Springer Semin Immun (2003) 25:S5–S60
DOI 10.1007/s00281-003-0151-1

ORIGINAL PAPER

Peter A. Miescher · Laura Zavota · Alejandra Ossandon · Bruno Lagana

Autoimmune disorders: a concept of treatment based on mechanisms of disease

Published online: 6 November 2003

Introduction

The history of autoimmunity is almost as old as the history of immunology. In 1890 Behring and Kitisato [1], working in Koch's institute, demonstrated that the serum of animals immunized against attenuated diphtheria toxins can be used as a preventive or therapeutic inoculation against diphtheria in other animals through a specific neutralization of the toxin of the disease. This discovery initiated the long history of the elucidation of the immune response. Just 10 years later Metalnikof [2] challenged the possibility of an immune response against self-constituents of the organism on the basis of his studies on guinea pigs immunized with spermatozoids. A few years later Chauffard and Vincent [3] reported the first case of autoimmune hemolytic anemia (AIHA) and Fiessinger [4] described anti-liver antibodies in patients suffering from chronic active hepatitis (CAH). Nevertheless, in the 1950s the idea of the immune apparatus being used against self-antigens was still considered absurd. The very existence of autoimmune disorders was questioned. Until recently, multiple sclerosis (MS) was considered a viral disease, and rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) to be caused by infectious agents, especially of the mycoplasma family. Interest in autoimmune diseases grew slowly but constantly in view of the intensive research being carried out in experimental animals as well the clinical research being carried out on thyroid disorders, RA, SLE, and MS.

It is now known that autoimmunity is not a rare event as was thought 50 years ago but is a frequent condition with over 40 recognized autoimmune diseases affecting more than

P. A. Miescher (✉)
Professor emeritus, Geneva University, Geneva, Switzerland
e-mail: Jean.Ringrose@hcuge.ch

L. Zavota
Division of Pediatrics,Parma University Hospital, Parma, Italy

A. Ossandon
Cattedra di Reumatologia, Dipartimento di Clinica e Terapia Medica Applicata,
Università di Roma La Sapienza, Rome, Italy

B. Lagana
Dipartimento di Medicina Clinica, Università di Roma La Sapienza, Rome, Italy

6% of the general population. Despite this development treatment of patients suffering from autoimmune diseases remains a very difficult domain. First, it is not easy for clinicians to follow the remarkable progress made in the field of molecular and cellular immunology. Furthermore, new discoveries in experimental immunology have made it necessary to change "accepted concepts" every 5 or 10 years. Secondly, autoimmune diseases are spread over 12–15 different clinical compartments, each concentrating on its own territory and not taking advantage of progress made in the other compartments. In addition, new immunomodulatory drugs are made available to the clinician only for specific indications and not for the inherent use of immunomodulation. For all these reasons we are still a long way from formulating a "unified" concept in the treatment of autoimmune diseases.

This review is intended for clinicians. Indeed, the development of our knowledge in the field of immunology has reached a level which is unattainable by the majority of practicing clinicians; yet it is the clinician who must take care of patients suffering from disorders of the immune system. Accordingly, we have revisited many areas which may appear trivial to the modern immunologist, but which need to be made clear to the clinician who must understand the complex mechanisms involved in immunopathology in order to offer an effective therapy to his patients.

This review begins with a short history of the events leading to today's molecular understanding of the immune response. From the plethora of scientific information we have chosen those crucial discoveries which help the clinician in the management of autoimmune disorders. In particular, the clinician must consider the type of immune response involved in the autoantigen-driven immunopathology and be aware of the kinetics of molecular and cellular events in the attempt to attenuate, correct, or limit the aberrant autoimmune response. Furthermore, one must bear in mind the clonal expansion of engaged T and B lymphocytes, essential for fighting infection but potentially detrimental in the course of the autoimmune response. Early intervention is indicated not only for this reason but also to prevent irreparable tissue damage. Furthermore, treatment must consider causative and disease-modifying environmental factors. With today's understanding of the immune response, its genetic background, and the importance of environmental factors, it has now become possible to establish a rational treatment regimen for the management of autoimmune diseases.

The second half of this review summarizes our personal experience in treatment of autoimmune diseases over the past 50 years, during which time the number of immunomodulating drugs has substantially increased. The great number of drugs with different mechanisms of action has enriched the overall therapeutic potential. The choice of drugs is crucial for treatment success. We discuss the selection of approriate immunosuppressive drugs and strategies of treatment (early intervention, remission-induction regimens, long-term maintenance treatment), monitoring of drug toxicity, and importance of environmental factors.

A short history of immunology

For the first 50 years of its development our knowledge concerning immunity was restricted to *humoral* immunity, i.e., immunity mediated by serum antibodies. Ten years after the discovery of antisera by Behring and Kitisato, Bordet [5] described a heat labile factor necessary for bacteriolysis to occur with specific antisera. This factor, called complement,

proved to be most relevant both for the defense mechanism and for the pathogenesis of autoimmune diseases. The question as to which cells produce antibodies remained unanswered until 1948 when Fagreus [6] discovered the plasmacellular origin of antibodies. Eight years later Glick et al. [7] discovered the role of the bursa of Fabricius in the development of humoral immunity in the chicken, a breakthrough which led to the term B lymphocytes. In humans B lymphocytes have a function similar to that of B lymphocytes in the chicken, although no certain equivalent specific B cell organ has been found.

In 1945 Chase [8] opened the field of *cellular* immunity with his experiments on the cellular transfer of cutaneous hypersensitivity to tuberculin. This cellular immunity was considered to represent "cell-bound antibodies," similar to serum antibodies but still fixed to the surface of the lymphocyte. Fifteen years later Benacerraf and Gell [9] came to a different conclusion. The results of sophisticated experiments in guinea pigs indicated that the differences in immunological specificity between delayed hypersensitivity and antibody-mediated reactions reflect fundamental differences in the nature of the recognition process. The sensitized cells recognize antigen by a mechanism other than having specific antibody on their surface; an antigen-specific receptor was thus postulated. The role of the *thymus* in the development of immunological capacity, first discovered by Miller [10] in 1961, opened the door to the recognition of the lymphocyte population responsible for cellular immunity (T lymphocytes; see [11]).

