, 302
  complex-coated platelets 266
  complex glomerulonephritis 223
  complex–mediated glomerulonephritis 25
  complex-related 33
  endocrine interactions 75
  neuroendocrine responses 185
  neuroendocrine system 185
  regulatory cytokine 30
  regulatory genes 63
  stimulatory endocrine factor 30
  system 75, 231
immunobead test 161
immunoblotting 85
immunofluorescence 85
immunoglobulin (Ig) 33, 301
immunosuppressive drugs 90
immunosuppressive effect 39
impaired wound healing 236
implantation 163
implantation failure 159, 166
implanting syngeneic pituitary glands 244
in vitro fertilization (IVF) 158, 166
inactive SLE 35
inactive X chromosome 24
incomplete type 2 MAS 142
increased transaminases 270
index of internal organ disease 36
indole-3-carbinol 243
indomethacin 164
INF 84
infarction 96, 192
infarcts 162
infection 29, 77, 84, 237, 285
infertility 103, 114, 116, 157, 161, 163
infiltration 83
inflammation 13, 29, 84
inflammatory 3, 38
  bowel disease (IBD) 164, 251, 293, 295
  cytokines 4, 13–14
  pain 257
  polyarthritis 164
infliximab 261, 301
inhibin A 191
inhibins 193
inhibitory antibodies 77
inhibitory hypothalamic dopamine effect 36
innate immune activation 194
innate immune system 188
insect cytogenetics 116
insomnia 237, 286
instant blood-mediated inflammatory reaction (IBMIR) 284
insufficiency 149, 152
insulin 38, 47–48, 50, 52–54, 121, 123, 159, 212, 287
  dependent diabetics (IDD) 211
  derived epitopes 214
  free 280
  independence 276
  induced hypoglycemia 3, 232
  induced hypoglycemia test 98
  producing β-cell 45
  receptor 178
  resistance 45
  secretion 45
  treated diabetes 123
  VNTR genotype 50
insulin-like 192
  growth factor I (IGF-1) 173, 305
insulinoma cell line (IA-2) 212
insulin-producing cells 279
insulitis 51, 177, 214
intercellular cell adhesion molecule-1 162
interferon 30, 205
interferon α 77
interferon (IFN)-γ 23, 188, 192–193, 244, 252, 293, 301
interferon regulatory factor-1 (IRF-1) 244
interferon-producing 205
interleukin (IL)-1 95, 106–107, 191, 230, 301
interleukin (IL)-1β 76, 84, 188, 252–253, 301
interleukin 6 (IL-6) 230
interleukin-10 (IL-10) 17, 24, 33, 107–108, 187, 190–294
interleukin-12 24, 31, 76, 187, 191
interleukin-2 24, 33, 64, 76–77, 108, 293
interleukin-3 (IL-3) 162, 193, 244
interstitial lung disease 233
interstitial pneumonitis 285
intestinal epithelial cells 75
intestinal infections 141
intestinal lymphangectasia 141
intra-articular GC injections 231
intracytoplasmic sperm injection (ICSI) 159
intraocular pressure 237
intra-portal thrombosis 278
intra-thyroid bone marrow–derived cells 75
intrauterine fetal death 189

intrauterine growth restriction (IUGR) 189, 192–194
intrauterine lethality 118
intravenous
  GC pulse therapy 231
  immunoglobulin (IVIg) 158, 165, 266, 268
intronic polymorphism 205
IRF-1 deficient (-/-) 244
IRF-1 is required for Th-1 immune responses 244
IRF-1 positive (+/−) 244
iris 115
irritable bowel syndrome 123
islets 278
  after-kidney (IAK) transplantation 281
  allotransplants 275
  amyloid polypeptide 214
  autograft function 282
  cell antibodies (ICA) 34, 37, 48, 87, 177
  cell autoantibodies (ICA) 212
  isolation 277
  specific antigens 48
  specific glucose-6-phosphatase 214
  transplant alone (ITA) 279
  transplantation 275
  transplantation networks 277
isolation teams 276
itching 270
IVGTT 53

J

Jaccoud's arthropathy 37, 175
JAK 2/STAT pathway 30
JAK/STAT/SOCS 84
JAK3 inhibitors 284
Janus kinase inhibitors 284
Janus kinase/signal transducer (JAK) 30
juvenile
  chronic arthritis 124
  idiopathic arthritis (JIA) 192, 204
  onset 157
  rheumatoid arthritis (JRA) 218

K

150–170 kDa (big big PRL, called macro-PRL) 35
23 kDa (monomeric PRL) 29, 35
60 kDa (big PRL) 35
kallikrein 269
karyotypes 117
karyotype 45,X/46,X,i(Xq) 128
karyotypically 114
ketoacidosis 54, 275
ketosis 211
kidneys 158
  islet transplantation 275, 278
Klinefelter's syndrome 113, 115–117, 123, 180
γ-knife radiosurgery 90

L

L-4 187
La (or SSB) 201
LA 193
laminin-alpha1 160
laminin-alpha1 chain G domain 160
Langer mesomelic dysplasia 119
late endosomes 150
latent autoimmune diabetes in adults (LADA) 48
late-onset spondyloarthropathy 258
late-slow onset (LADA) 211
Latin America 211
leptin 123, 305
Leri-Weill dyschondrosteosis 119
lethargy 87, 150, 270
letrozol 23
leucopenia 285
leukemia 115
  inhibitory factor (LIF) 84, 186
leukocyte subpopulations 107
leukocytes 229
Leydig cells 116
liberase-CI 277
liberase-HI 277
LIF deficiency (LIFKO) 84
limb extremity varicose veins 114
linear growth in children 236
linkage disequilibrium (LD) 63
lipids 205
  lowering drugs 285
  profile 236
lipopolysaccharide (LPS) 85, 230
liver 278
  disease 114–115
  enzymes 253
  function tests 284
long-term steroid therapy 260
low estrogen/low prolactin 24
low molecular weight heparins 158
low-calcemic vitamin D 295
low-dose 193
  acetylsalicylic acid 192
  bromocriptine therapy 35
low-grade fever 257
low vitamin D intake 295