The next breakthrough was made in 1972 by Benacerraf and McDevitt [12] with the discovery of the *immune response genes*. The monocytes emerged finally from a "labor status," only passively involved in defense reactions, to a fundamental and primary status, essential for initiating a specific immune response, including autoimmune responses. These immune response genes belong to the major histocompatibility complex (MHC) gene. Five years later Zinkernagel and Doherty [13] described the MHC requirement for T cell mediated lysis of target cells infected with lymphocytic choriomeningitis virus.

With the help of surface markers it became possible to differentiate various T cell subpopulations. A first separation was made with two different anti-leukocyte antibodies, anti-Leu2a and anti-Leu3a. The Leu3a population was subsequently named "T4 helper cells" and the Leu2a population "T8 suppressor/cytotoxic T cells." Further systematic search for additional surface markers of lymphocytes, monocytes, granulocytes, and endothelial cells was carried out using monoclonal antibodies. The ensuing CD (cluster of differentiation) system today encompasses more than 100 different markers. Of special interest to clinical immunology are the CD4 (T4) helper cells which recognize the antigenic target in association with immune response gene markers of the MHC class II type, and CD8 (T8) cells which recognize the antigenic target in association with the MHC class I type. With regard to B lymphocytes, the CD20 marker has become a specific target in the treatment of B cell lymphoma and possibly in the treatment of B cell dependent autoimmune disorders (Rituximab, a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen present on the surface of normal and malignant B lymphocytes).

Research on cytokines, which are involved in cellular development and differentiation, led to another fundamental discovery. In 1986 Mosmann and Coffman [14] discovered two different subsets of T helper (Th helper) cells. Depending on the cytokines involved in the immune response, one of the two types prevails. In general, it has been considered that Th1 and Th2 cells are fundamental for cellular and humoral immunity, respectively. The role of Th1 cytokines in the switch from IgM to complement-fixing IgG antibodies

additionally emphasizes the implication of Th1 immune responses in the generation of cytotoxic antibodies.

Another development relevant to clinical immunology concerns *transplantation immunology*. In 1953 Billingham et al. [15] recognized that antigens responsible for graft rejection reside in leukocytes. Actively acquired tolerance with regard to incompatible skin grafts was obtained by the injection of spleen cells of the donor's A strain into mouse embryos of the recipient's CBA strain in utero. The tolerance thus induced was specific since CBA mice tolerant of A-strain tissues easily reject allografts from other unrelated strains. In 1955 Gorer [16] reported leukoagglutinins developing in the recipients of skin allografts which reacted with cells of the donor strains. These results created interest in the MHC in human leukocytes. The first evidence of different leukocyte groups in humans emerged in 1954 [17]. Much work was subsequently carried on this topic by Dausset [18], leading to the definition of the human leukocyte antigen (HLA) system. It is not surprising that the genes controlling the HLA network are the same as the immune response genes of Benacerraf, which proved to be fundamental in the genetic predisposition to autoimmune diseases.

The immune response

The immune response is the key event in the development of immunity. It involves a most sophisticated interaction between the three pillars of immunity: the antigen-presenting cells (APC), T lymphocytes, and B lymphocytes (Fig. 1). In a first step the antigen in question (usually a protein) must be captured and metabolized into peptides by the APC. If a peptide of the metabolized antigen is recognized by a molecule of the MHC class II

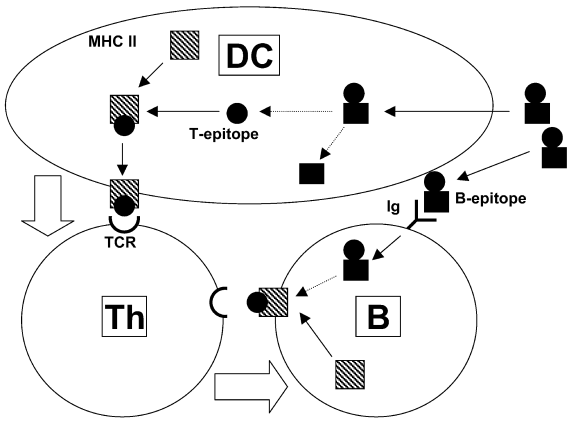

Fig. 1 Schematic representation of antigen recognition in T-dependent B cell activation. The dentritic cells (DC), the antigen presenting cells for the T cell activation, internalize antigen and, by enzymatic processing, generate peptides which bind to MHC class II molecules, i.e. peptides which serve as T-epitopes. Recognition of the MHC-peptide complexes by the T cell antigen receceptors (TCR), together with the costimulatory signals generated by the activated DC, leads to T helper cell activation. The B cell antigen receptors (membrane immunoglobine, Ig) recognize B-epitopes of the antigen. Ig-bound antigen is internalized by the B cell and, like in DC, processed. If a B cell presents an MHC-peptide complex recognized by an activated T helper cell, it receives stimulatory signals for its own activation.

complex, this antigenic peptide in association with MHC class II is transported at the surface of the APC and presented to CD4 T lymphocytes exhibiting the specific receptor for the antigenic peptide-MHC complex. This interaction, with the help of costimulatory molecules (B7 on APC and CD28 on T cells), activates the CD4 T lymphocyte resulting in clonal expansion. B lymphocytes specifically recognize the antigen by the B cell antigen receptor, followed by its internalization and subsequent degradation, as in the case of APC, the B lymphocytes themselves assuming the role of the APC. Such an antigen-specific B lymphocyte must be activated through the interaction with specific CD4 T lymphocytes in order also to enter the clonal expansion cycle. By this mechanism the organism builds up an impressive army of antigen-specific T and B cells in 7–10 days, necessary to fight an infectious agent. The nature of this army depends on a number of factors.

If the initial step of the immune response triggers and favors the production of interleukin (IL) 12 by APC such as dendritic cells (DC), which probably depends on the type of invading pathogen, development of Th0 into Th1 lymphocytes is induced (e.g., in tuberculosis), with only minor development of Th2 lymphocytes. These Th1 cells release interferon (IFN) γ, IL-2, and tumor necrosis factor (TNF) α, resulting in an explosive battle with the aggressive microbe by activating macrophages, promoting cytotoxic CD8 T cell responses, and inducing complement-binding cytotoxic IgG antibodies, at the cost, however, of damage to the surrounding tissue. If IL-12 production by DC is limited in the initial stages of the immune response, IL-4 production (although its cellular origin remains to be determined) favors the development of Th2 immunity. Activated Th2 cells preferentially secrete IL-4, IL-5, and IL-13, leading to the production of non-complement binding IgG and IgE.

The type of Th development also depends on the localization of the immune response. In particular, DC engaged in mucosal immunity play an important role in the regulation of the immune response. The intestine is home to a complex microflora with 300–400 different bacterial species. The mucosal immune system is the primary defense against numerous pathogens, not only in the gastrointestinal tract but also in the respiratory tract. The defense within such a vulnerable environment needs a very fine tuning of CD4 T lymphocytes with limited inflammation. On the one hand, DC in Peyer's patches apparently play an important role in directing Th development in three directions: the development of the Th1 pathway, the development of the Th2 pathway, and the induction of IL-10 mediated mucosal tolerance by regulatory T lymphocytes. On the other hand, IgA specifically secreted in the mucosal immune system permits the neutralization and elimination of antigen (without relevant inflammation).

The distinction between Th1 and Th2 immune responses has contributed greatly to our understanding of immunopathological conditions, but it should be borne in mind that we are dealing with a very dynamic biological system which involves parallel Th1 and Th2 pathways. In the case of childhood idiopathic thrombocytopenic purpura (ITP) Mouzaki et al. [19] have shown this flexibility of the expression pattern of Th1 and Th2 cytokine genes. In their series of patients the Th1 dominant form has been shown to be more resistant to high-dose Ig treatment than the Th2 dominant form.

In the Th1 immune response the CD4 T cells involved in the afferent pathway can also be engaged in the efferent pathway, i.e., in the cellular defense mechanism with MHC class II dependent antimicrobial activity. However, cellular immunity involves mostly cytotoxic CD8 T lymphocytes, i.e., cells with a different T cell receptor (TCR), and with histocompatibility class I dependency. It has been shown that the immune response can also

be initiated through the interaction of CD8 T lymphocytes with antigens presented by MHC class I molecules without the help of CD4 T lymphocytes.

In the field of autoimmunity one deals with similar pathways of the immune response. Some autoimmune phenomena show a MHC class II dependence and others a class I dependence, with the involvement of either CD4 or CD8 T cells.

Autoimmunity

The possibility of misusing the immune system against self-constituents was considered as early as the first few years following the discovery of the humoral immune response. Nevertheless, it was several decades before physicians in the fields of research and clinical medicine accepted this type of pathology, except for extremely rare instances such as certain forms of hemolytic anemia. After the introduction of the antiglobulin test by Coombs et al. [20] in 1945 AIHA finally entered hematology as a recognized autoimmune condition. Other, more frequent conditions, such as RA and MS had to wait many more years before acquiring such recognition.

In 1942 Klemperer et al. [21] published their concept of "collagen disease," encompassing various pathologies all exhibiting significant alterations in the connective tissue, especially fibrinoid necrosis within the walls of arterioles and small arteries but also membranes such as the pleura, pericardium, and joint capsules. Subsequent analysis showed these structures to be rich in gammaglobulins, suggesting a hypersensitivity condition. This concept was stimulating and appealing to clinicians who rapidly accepted this term to designate a specific pathology, rather to the dismay of Klemperer et al. who had intended it to be used in purely topographic terms. However, it proved to be a most constructive concept with growing evidence of autoimmunity as the prevalent factor in the pathogenesis of most of these disorders.

The discovery of the "LE cell phenomenon" in 1948 by Hargraves et al. [22] paved the way for more specific research on SLE, a "prototype" of collagen disease. In particular, the factor responsible for the LE cell was shown to be a gammaglobulin. The fact that this factor is absorbable on isolated cell nuclei, but not on intact cells, revealed its anti-nuclear antibody nature [23]. Since it does not react with intact cells, the LE factor cannot be directly responsible for cellular or tissue damage. The target of the LE cell factor proved to be deoxyribonucleoprotein. Further work led to the detection of antibodies against the constituents of nucleoprotein, DNA [24] and histone [25]. While these antibodies are not directly implicated in the mechanisms of tissue damage, they were later found to be implicated in the "immune complex pathology."

The discovery of the rheumatoid factor (RF) opened another large area of research on RA, the most important of the collagen diseases. In 1940 Waaler [26] reported a factor in human serum which activated the specific agglutination of sheep erythrocytes. In 1948 Rose et al. [27] studied in greater depth this phenomenon of differential agglutination of normal and sensitized sheep erythrocytes by sera of patients with RA. In subsequent studies this RF proved to be an autoantibody against "aggregated" IgG. Clinicians believed the RF to be a causative element for the disease, despite observations that this factor appears, if ever, only months after onset of the disease. It may also be present without RA. Nevertheless, the discovery of the RF proved very stimulating for research on the structure of immunoglobulin. All attempts to find a causative infectious agent for RA failed. After dec-

ades of intense research, RA is now recognized as a chronic inflammatory autoimmune disease, targeting articular structures (collagen II or a degradation product?) on the basis of a complex genetic disorder. In particular, immune response genes of the HLA-DR4 family are involved. Today one recognizes the correlation between various clinical forms of synovitis and disease-associated HLA alleles. Polymorphism of the HLA-DRB1 genes appears to predict clinical manifestations of the disease [28]. In fact, RA is not a distinct, well-defined disease, but rather a spectrum of different subtypes with erosive and nonerosive forms and with various patterns of joint involvement. Similar conclusions can be applied to SLE [29]; various clinical phenotypes occur in both conditions.

Recent decades have witnessed a much wider acceptance of the existence of autoantibodies. The reasons for this are partly technical, partly conceptual. On the technical side the sensitivity of methods for the detection of antibodies has increased tremendously, resulting in an unexpectedly large number of different autoantibodies in conditions thought to be due to autoimmunity. Side by side with the progress in the detection of autoantibodies, the concept of autoimmunity has become increasingly accepted. Indeed, the list of "accepted" autoimmune diseases has grown to more than 40 different entities (see "Appendix"). In most of them we do not know whether the autoantibodies are directly involved in the pathogenesis of the disease, or whether they are the consequence of the immunopathological processes involved. However, the detection of these antibodies has become very useful in diagnostic terms, much less for the assessment of disease activity. Furthermore their absence does not exclude the existence of autoimmune disease.