lumbar spine BMD 253
lung 205, 285
  disease 205
  involvement 35
lupus 64, 106, 157
  activity 23, 188
  anticoagulant (LA) 96, 150, 178, 192–193, 224
  like illness 201, 204
  like syndrome 23, 25, 223
  nephritis 22–23, 158, 165, 225, 296
  nephropathy 175
  prone mice 21
  susceptibility locus Sle1 25
  susceptibility locus Sle3 21
luteal phase 186
luteal phase deficiency 185
luteinizing hormone (LH) 97, 265
lymph 47
lymphocytes 35, 159, 244, 302
  derived PRL 34
  function 165
  maturation and activation 22
  proliferation 253
lymphocytic
  adenohypophysitis (LAH) 87
  adrenalitis 138
  hypophysitis (LYH) 33, 38, 85–87, 179
  infiltrates 199
  infiltration 77
  infundibulo-neurohypophysitis (LINH) 86–87
  infundibulo-panhypophysitis (LIPH) 86–87
lymphoid 76
  tyrosine phosphatase (LYP) 50, 66, 121
lymphokine-activated killer (LAK) cells 188
lympholytic drugs 90
lymphoma 286
Lyonization 113
lysosomal bodies 88

M

macrophage 8, 30–31, 106–107, 159, 249, 252–253, 293, 301–302
  activity 253
  colony-stimulating factor (GM-CSF) 77, 294
  phagocytic activity 108
macroprolactin 180
macroprolactinemia 180
macrovascular disease 152
Madelung deformity 119
magnetic resonance 257
  imaging (MRI) 38, 287
major histocompatibility complex (MHC) genes 63, 120
major surgical stress 176
male 266
  hypogonadism 180
  infertility 164
malignancy 286
mammalian targets-of-rapamycin (m-TOR) 283
marginal zone
  B-cells 243
  cells 15
  phenotype 22
MAS type 1 138
β-mass 53
mass effect symptoms 87, 88
maternal hypothalamic 191
maternal–fetal complications 189
materno–fetal interface 186
matrix 35
  metalloproteinases (MMPs) 3, 186, 252, 301
  proteins 158
mature B cells 32
MCH class II alleles 177
4-methoxylated 251
2-methoxylated estrogens 251
membrane 162
  boundGCR (mGCR) 230
  cofactor protein (MCF) 188
memory 237
menopause 13–14, 17
menorrhagia 223
menstrual cycle 13–14, 186
menstrual irregularities 158
mental retardation 115
6-mercaptopurine (6MP) 164
mesenteric venous 278
metabolic syndrome 124
metalloproteinase 35, 252
metaplasia of peritoneal cells (coelomic metaplasia) 103
methamizole 200
methotrexate (amethopterine) 90, 164, 234, 261, 268
methylprednisolone 176, 229, 260
mGCR-mediated non-genomic effects 230
MHC 30, 215
MHC-II 294
MHC II molecule 67
MHC polymorphisms 120
MIC-A gene 143
mice 191
microadenoma 37
microprolactinoma 38

migraine headaches 224
mild hyperprolactinemia 35
mild-to-moderate 23
minor 176
Mip1a 35
miscarriage 161, 194
Mitochondrial dysfunction 117
mitral valve prolapse 201
mixed agglutination assay 161
mixed connective tissue disease 199
moderate surgical stress 176
molecular mimicry 161
monocytes 30, 179, 230, 301
mononuclear phagocyte system 266
monozygotic (MZ) twins 62, 104, 215
mosaicism 116, 118
mouse 51, 218
  model 296
  monoclonal antibodies to cardiolipin (ACL) 192
mouth ulcers 285
MRI diagnostic criteria 88
MRL/lpr mice 23, 95, 191, 223, 296
mRNA 66, 160, 287
mTOR inhibitor-free 284
mucosal cancer 141
multinucleated giant cells 88
multiple 159
  autoimmune syndromes (MAS) 136
  lentigenes 114
  sclerosis (MS) 32–33, 37, 105–106, 179, 204, 217, 293, 295
muscle bulk and strength 116
mutation 141
myalgia 105, 270
myasthenia gravis 64–65, 143–144, 159
mycophenolate mofetil 165
myeloid cells 76
myeloid stem cells 128
myeloma 258
myocardial infarction 152, 224
myopathy 235
myositis 233
myositis-specific 206

N

^{13}N 287
nafoxidine 22
nail dystrophy 141
nano-particles of iron 287
naproxen 164
natural killer (NK) cell activity 30, 76, 107, 163, 187
nausea 96, 150, 286
neck webbing 114
necrosis 162
negative Gram staining 278
neonatal diabetes 47
neonatal lupus 189, 201
nephritis 64, 177
nephritogenic anti-DNA antibody 243
nephrotoxicity 284
nervous system antigens 158
networks 186
neuroendocrine 85
  axes 3
  immune responses 84
  system 75
neurogenic bladder 153
neurohypophysis 87
neuroimmune interface 84
neuronal
  deaths 117
  destruction 88
  tissue 85
neurons 85
neuron-specific enolase (NSE) 85
Neuropeptide Y (NPY) 5, 306
neuropeptides 84
neurotransmitters 84
NF-κB 17, 252, 283
nimesulide 164
NK cells 31, 165
non-autoimmune C57BL/6 mice 223
non-disjunction 116, 118
nonerosive asymmetric polyarthritis 257
non-exposed (cryptic) antigens 120
non-genetic factors 215
non-invasive pituitary imaging 86
non-obese diabetic (NOD) 50, 218, 296
non-obese diabetic mouse 138
non-organ specific 123
non-pathogenic aPL 151
non-pathogenic IgA aPL 151
nonpregnant female 266
non-secreting pituitary adenomas 83
non-specific non-genomic effects 231
non-specific polyclonal B-cell activation 106
non-steroidal anti-inflammatory drugs 164, 258
non-twin siblings 48
noradrenaline 7–8, 85
norepinephrine 7, 187, 306
NSAIDS 164
nuclear antigens 158
nuclear factor of activated T-cells (NFAT) 283

nuclear factor-kappaB (NFκB) 230
nucleotides 160
Nurses' Health Study 295
nursing 32
NZB/NZW F1 mouse 21, 23, 96, 180, 223, 245
NZB/W F1 mice 24
NZM2410 lupus-prone mice 25

O

^{15}O 287
obesity 52
oblique eye fissures 115
obstetrical
  antiphospholipid syndrome (O-APL) 192
  rheumatoid arthritis (O-RA) 190
  systemic lupus erythematosus (O-SLE) 188
ocular abnormalities 119
ocular movements 90
1,25$(OH)_2$D 293, 296
25(OH)D 293
25(OH)D levels 295
25$(OH)D_3$-1–hydroxylase 293
21-OH antibodies 34
21-OHAb 87
21-OHAbs 139
OKT3 283, 286
older obese children 45
oligospermia 116, 164
oncocytic changes 88
Oncostatin M (OSM) 84
oocyte cryopreservation 225
oocytes 159–160
oophoritis 159
ooplasm 159
open-angle glaucoma 237
ophthalmopathy 77
optic nerve 37
oral contraceptives 13–14, 190, 224
oral glucose tolerance testing (OGTT) 45, 53
organ retrieval 276
organ transplantation 164
organ-specific 123
organ-specific autoimmune diseases 83
osteoarthritis (OA) 7, 204, 250
osteoarthritic joint 233
osteoblasts 252–253
osteocalcin levels 253
osteoclast bone resorption 253
osteoclastogenesis 302
osteoclasts 253
osteonecrosis 235
osteopenia 253
osteoporosis 22, 114, 116, 225, 235
osteoprotegerin 253
otitis media 114
ovarian
  biopsy 160
  cyst 104, 286
  failure 158, 225
  follicles 159
  function 225
  proteins 159
  reserve 185
  resistance 194
  tissue 225, 160
  vein thrombosis 153
ovariectomized NZB/W F1 mice 23
ovariectomy 22
overt diabetes 54
overweight 116
ovulation-inducing agents 163
ovulatory dysfunction 161

P

5p13 30
10p15 (rs2104286) 217
P450 side chain cleavage enzyme (P450sccAbs) 139
P450c11 303
P450c21 303
P450ssc 303
P52T SNP 67
pancreas glands 277
pancreas transplant alone (PTA) 280
pancreas-after-kidney transplantation (PAK) 281
pancreatitis 235, 276
pancreatic
  autoantibodies 141
  biopsy 214
  β-cells 123
  insufficiency 141
  islet 45
paracrine/autocrine PRL secretion 38
parathyroid glands 139, 175
parathyroidectomy 153
parathyroid-related peptide (PTHrP) 175
parental age 118
pathological antibodies 165
peliosis hepatitis 270
pelvic
  EM 106
  girdles 257
  pain 103

pemphigus 251
peptidergic 7
peptides containing citrullinated arginine 203
percutaneous trans-hepatic portal 278
perforin 187
perinatal mortality 189
peripheral tolerance 47
peripheral vascular 35
peritoneal fluid 106
pernicious 159, 216
pernicious anemia 87, 144
persistent ductus arteriosus 115
PGE2 84
phaeochromocytomas 177
phlyctenular conjunctivitis 141
Phogryn 214
phosphatidylethanolamine 166
phosphatidylserine 160, 166
phosphodiesterase inhibitors 194
phospholipase A2 and phospholipase C stimulation 192
phospholipid-associated antigens 158
phospholipids 159, 162
physical or psychological stress 3
phytoestrogen-free diet 243
pinopodes 186
pitting edema 257
pituicytes 88
pituitary 38, 85, 152
  acini 88
  adenomas 244
  adenomectomy 177
  adenylate cyclase-activating polypeptide 85
  adrenal function 176
  adrenal suppression 176
  autoimmunity 83
  cells 84
  micro-adenoma 37
  surgery 90
  transcription factor Pit-1 30
  tumors 85
placebo-controlled study 35
placenta 163–164, 188, 191, 193
  abruption 192
  CRH 191
  damage 192
  explants 159
  hormones 32, 191
  insufficiency 193
  ischemia 186
  placental compromise 17
  tissue 85
placentation 163
plasma cells 88, 159
plasma proteins 158
PMR/GCA 98
POF 159
polyclonal lymphocytic infiltration 88
polycystic ovarian syndrome 185
polycystic ovary syndrome 157
polyendocrine autoimmune syndrome (APS) 37
polygenic 215
polygenic model 127
polyglandular autoimmune syndrome 199
polyglutamine tract 124
polymorphic markers 62
polymorphisms 50, 98, 121
polymyalgia 98
  rheumatica (PMR) 98, 232, 257
polymyositis 205, 258
polymyositis-like 205
polyneuropathy 178
polynucleotides 159
poly-X variants 114
POMC 84
portal vein 278
position 36 (D36H) 67
position 52 (P52T) 67
positive pregnancy test 224
positron-emission tomography (PET) 287
post-implantation embryos 160
post-implantation loss 164
postpartum 86, 152
  flares 188, 190
  Graves' hyperthyroidism 77
  hyperthyroidism 77
  period 13–14, 32
post-term labor 191
post-transplant 280
prediabetic period 45
prednisolone 176, 229, 234, 303
prednisone 194, 229, 258
pre-eclampsia 158, 186, 189, 191–192
pre-embryonic 192
pregnancy 13–14, 17, 32, 86, 185, 188, 193, 278
  loss 157, 188, 192
  lupus flares 158
pregnant SLE 17
pregnant women 217
pregnenolone 5
pre-implantation 160
  loss 164
premature
  aging 128, 162
  birth 189