At first, clinicians thought that the presence of autoantibodies implied their direct role in the disease process. Today we are more critical, especially in view of the question as to whether a disease process, thought to be autoimmune in nature, is mediated by a humoral or a cellular immune process. Answering this question is rendered difficult by the lack of diagnostic T cell markers. Extensive studies with animal models of autoimmune diseases have shown that T cell immune processes are relevant in most organ-specific autoimmune conditions such as diabetes, arthritis, experimental autoimmune encephalitis, and experimental autoimmune thyroiditis. Disease specific T cell markers had to be postulated, and well documented in some animal models, particularly in autoimmune encephalitis.

Mechanisms of cellular and tissue damage in autoimmune diseases

Antibody-mediated conditions

Since antibodies are the product of B lymphocytes, clinicians have been tempted to consider autoantibody-mediated conditions as "pure" B cell pathologies. However, research on the immune response has clearly demonstrated the T cell dependency of B cell expansion. B lymphocytes have the potential to develop antibody-specificity to an almost unlimited extent, including against self-constituents, in part due to the mechanism of somatic hypermutation of the genes responsible for antibody specificity. It is exactly this T cell dependency of B cell activation which protects the organism from the expansion of potentially dangerous autoreactive B cells. Genes responsible for the T cell receptor do not undergo somatic mutations; on the contrary, they remain stable throughout life. Potentially dangerous autoreactive T cells are eliminated by the thymus at the very beginning of development of the immune system. An interesting example of an aberrant thymic function lead-

ing to pathogenic autoantibodies is myasthenia gravis (MY), an antibody-dependent disease which responds in some thymoma patients to the elimination of the thymic tumor. T cells are thus of primary importance also in antibody-mediated pathologies, and must be included in the treatment strategy of autoantibody-mediated conditions.

Antibodies can exhibit pathogenicity by acting directly on target cells (cytotoxic activity) or target structures (e.g., acetylcholine receptor), or they may act indirectly in the form of antigen-antibody complexes. Autoimmune hemolytic anemia, thrombocytopenia and leukopenia are classical examples of antibody-mediated cytotoxic conditions. The pathogenicity of these antibodies depends on various features such as binding strength, complement fixation, and interaction with IgG Fc receptors as well as on the antigenic determinant of the target cell. Thus, patients may develop antibodies to a red cell constituent (usually the basic Rh antigen), initially without hemolytic anemia, with low-affinity antibodies bound to red cells and eventually with increased antibody production of increasing binding strength, finally with excess "free" autoantibodies detectable in the serum of these patients. Such a development reflects the phenomenon of clonal expansion of autoreactive B cells with increasing binding affinity, stressing the conclusion that we are dealing with an autoantigen-driven immune response.

In 1924 in his studies on the Arthus phenomenon, Opie [30] was the first to demonstrate that antibodies indirectly produce inflammatory tissue lesions. Twenty-three years later Germuth and McKinnon [31] demonstrated the role of antigen-antibody complexes in the experimental serum sickness model, which has become the prototype of immune complex disease [32]. At the same time Miescher et al. [33] demonstrated the action of soluble immune complexes on leukocytes and thrombocytes, leading to leuko- and thrombocytopenia of experimental serum sickness. In 1957 Mellors et al. [34] demonstrated Ig deposits in lupus nephritis. Ever since then an immune complex pathology has been believed to play a major part in conditions in which Ig deposits are detected in the affected tissues (SLE, panarteritis nodosa, certain types of cutaneous vasculitis).

T cell mediated pathology

All the serological parameters for the diagnosis of autoimmune disorders belong to humoral immunology, i.e., to the B lymphocyte lineage. With these serological parameters to hand, clinicians were tempted to consider their role in pathogenetic terms. However, with regard to collagen diseases these serological parameters are not directly implicated in the pathogenesis of disease but are more likely the consequence of the immune conflict. This does not exclude their participation in the disease process.

With the lack of diagnostic procedures for the detection of disease-specific T cell receptors T cell pathology has long been neglected. In this respect the study of experimental models that demonstrated the primordial role of cells, for example, collagen-induced arthritis, autoimmune encephalitis, autoimmune thyroiditis, and autoimmune diabetes, has been of great help. These pathologies were attributed to "cellular immunopathology," in contrast to "humoral immunopathology." The histological features of these conditions attributable to cellular immunopathology show a granulomatous pattern with the participation of macrophages, T and B lymphocytes. With the recognition of two different Th cells, Th1 and Th2, the question of cellular immunopathology had to be reassessed. Th1 and Th2 pathways involve different cytokines and different antibody responses, responding differently to immunodulatory treatments. Different treatment regimens must thus be de-

signed for Th1 and Th2 pathologies. In particular, the localized Th1 immune conflict in patients with RA liberates TNF in sufficient concentrations to produce inflammation, pain, and tissue damage.

Similar events occur in other granulomatous inflammatory conditions such as Crohn's disease, ulcerative colitis (UC), and ankylosing spondyloarthritis (AS). In these conditions a positive response to anti-TNF treatment is limited to acute phases of the disease. In the attempt to separate Th1 from Th2 pathology it must be remembered that a given immune response is rarely limited to one of the two pathways. For example, in RA, a preferential Th1 condition, the periarticular pannus contains T and B lymphocytes, while immune complexes are detectable in the synovial fluid.

IL-10 has become an important target of research on the Th1/Th2 balance. The key function of IL-10 is to inhibit cytokine synthesis of Th1 cells, thus exerting a suppressive function on the Th1 pathway. Furthermore, IL-10 has been shown to be involved in the development of mucosal tolerance. A classical example of this role of IL-10 is seen in patients with RA: diminution of IL-10 blood levels reflects disease activity; administration of IL-10 attenuates the disease symptoms, as should happen in a Th1 pathology. Nevertheless, we cannot conclude that conditions with an elevated serum IL-10 level necessarily belong to the Th2 pathology.

In the case of SLE IL-10 levels are increased and are correlated with disease activity. Furthermore, anti-TNF treatment, beneficial in the Th1 pathology of RA, is not of benefit in SLE patients. For these reasons SLE has been considered a disease with a Th2 profile, dominated by antibody-mediated responses [35]. However, the question is not whether we are dealing with a Th1 or Th2 pathology, but rather whether and how Th1 and Th2 pathologies are involved consecutively or simultaneously in the course of SLE as well as other immune complex-mediated disorders.