cardiovascular disease 178
ovarian failure 157, 159
rupture of membranes 189
pre-ovulatory follicles 159
pre-ovulatory oocytes 159
preterm 191
prevention 53
primary 159
adrenocortical insufficiency 175
amenorrhea 114
APS (PAPS) 149
biliary cirrhosis 87, 127
Cushing's syndrome 177
follicles 159
hyperaldosteronism 177
primary adrenal 149
Sjögren's syndrome (pSS) 151
150-kDa PRL (big big PRL) 190
PRL genes 34, 303
PRL levels 37, 193
PRL receptors (PRL-Rs) 29, 186, 190
PRL secretion 36
PRL upregulation 32
PRL-R 30, 36
PRL-releasing 38
PRL-secreting tumors 179
proapoptotic effects 17
progesterone (P) 5, 13, 22, 32, 159, 185, 189, 194, 224
dependent immunomodulation 187
levels 17
progressive
sensori-neural deafness 114
systemic sclerosis 181, 124, 251
pro-inflammatory
cytokines 160
estrogens 251
proinsulin 214
prolactin (PRL) 21–22, 29, 32, 37, 179–180, 185, 189–190, 192, 194, 241–243
autoimmune diseases 32
induced lupus 25
leads to the production of 23
receptors 30, 244
treated mice 15
prolactinoma 37
proline 67
prolonged partial thromboplastin time 149
propyl pyrazole 242
propylthiouricil 200
prostaglandin E2 294
protein 50, 192
tyrosine phosphatase (PTPN22) 201
tyrosine phosphatase-22 (PTP-22) 121
tyrosine phosphatase-22 (PTPN22) gene 66
tyrosine phosphatase-like autoantibodies (IA-2A) 212
17-kDa protein 301
proteinuria 23
proximal muscle weakness 205
P-selectin 162
pseudopregnancy 245
pseudopregnant state 190
psoriasis 123
psoriatic arthritis 301, 305
psychosis 181, 235
PTH 153, 293, 296
PTPN22 1858 T 217
PTPN22 gene 50, 63, 204, 215–216, 218
PTU 201
puberty 116
puffiness of the hands and feet 114
pulmonary embolus 224
pulse intravenous steroid 260
purine synthesis 164
purpura 159, 236

Q

18q12-q21 217
21q21q translocation 117
14q21q translocation 117
Q358X 141

R

R139X 141
R257X mutation 141
R2A g2b BALB/c mouse model 244
R4A γ2b C57BL/6 mice 245
RA 33, 36, 98, 296, 232, 250, 254
rabbit synovial cells 252
radioimmunoassay 212
radio-ligand assay 85
radiologic damage 234
radiotherapy 90
raloxifene 22
RANK ligand 302
rapamycin 283
rat embryos 162
rat islets 275
receptor 199 120
receptor modulator (SERM) 22
rectovaginal endometriosis 104

regulatory factors-1 (IRF-1) 30
rejection 32
relaxin 191
renal disease 114, 178
renal vasculitis 223
renin 177
restrictive 205
  lung disease 114
reticuloendothelial system 266
retinopathy 178
retrograde menstruation/implantation theory 103
rheumatoid 32, 179
  arthritis (RA) 3, 13, 35, 66, 77, 85, 95, 104, 123–124, 151, 159, 164–185, 192, 199, 203, 216–217, 230, 260, 267, 301
  factor (RF) 107, 181, 203, 301
RNA receptor 21
Ro (or SSA) 201
Robertsonian translocation 116
RT-PCR 287

S

sarcoidosis 99
Schmidt's syndrome 141
scleroderma 199, 205
scleroderma renal crisis 233
sclerosis 159
second
  meiotic division 116
  trimester miscarriages 163
  X 45,X/46 118
  X chromosome 113
secondary amenorrhea 114
secondary Cushing's syndrome 177
selective GC receptor agonists, SEGRAs 230
SELENA-SLEDAI 224
self-reactive T cells 120
self-tolerance 161
sellar mass 90
semaphorin 3C 8
serositis 158
serum
  complement levels 160
  estrogen milieu 14
  free 180
  prolactin levels 23
  T4 205
severe EM 104
sex hormones 13, 21, 231
sex hormones and autoimmune diseases 13
sex hormones in the pathogenesis of lupus 24
sexual hair growth 116
SF estrogen concentrations 15
Sheehan's syndrome 152
sheep's pancreas 275
short stature 114
short wide neck 114
shoulder 257
SHOX gene 119
SHP-1 243
shrunken 116
sialadenitis 223, 242
signal 1 MHC/T-cell receptor pathway 283
signal 2 pathway 283
signal 3 pathway signal transduction pathway 283
silent autoimmune thyroid diseases 151
silent thyroiditis 78, 174, 204
simultaneous islet/kidney transplantation (SIK) 281
simultaneous pancreas/kidney transplantation (SPK) 281
single-nucleotide polymorphisms (SNPs) 62–63, 67, 204, 217
sirolimus/FKBP12 282–283
Sjögren's syndrome (SS)33 36–37, 95, 98, 104, 106, 179, 192, 199, 201, 218, 243, 254
skin rash 270
skin sclerosis 35
Sle1gene 242
Sle3/5 25
Sle3gene 242
SLEDAI 96
sleep 306
small bowel 285
small jaw 114
small nose 115
smoking 77
SOCS 1 and 3 38
solid tumours 258
soluble factors 107
SOX9 139
SOX10 139
spaced nipples 114
specific organ involvement 23
sperm 106
spermatogenesis 159
sphingocine-1-phosphate receptor antagonists (FTY720) 285
sphingosine-1-receptor blocking drugs (e.g., FTY720) 284
spleen 47, 244
splenectomy 266, 268
splenic
  dendritic cells (DCs) 75
  macrophage Fc (IgG) receptors 265
  macrophages 266