Causes of autoimmunity

Autoimmune diseases are caused by multiple genetic factors with unknown modes of inheritance that interact with multiple environmental or stochastic factors. No particular genetic or environmental factor is sufficient or necessary to generate disease. Moreover, the mutual interaction between the various factors is extremely complex and unpredictable. Thus the predictive value of any one predisposing mechanism may vary from one patient to another and from one disease to another.

Genetic predisposition

The important role of genetics is clearly demonstrated by epidemiological studies. The recurrence of autoimmune diseases within families and the concordance rate of disease in monozygotic twins indicate a substantial contribution of the shared genetic inheritance in the development of the disorder. A study on 13,299 pairs of twins in Finland, for example, showed a 12% concordance rate for RA in monozygotic twins compared with a 4% concordance rate in dizygotic twins [36]. These results assume even more significance if we consider that the background disease prevalence in nonrelatives was approximately 1%.

Inheritance of susceptibility is generated by the effect of multiple genes which encode fundamental immune system molecules. A series of linkage analyses have been performed

over a period of over 10 years to identify the specific alleles involved. Unfortunately, given the extensive genetic heterogeneity and epistatic interactions among multiple genes it has often been difficult to interpret the results obtained. Moreover, the immune system genes identified are not specific for a given autoimmune disorder and affect susceptibility to many different forms of autoimmunity. It is common in clinical practice to encounter the coexistence of more than one autoimmune disease within the same family, for example, a mother with Hashimoto's thyroiditis and a daughter with SLE.

MHC genes

The genes of the MHC, located on chromosome 6, are highly polymorphic and seem to play a central role in generating susceptibility to different autoimmune disorders. Predisposition to RA, for example, is clearly linked to the presence of the HLA-DR4 genotype, which is found in approximately 70% of white rheumatoid patients, compared to 28% of nonrheumatoid patients, conferring a relative risk for the individual of 3.5 [37]. These data have not been confirmed in other ethnic groups such as native Americans, in whom susceptibility to the disease seems to be correlated with the HLA-DR9 genotype. Among the different HLA-DR4 subtypes the ones associated with the disease are Dw4 and Dw14, encoded by the DRB1*0401 and the DRB1*0404 alleles, respectively. These genes contain a specific sequence called "shared epitope," which is also present in other RA-associated HLA haplotypes such as DR1, DR6, and DR10, while it is absent in the MCH alleles not associated with RA. The "shared epitope" encodes for a specific amino acid motif situated between amino acid 70 and 74 of the class II MHC β_1 chain and constitutes an important binding site for the processed antigen and for the TCR. Therefore this region may be directly implicated in the pathogenesis of RA [38].

Linkage analysis studies regarding class II MHC alleles have also been performed in several other autoimmune disorders such as SLE, juvenile RA, and Sjögren's syndrome with less definite results. Class I MHC molecules have also been associated with autoimmune disorders. The best characterized is HLA-B27. The associated diseases, characterized by the involvement of the spine as a major target of inflammation, are ankylosing spondylitis, reactive arthritis, psoriatic arthritis, and inflammatory bowel disease with spondylitis. The strongest HLA-B27 association is with ankylosing spondylitis, and the allele can be found in about 95% of the patients, independently of ethnic origin. The direct involvement of the HLA-B27 molecule in the pathogenesis of these diseases is highly probable [39].

HLA-B51 is a class I MHC molecule strongly associated with Behçet's syndrome (BS) in patients from the Mediterranean Basin, while this association is not found in other geographical areas. These data underline another important point: for a single disease there may be different predisposing susceptibility alleles in the different ethnic groups [40].

Genetic background not only predisposes to disease but also influences the severity of its clinical expression. In RA, a mild disease course with late or absent erosions and good control with nonaggressive treatment is associated preferentially with HLA-DRB1*01 or with non-RA linked haplotypes. Conversely, early progressive and destructive disease refractory to common therapy is more frequently found in HLA-DRB1*04 patients [41].

Various hypotheses concerning the mechanisms by which the molecules encoded by the MHC genes may lead to an autoimmune response have been proposed. (a) The first focuses on the role of the MHC molecules in shaping the individual antigen-specific T cell

repertoire during thymus ontogenesis. The presence of a particular haplotype may lead, through positive and negative selection processes, to the final survival of autoreactive T lymphocytes, thus predisposing to a future disruption of immunological tolerance. (b) The second hypothesis focuses on MHC molecules as key structures in the selection and presentation of peptides to T lymphocytes by APC during immune responses. The size, shape, and biochemical properties of the peptide cleft located at the distal end of the MHC molecules effectively enhance or restrict selection of antigenic epitopes which must be presented to autoreactive T cells in the APC groove. In animal models mutation experiments that alter the amino acid sequence of this distal end of the MHC peptide have indeed been shown to have an impact on the selection of antigenic epitopes and their presentation to autoreactive T cells. (c) The third hypothesis identifies the MHC molecule itself with the recognized autoantigen. Break of tolerance would occur as a consequence of the resemblance (molecular mimicry) between amino acid sequences of the HLA molecule and antigenic peptides derived from infectious agents. The normal defensive immune response against exogenous peptides would thus turn into an autoimmune response to self-HLA determinants.

The fact that autoimmunity-associated MHC haplotypes are also found with high frequency in individuals who never develop an autoimmune disease reinforces again the concept that several genetic loci are involved, and that environmental factors certainly play an important role.

Complement

The complement system belongs to the innate immune system and is made up of more than 30 serum and membrane components mediating a multifaceted array of functions. The most important are chemotaxis and activation of leukocytes, opsonization and phagocytosis by polymorphonuclear leukocytes and macrophages, B cell activation, and clearance of immune complexes and apoptotic cells.

Inherited abnormalities in complement factors and complement receptors have been described in SLE, RA with vasculitis, mixed cryoglobulinemia, and other systemic vasculitis. Among these diseases, the association between complement deficiencies and lupus is the best characterized and mainly involves the C4, C1q, and C2 components [42]. In general, homozygosis of the defective alleles is rarely seen but confers a higher susceptibility than heterozygosis. C4 homozygous deficiency requires the absence of a functional gene product from both of the C4 genes, C4A and C4B. Indeed, when it occurs, 75% of patients are affected. Conversely, heterozygosity of the C4A null allele is much more common, and it may not only increase susceptibility to SLE but also influence its severity of expression. Of note, the C4A null allele is in strong linkage disequilibrium with HLA-DR3, a class II MHC haplotype associated with the disease, since they are both located in the p21 region of chromosome 6. The importance of the C4A gene product resides in its efficiency in forming links with amino groups particularly relevant for solubilization and opsonization of immune complexes. C2 homozygotic deficiency accounts for a relative risk of developing SLE of 3.5, and it has been associated with prominent cutaneous manifestations, while C1q homozygotic deficiency is correlated with severe glomerulonephritis and typical autoantibodies.

Several hypotheses have been proposed to explain the role of genetic complement deficiencies in the pathogenesis of lupus. The hypothesis focusing on the role of complement

in the solubilization and clearance of immune complexes and apoptotic waste material from tissue and circulation is the most accepted. Marked impairment of this function may result in a cascade of events that ultimately lead to inflammation and tissue destruction [43].

Other genes

Considerable effort has been devoted to identifying other genes capable of predisposing to autoimmune disorders. Results are controversial for most of them. Immunoglobulin genes, TCR genes, cytokine genes, genes involved in apoptosis (e.g., Fas/Fas ligand and bcl-2) have all been considered. Further studies are needed to establish more definite associations.

Viruses and bacteria

The role of infection in the development of autoimmunity has been extensively studied over the years but without any final conclusion being reached. Clinical experience suggests a causal linkage since it is common to detect an infectious episode preceding or accompanying the burst of an autoimmune disease in genetically susceptible patients. Tremendous effort has been put into identifying a viral or bacterial agent that could play a causal role. Unfortunately, to date only few examples of a clear association between infectious agents and autoimmune disorders have been found. Moreover, the mechanisms by which they may initiate the autoimmune process are still under debate. The various theories that have been proposed are based upon the assumption that the host who develops an autoimmune disease possesses autoreactive lymphocytes which have escaped the mechanisms of central and peripheral tolerance by avoiding deletion in the thymus and in the peripheral immune system. The activation and subsequent proliferation of these cells by different triggering mechanisms would lead to the disease [44].

1. Molecular mimicry is the most popular hypothesis. Microbial antigens which share homologies with host antigens drive the initial immune response directed against the infectious agent to an autoimmune reaction against the immunological similar self-peptides. Cross-reaction may involve linear or conformational epitopes so that there is no need for an exact amino acid sequence correspondence between self and environmental peptides. Cellular and/or humoral responses may be involved, and once the autoimmune reaction has been triggered, microbial antigens may or may not still be present.

2. In the bystander activation model the activation of autoreactive T cells, specific for an autoantigen X, occurs during an immune response, triggered, for example, by a viral infection, against the antigen Y. The inflammation generated by the infection may, by tissue destruction, uncover previously sequestered (cryptic) autoantigens which are then presented to quiescent autoreactive T cells with their consequent activation. Alternatively or concomitantly, inflammation may create an appropriate local milieu in which upregulation of MCH molecule expression on APC and promotion of cytokine secretion favors the activation of “bystander” autoreactive T cells.

3. Superantigens are peptides produced by various pathogens and are characterized by the ability to activate T cells by binding to the T cell receptor and the MHC outside the peptide-binding groove. Therefore they are not MHC restricted, do not undergo peptide

APC processing, and do not rely on specific T cell recognition since they bind to nonpolymorphic variable T cell Vβ chains. Superantigens, which usually recognize more than one Vβ, can therefore activate up to 100,000 times more T cells than those activated by specific peptide recognition. Consequently during infection it is possible that among the polyclonal T cells superantigens also activate the autoreactive ones, thus breaking immunological tolerance. This theory has been proposed for various autoimmune diseases, including RA and MS.

The complex issue of whether an infectious agent is able to trigger an autoimmune disease is further complicated in some cases by the difficulty of differentiating between a viral disease with autoimmune features and a truly autoimmune disease triggered by a virus. Autoimmune hepatitis (AIH), for example, has been associated with hepatitis C virus (HCV) following the discovery of a high prevalence of anti-HCV antibodies in patients with anti-liver-kidney microsomal (LKM) 1 autoantibodies, which are associated with type 2 AIH. Moreover, molecular mimicry has been proposed between the HCV genome and cytochrome P450 2D6, which is the major epitope recognized by anti-LKM-1 antibodies. However, not all patients suffering from AIH have anti-HCV antibodies and clinical and serological differences seem to exist between this group of patients and those who have encountered the virus (e.g., age, sex prevalence, anti-LKM antibody titer). Thus, whether this group of patients has an AIH triggered initially by HCV or an HCV hepatitis with autoimmune features remains controversial. Indeed, the answer may have clinical relevance since "pure" AIH may respond to immunosuppressive treatment and may be exacerbated by IFN-α therapy, while virus-associated autoimmunity may benefit from IFN-associated antiviral treatment [44].

HCV has also been associated with other autoimmune diseases such as type II mixed cryoglobulinemia, a multifaceted vasculitic disorder characterized by the presence of monoclonal IgM antibodies with RF activity that precipitate IgG at cold temperatures and redissolve on rewarming. The majority of patients are positive for anti-HCV antibodies, HCV RNA, and HCV core proteins. The virus is thought to be responsible for the production of RF and other autoantibodies by B cell activation, formation of immune complexes responsible for vasculitic manifestations (as demonstrated by the presence of HCV RNA in the lesions and in the cryoprecipitate), and the generation of the lymphoproliferative disorders associated with the disease by chronic stimulus of B cell expansion. Moreover, the pathogenetic importance of the virus is also demonstrated by the clinical response obtained with agents that inhibit viral replication and by the fact that remission of vasculitic lesions parallels the disappearance of HVC RNA in the cryoprecipitate [45].