spliceosome complex 199
spontaneous abortion 189
SP-positive nerve fibers 8
squamous epithelial cancers 286
SS-A and SS-B 151
SSc 35, 37
stanozolol 270
STAT-5 30, 35
status epilepticus 149
steroid 84, 96, 158, 161, 163, 282
  acne 236
  based immunosuppression 276
  free sirolimus/low-dose tacrolimus 281
  hormonal replacement 14
  hormone 13, 258
  induced diabetes (SID) 179
  myopathy 235
  producing cell antibodies (StCA) 139
  psychosis 235
  resistant patients 261
  side effects 260
  sparing agent 261
  sulfatase 249
  withdrawal syndrome 97–99
steroidogenesis 161
stiffness 257
sTNFR 192
stress 29, 77, 84, 95, 121, 231
  adaptation molecules 30
  response system 13, 231
stressful 3
striae 236
stroke 125
α sub 1 301
β sub 2 integrin 301
sub-clinical hypothyroidism 78
substance P 7
sub-villus crypt regions 75
sulfate (DHEAS) 14
sulfate ester DHEAS 249
suppression of cytokine signaling (SOCS) genes 30
suprasellary 38
surgery 90, 176
surgical
  laparotomy 278
  oophorectomy 241
swelling of the hands 257
sympathetic
  activity 5
  nerve fibers 7, 306
  nervous system (SNS) 13, 231, 306
  neurotransmitters 306
  tone 306
synctial knots 162
syncytial fusion 192
syncytiotrophoblast 162
syndrome of inappropriate secretion of antidiuretic hormone (SIADH) 181
Synovial
  endothelial cells 302
  fibroblasts 249, 301
  fluid (SF) 5
  hypervascularity 302
  tissue 249
synovitis 257
systemic
  autoimmune diseases (SAD) 95, 99
  granulomatous diseases 90
  lupus activity measure (SLAM) 35
  lupus erythematosus (SLE) 13, 17, 21, 23, 32–33, 35–37, 65–66, 77, 95–99, 104, 114, 123, 149, 173, 179, 185, 190, 199–200, 215, 218, 223, 230, 241, 251–252, 254, 258, 267, 269, 295
  lupus erythematosus, autoimmune hepatitis 87
  lupus erythematosus, APS 193
  lupus erythematosus, Klinefelter's syndrome (KS) 180
  manifestations 37
  pregnancy 190
  sclerosis (SSc) 33, 35, 95, 98, 127, 151, 179, 192, 205, 233
  vasculitis 98, 233
systolic blood pressure 224

T

T and B lymphocytes 75
T cell 30–31, 64, 88, 120, 143, 205, 278
T cell receptor activation 121
T cell tolerance 120
T-cell 47, 138, 159, 161, 177, 179–180, 214, 223, 301
  activation 66
  receptor (TCR) 47, 50, 66
  receptor activation 64
T cell–dependent 23
T cell–mediated immune responses 64
T helper cells 107, 205
T lymphocytes 33, 211
Tÿ*$_2$-weighted MR images 287
T1 B cell 22
T1/T2 ratio 245
T1D 45, 54–55, 66
T1–T2 interface 22
T3 76, 200

T3 receptor 76
T4 76, 200
T- and B-cells 293
tacrolimus 283
tamoxifen 22, 242–243
tandem repeats (INS VNTR) 121
tendon reflex relaxation phase 205
tendonitis 257
teratogenic 162, 164
testicles 116
testicular hyalinization 181
testosterone (T) 5, 14, 17, 120, 123, 127, 225, 250, 253, 303
  receptors 120
  therapy 181
tetralogy of Fallot 115
Tg amino acid variants 67
Tg gene 67
TgAb 37
TGF-β 294
Th cells 31
Th1 31–32, 36–37, 123, 185, 244, 293
  dominance 33
  function 32
  related autoimmune disease 35
Th1/Th2 31–32
Th1/Th2 cytokine balance 192
Th-1/Th-2 cytokines 162
Th2 cells 32, 37, 123, 185, 244, 293
Th2 profile 33
Th2-related autoantibody production 32
T-helper 1 (Th1) 30
thioamides 201
thoracic aortic dissection 114
Thr > Ala substitution 65
threonine 67
thrombocytopenia 96, 151, 158, 267
thrombocytopenic 159
thrombosis 96, 150, 153, 162, 192
thromboxane prostacycline 162
thymic 30
thymic involution 253
thymocytes 30
thymoglobulin 284
thymus 47, 244, 294
thyrocyte 38, 61
thyroglobulin (Tg) 63, 65–67, 121, 135, 199
thyroglobin antibodies 122, 126
thyroid 115, 124, 173, 205
  antibodies (TAbs) 61–62, 65, 122, 126, 142, 161
  antigens 61, 174
  atrophy 138
  autoantibodies (ATA) 161, 166
  autoimmunity 77, 85, 121
  cells 199
  dermopathy 77
  disease 87, 126, 185
  dysfunction 77, 114
  function 75
  gland 77
  hormone 199
  hypofunction 61
  infiltrating T cells 61
  peroxidase antibodies 65, 122
  peroxidase 126, 199
  specific antibodies (aTPO, TGAbs) 77, 217
  specific genes 66
  stimulating antibodies 204
  stimulating hormone receptor (TSHR) 121
  stimulating hormone (TSH) 75, 217
  stimulating thyrotropin receptor antibodies 77
  stimulation 77
thyroiditis 99, 122
thyroperoxidase (TPO Ab) antibodies 34
thyrotroph 86
thyrotropin receptor (TSHR) stimulating antibodies 61
thyrotropin-releasing hormone 38
thyroxine 122
tissue 188
  injury 84
  transglutaminase antibodies 123
TLR7 21, 24
T-lymphocyte proliferation 293
TNF 251–253, 301
TNF-α 33, 35, 84, 162, 107, 232
TNF-α blocking agents 261
TNF-ÿγ 108
TNF blockers 234
TNF inhibitors 301
TNF-α inhibition 261
TNF-308A 217
TNF-suppressing drugs 204
tolerance to self 47
topoisomerase I (anti-Scl70) 205
total body irradiation 76
TPO antibodies 37–38, 62, 106
TPO gene 62
transactivation 230
transaminases 284
transcription 5 (Stat5) 193
transforming growth factor-β (TGF-β) 107
transgenic DQ8 + /mII-/mIE- mice 214
transglutaminase antibodies (tTGAb) 34
transjugular intrahepatic techniques 279
transplanted islet imaging 287