HCV has been associated with other autoimmune diseases such as autoimmune thyroiditis, RA and sicca syndrome, but in these cases the data seem to be of less certain significance. The role of Epstein-Barr virus (EBV) as an initial triggering agent in autoimmune diseases has been investigated in RA and SLE, and recently an interesting work has shown evidence of cross-reactive responses between self-HLA epitopes and EBV proteins in juvenile RA. Healthy subjects showed cytotoxic responses to EBV derived peptides and not to homologous sequences of self-HLA-derived peptides while juvenile RA patients responded to both. The authors hypothesized that molecular mimicry is responsible for the conversion from a normally self-limited response to EBV infection to a perpetuating autoimmune response in juvenile RA patients with class II self-MHC molecules serving as autoantigens in a disease- and allele-specific manner [46]. *Proteus mirabilis* has been linked to RA because of homologous sequences between hemolysin and the "shared epitope" of

the class II MHC molecule which is responsible for the genetic susceptibility to the disease. Antibodies against the bacterial enzyme and against the self-HLA peptides have been detected in patients. In ankylosing spondylitis amino acid sequence mimicry between HLA-B27 and *Klebsiella pneumoniae* nitrogenase reductase and pullunanase enzymes has been investigated [47]. These are merely a few examples among the myriad reports on this subject. Taken together, the data published so far certainly support a role for viruses and bacteria in the pathogenesis of autoimmune disorders. Further studies are warranted to better elucidate the pathogenetic mechanisms involved and whether there is a specificity of events for a single micro-organism and in a single disease.

Autoantigens

Autoantigens are a crucial issue in autoimmune disease pathogenesis. Several studies have been carried out to identify the sequences and the molecules against which the immune response is directed. The main question is whether the autoantigens identified so far are really involved in the initiation of autoimmune reactions or are only a secondary phenomenon. However, they will certainly help us to better understand the immunological processes involved. We discuss below some of the possible mechanisms [48].

Sequestered autoantigens

This theory is based upon the presence of sites such as the eye and testis in which autoantigens are hidden from the immune system by anatomical barriers. Therefore during the development of tolerance against self-molecules (e.g., negative selection of T cells in the thymus), the unavailability of these antigens would prevent deletion or anergy of the specific autoreactive cells. Following tissue damage the previously sequestered antigens would be released and the immune system would react against them as it would with foreign substances encountered for the first time. Orchitis after vasectomy and sympathetic ophthalmia after eye injury are two examples of organ-specific autoimmune disorders which are thought to be generated through these mechanisms.

Cryptic autoantigens

Another interesting theory relates autoimmunity to the release of antigens “sequestered” within a molecule rather than behind anatomical barriers. Every protein is thought to have a limited number of dominant determinants against which tolerance develops. “Hidden inside the molecule” there are a number of subdominant/cryptic antigens which do not normally induce immune responses. Thus a sort of hierarchy in the immunogenicity of determinants within a molecule seems to exist. Infectious micro-organisms or other noxious agents may initiate autoimmune responses by stimulating the presentation of these cryptic determinants either by molecular mimicry mechanisms or by cytokine-induced upregulation of MHC molecules on APC. Moreover, a response to a cryptic antigen may induce additional responses to other determinants within the same molecule or in other molecules by a phenomenon named “epitope spreading,” thus amplifying the autoimmune reaction.

Modified autoantigens

Another potential source of autoantigens is posttranslational modification of self-proteins during several physiological and pathological processes. The "remnant epitopes generate autoimmunity" model focuses on the proteolysis of local proteins occurring during acute inflammation by extracellular proteinases. According to this theory, the resulting cleaved peptides (remnant epitopes) are presented to T cells by APC generating autoimmune responses. In RA, for example, neutrophils attracted into the synovium during the active phases of the disease produce matrix metalloproteinases able to degrade the extracellular matrix. The cleavage of local proteins would generate neo-autoantigens. Among matrix metalloproteinases, gelatinase B, which is increased in the joints of RA patients, cleaves type II collagen in specific sites with the subsequent release of immunodominant epitopes. Gelatinase B is also increased in the cerebrospinal fluid of MS patients and cleaves human myelin basic protein into immunodominant peptides recognized by the immune system [49].

Proteolysis and several other posttranslational modifications of self-molecules occur during apoptosis, as explained in detail below.

Apoptosis

Apoptosis is a form of cell death which occurs in several physiological processes to control excessive cell proliferation and to eliminate damaged or unwanted cells. Therefore it is involved in limitation of immune responses after successful infection control, deletion of self-reactive T and B lymphocytes during central and peripheral tolerance processes, regulation of cell proliferation during embryogenesis, and tissue healing. Apoptosis can be triggered by various noxious stimuli or by a deficit of survival signals and consists of a multistep process in which the apoptotic cell goes through morphological and biochemical changes. Clearance of dying cells is rapidly performed through engulfment phagocytosis by macrophages and semiprofessional phagocytes (e.g., kidney mesangial cells) and is not associated with release of proinflammatory mediators.

Dysregulation of apoptosis is thought to play an important role in the generation of autoimmunity by different mechanisms, as demonstrated by experimental evidence:

1. Mutations of genes involved in the induction of apoptosis (Fas and Fas ligand) result in a rare autoimmune-lymphoproliferative disorder termed Canale-Smith syndrome and characterized by an alteration in lymphocyte survival. Patients with this syndrome present an abnormal elevation in undifferentiated "double negative T cells," chronic lymphadenopathy, splenomegaly and various manifestations of autoimmunity such as hemolytic anemia and thrombocytopenia [50].

2. During cell apoptosis many intracellular autoantigens such as nucleosomal DNA, Ro, La, U1-snRNP, and other molecules recognized by autoantibodies in autoimmune diseases are relocalized and clustered on the cell surface in apoptotic blebs and bodies, becoming available for recognition by autoantibodies. Moreover, these molecules go through posttranslational modifications by caspase cleavage, deubiquitination, phosphorylation, transglutamination, and other as yet unidentified pathways, with potential creation of neoepitopes to which T and B cells have not been rendered tolerant [51].

3. Defective clearance of apoptotic cells has been found in patients with SLE. This may be due to overabundant apoptotic material which exceeds the phagocytic capacity of the

reticuloendothelial system (e.g., after ultraviolet exposure or infection) or to intrinsic defects in the mechanisms of engulfment phagocytosis. The latter may be associated, for example, with C1q complement deficiency since C1q acts as an opsonizing receptor favoring phagocytosis. Abnormal accumulation of apoptotic cells may represent a continuous source of highly concentrated and altered autoantigenic material [52].