transportation 276
transrepression 230
trans-sphenoidal surgery 86
treatment-naïve SLE patients 96
TRH 35
TRH-induced secretion 37
Trial-type 1 53
triglycerides 205
triphasic 35*g ethinylestradiol/0.5–1 mg norethindrone 224
tRNA synthetases 199
tRNA transferases 205
trophoblast 162, 186, 192
trophoblast cells 186
tryptophan 50, 66, 121
TSH 77, 122, 142
  receptor 63, 76–77, 199–200, 202, 206
  receptor antibodies 122
  receptor-blocking 77
  receptor defective (C.RF-hyt/hyt) mice 76
  receptor (TSHR) gene 67
TSHR autoantibodies 67
tumor necrosis factor (TNF) blockers 5, 187–188, 191, 193–194, 249, 301
tumor necrosis factor gene 143
tumor necrosis factor TNF-α 106, 230
Turner karyotype 45 X 113
Turner's syndrome 113–114, 118–119, 122, 126, 128
Type 1 diabetes 33, 37, 124–125, 158, 211, 296
  A diabetes 45, 48
  B diabetes 45, 48
  BioBreeding (BB) rats 138
  mellitus (DM) 87, 121, 123, 141, 143–144, 204, 293
  multiple autoimmune syndrome (MAS) 138
  polyglandular autoimmune failure 205
type 2 APS 141
type 4 APS 87
type 2 diabetes (T2D) 45, 124, 214, 279
type 2 diabetes mellitus 86
type 2 MAS 137, 141, 143
type 3 MAS 137, 143–144
Type 4 MAS 144
type B insulin resistance 177
type I diabetes 191, 275, 279
type-I RR 215
type II collagen-induced arthritis 190, 244
type II RR 215
tyroglobulin (Tg Ab) 34
tyrosine
  hydroxylase 8
  hydroxylase autoantibodies 139
  phosphatase family 212
  phosphorylation 30

U

3′UTR 66
ulcerative colitis 123, 252, 254, 306
ulcerative colitis unexplained pregnancy loss 159
ultra-sonography 114, 257
uncontrolled diabetes 185, 224
unexplained vaginal bleeding 224
uNK cells 194
unstimulated peripheral blood monocytes (PBMs) 23
5′-untranslated region (5′UTR) 64
upper respiratory airways 285
uremia 280
urinary C 187
urinary excretion of hydroxyestrogens 16
urinary tract obstruction 244
urticarial vasculitis 37
uterine bleeding 286
uterine blood flow 164
uterine endometrium 161
Uterine NK (uNK) 187
uteroplacental vasculature 192

V

vaccinations 52
vaginal dryness 224
vaginal infection 286
valganciclovir 285
variable number of tandem (VNTR) 1, 45, 50
vascular cell adhesion molecule-1 162
vascular endothelial growth factor (VEGF) 108
vasculature 193
vasculitis 177–178, 181, 201
vasculosyncitial membranes 162
vasopressin 85
VDR 294
VDR gene BsmI polymorphisms 296
VDR-FokI polymorphisms 297
venous stasis 114
venous thrombosis 224
ventricular septal defects 115
vertebral 260
vertigo 270
vessel 96
villi 162
villous trophoblast 193
viral infections 52
viral protein gp70 190
virilizing side effects 270

visual
  acuity 90
  deficits 90
  fields 90
1α-vitamin D 296
vitamin D 139, 260, 293, 295
vitamin D receptors (VDRs) 293
vitamin D3 296
vitiligo 143–144, 159, 204, 215
VMAT2 287
voice change 270
vomiting 96, 150, 286

W

W78R 141
warm antibody-mediated AIHA 269
warm ischemia 276
wasting 211
weakness 96, 150
weight gain 270
weight loss 257
white spots 115
Wistar rats 164

X

45,X genotype 118–119
45,X karyotype 118
45,X/46,X+ 118
45,X/46,XX mosaicism 118
45,X/46,XX/47,XXX 118
45,X/46,XY 118
45,X/47,XXX 118
46,XX, karyotype 113
46,XY karyotype 113
47,XXX 118–119
47,XXY karyotype 114
47,XXY 118, 119
47,XXY/46,XY 118
47,XYY 118
47/XXY karyotype 180
X chromosome 21, 24, 47, 114, 118–119, 127
X chromosome long arm 128
X inactivation 127
X isochromosome 122
X monosomy cells 127
X sex chromosome 113
X 122, 124
Xi(Xq) 118
X-inactivation 118
X-linked autoimmunity genes 127
X-linked genes 118
Xq21.33-22 127
XY sperm 118

Y

Y85C 141
Y chromosome 21, 24, 113–114
Yaa 25
Yaa mutation 21
Y-linked autoimmune acceleration (Yaa) 24
yolk sac 162
young baboon pituitary glands 86

Z

zona pellucida 159, 161
zona reaction 161
zygote membrane 161

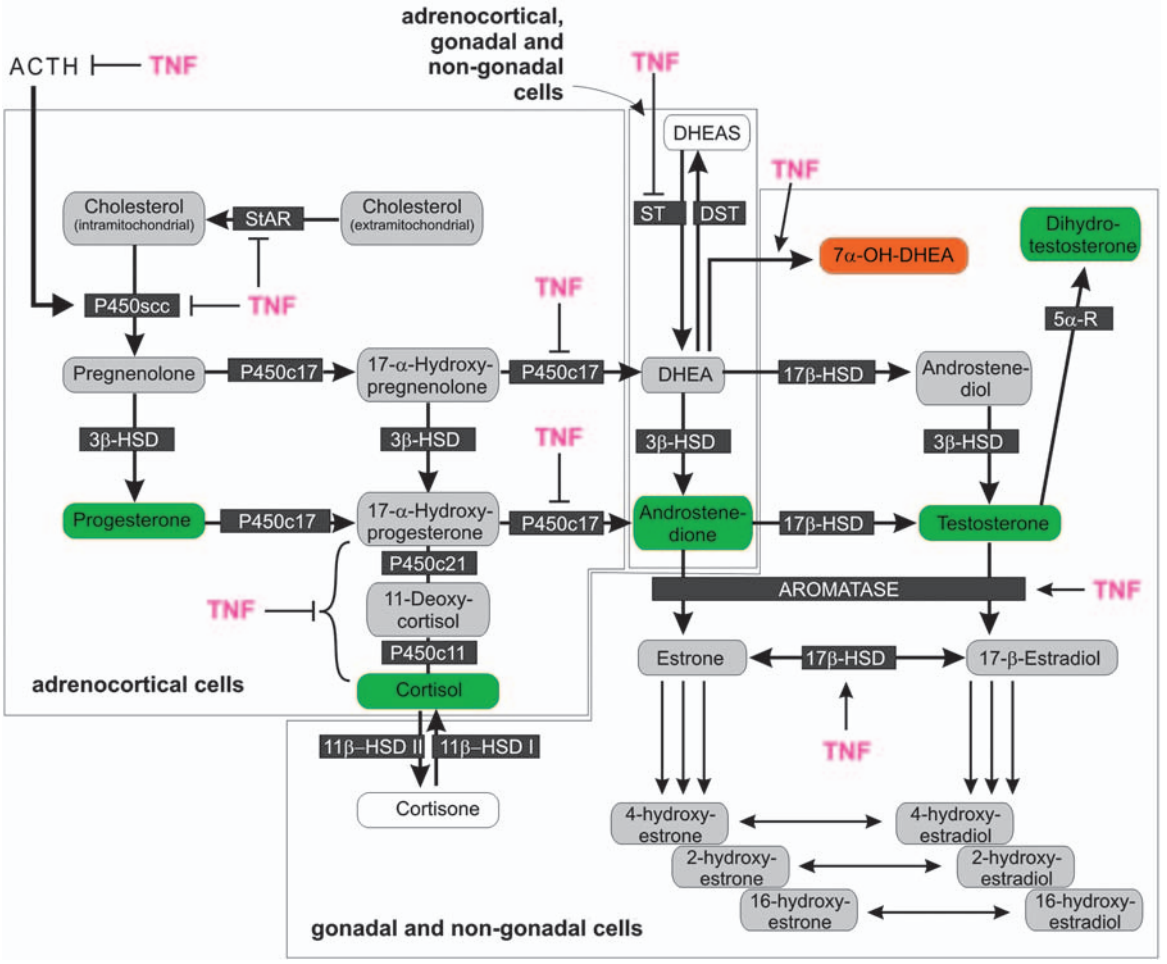

Plate 1. Hormonal conversion in gonadal, non-gonadal, and adrenocortical cells. Red (green) colors indicate proinflammatory (anti-inflammatory) factors. Arrows indicate a stimulatory effect whereas lines with a bar at the end indicate inhibitory effects. Abbreviations: DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; TNF, tumor necrosis factor; ACTH, adrenocorticotropic hormone; StAR, steroidogenic acute regulatory molecule; P450scc, cytochrome P-450-mediated cholesterol side-chain cleavage; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; 11β-HSD, 11β-hydroxysteroid dehydrogenase; 5α-R, 5α-reductase; ST, sulfatase; DST, DHEA sulfotransferase. (For Black and White version, see page 6.)

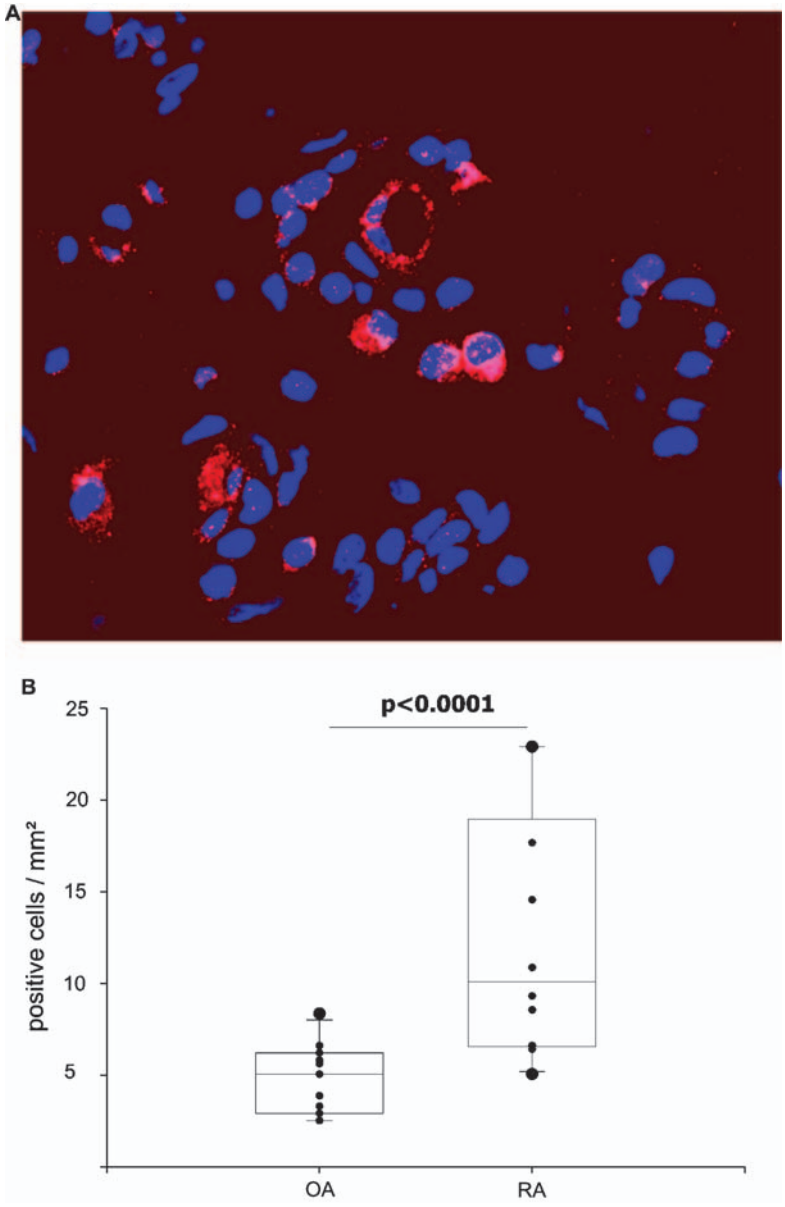

Plate 2. Tyrosine hydroxylase (TH) positive cells in synovial tissue. (A) Immunofluorescence staining of tyrosine hydroxylase (red staining) in synovial tissue from an RA patient, counterstained with DAPI (blue) as unspecific DNA staining. (B) Density of TH-positive cells in synovial tissue from 10 rheumatoid arthritis (RA) and 10 osteoarthritis (OA) patients. The density of TH-positive cells is significantly higher in synovial tissue of RA patients ($p < 0.0001$). (For Black and White version, see page 9.)

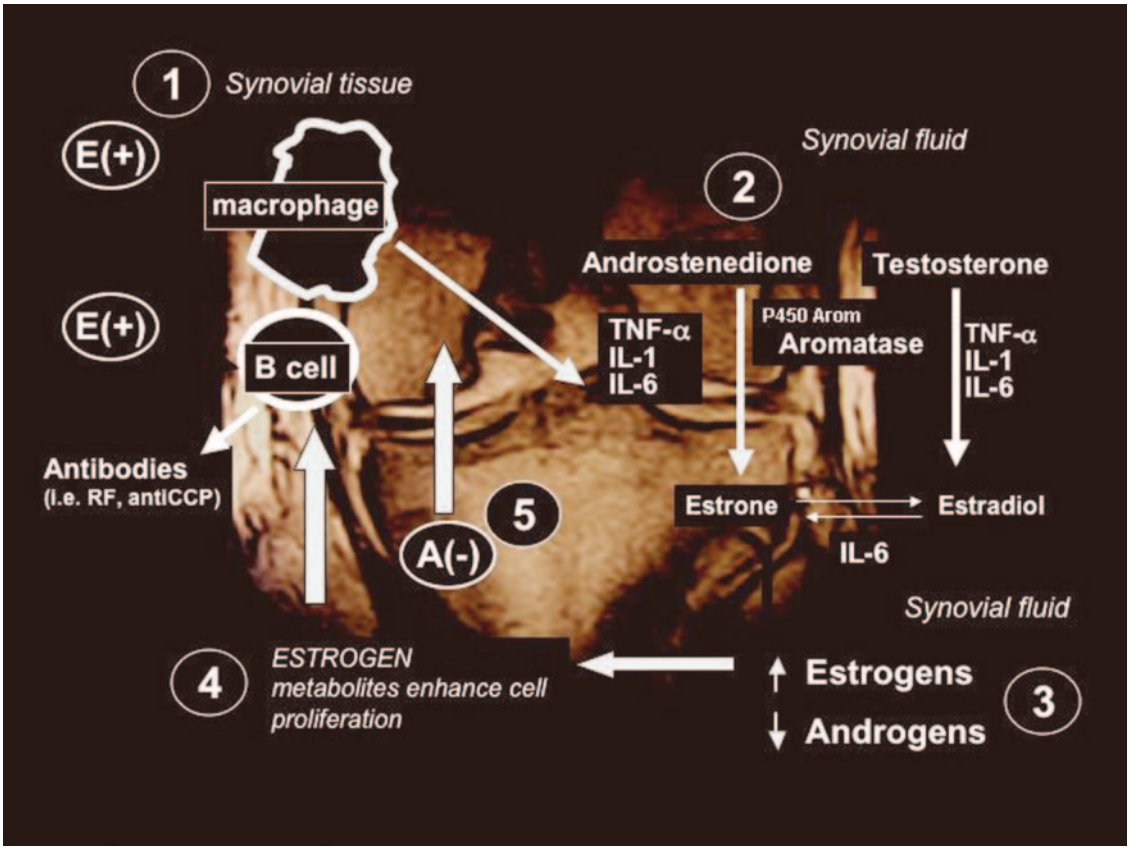

Plate 3. (1) Estrogens enhance the humoral immunity and proliferation of monocytes/macrophages. (2) The pro-inflammatory cytokines (e.g., TNFα, IL-1β, and IL-6) induce aromatases in synovial tissue, thereby accelerating the metabolic conversion of androgens to estrogens. (3) Increased concentrations of estrogens and low androgen concentrations are observed in synovial fluid of RA patients of both sexes. (4) Hydroxylated metabolites of estrogen hydroxylated metabolites affect mitogenesis and cell proliferation. (5) Androgens exert apoptotic and anti-proliferative effects. (For Black and White version, see page 14.)