4. Moreover, the unremoved cells may enter other dying processes such as secondary necrosis, which leads to the further generation of neoepitopes through new proteolytic processes which stimulate proinflammatory removal pathways that create an appropriate milieu for the development of autoimmune responses (e.g., release of cytokines and chemokines, expression of costimulatory signals, activation of DC, exposure of adhesion molecules) [53].

The cited mechanisms may not be mutually exclusive and probably interact in a multifactorial manner.

Triggering factors

There are several agents which are thought to have an impact on the course and exacerbation of autoimmune diseases. This issue is of major importance since patients often wonder whether changes in their life-style would contribute to a better management of their illness. Intercurrent infections, psychic and physical stress, diet, ultraviolet radiation, hormones, and cigarette smoking are among the triggering factors that have gained increasing scientific attention.

Intercurrent infections

We discuss above the potential role of viruses and bacteria in the development of autoimmune disorders and stress that to date no conclusive data have been presented definitely implicating a specific micro-organism. Another interesting issue is the effect that ubiquitous infectious agents determining common intercurrent infections may have on the course of the disease. It is not uncommon to encounter relapse of an autoimmune disease following upper respiratory or gastrointestinal infections. In this respect some autoimmune diseases are more susceptible than others. Berger's nephropathy with macroscopic hematuria, for example, often follows tonsillitis. Studies have suggested that stimulation of the mucosal immune system by infection leads to an increased IgA production and formation of immune complexes responsible for renal damage [54]. Other studies have proposed Gram-negative bacterial lipopolysaccharide, which is a polyclonal B cell activator and IL-1 and TNF-α synthesis inducer, as another potential trigger for the reactivation of autoimmune responses, especially in some autoimmune disorders such as Crohn's disease [55].

Stress

It is generally accepted that stress can influence immune responses. Clinicians can frequently trace a stressful event preceding the burst of an autoimmune disease or altering its course by aggravating or precipitating a flare. Several studies conducted on this issue have associated disease onset and relapse to specific events such as marriage or divorce, moving

house, pregnancy, business loss, and physical traumatic events such as a surgical intervention. Stress can also be generated by the illness itself. Patients affected by an autoimmune disease have to accept the fact that they suffer from a chronic illness, that they need to be under treatment and to face periodic laboratory and clinical controls throughout their lifetime, and that they must learn to cope with potential disability. Patients unable to cope emotionally with their disease often present a worst prognosis with poorer response to treatment [56]. The exact mechanisms by which stress influences the immune system are not fully understood. The organic reaction to stress involves peptide hormones, some of which, such as β-endorphin and substance P, have immunoregulatory properties and might be expected to modulate inflammatory reactions. Moreover, stress has been associated with a decreased immune lymphocyte responsiveness. Unfortunately, the intangible nature of stress often renders difficult the investigation, in controlled experimental trials, of its multiple consequences in immune processes.

Diet

The role of diet in influencing immune system function has long been a matter of debate. Most of the available studies come from autoimmune disease animal models and seem to support the hypothesis that moderate calorie restriction and low protein and fat intake can decrease morbidity and mortality. Beneficial effects of polyunsaturated fatty acids such as ω-3 fatty acid fish oil and linseed oil are well established. Results of studies on SLE and RA patients show serological and clinical improvement with a diet that is enriched in polyunsaturated fatty acids. Underlying mechanisms of action seem to involve modulation of inflammatory eicosanoids and cytokine levels. Moreover, ω-3 fatty acids appear to upregulate apoptosis of autoreactive T lymphocyte precursors in the thymus [57]. Other possible beneficial dietary compounds include vitamin E, vitamin A, and selenium [58].

Ultraviolet radiation

Sun exposure does not have the same effect on all autoimmune diseases. On some it has a detrimental effect, on others a beneficial one, but in the majority it does not seem to affect the disease course. Photosensitivity is part of the major diagnostic criteria in SLE, and ultraviolet radiation is able to induce cutaneous and visceral flares. Extensive work has been performed by researchers to determine the underlying mechanisms. It has been demonstrated that ultraviolet light induces apoptosis of keratinocytes. As a consequence of this several intracellular lupus autoantigens such as Ro, La, snRNP, and Sm, are modified and exposed on the dying cell surface and become available to be recognized by autoantibodies [59]. In addition to autoantigen overexpression, other immunological abnormalities might be involved in the formation of SLE cutaneous lesions. In fact, repeated or persistent ultraviolet exposure initiates a cascade of proinflammatory events in the epidermis and the dermis, with the release of IL-1, TNF-α and several other chemokines and cytokines which create the appropriate milieu for adhesion molecule induction, activation and migration of leukocytes, initiation of immune responses, and production of autoantibodies. In this context, dermal inflammation is followed by epidermal cytolysis through T cell cytotoxicity, antibody-dependent cell-mediated cytotoxicity, direct cytokine effects, and complement-mediated lysis [60]. Conversely, daily sun exposure has beneficial effects on another autoimmune disease, psoriasis. Ultraviolet radiation treatment significantly reduces the Psoria-

sis Area and Severity Index score, and interesting work has demonstrated that ultraviolet A plus methoxypsoralen induces a shift from a Th1 to a Th2 cytokine response [61]. This is particularly important since psoriasis is a Th1 cell driven disease, and changes in cytokine production may influence disease activity.

Cigarette smoking

There is increasing evidence that cigarette smoking is an important risk factor affecting the incidence and severity of many autoimmune diseases such as systemic sclerosis, RA, lupus nephritis, Graves' disease, and Goodpasture's syndrome. RA smokers, for example, tend to have higher levels of RFs, worse Larsen scores on radiography, worse functional disability, and an increased incidence of extra-articular manifestations such as rheumatoid nodules, interstitial lung disease, and vasculitis, probably partly mediated by a direct effect of smoke on blood vessels and angiogenesis [62]. Smoke has various effects on the immune system: it produces abnormalities in T lymphocyte function, reduces natural killer cells, depresses humoral and cell-mediated immunity, and inhibits prostaglandin synthesis. It also has direct effects on the endothelium, promoting leukocyte and platelet adhesion to endothelial cells and impairment of endothelial cell-dependent vasodilatation by alteration in nitric oxide biosynthesis and action. Furthermore, cigarette smoke produces several toxic components, including reactive oxygen and nitrogen species that cause oxidative and nitrosative stress, a phenomenon directly implicated in the pathogenesis of some autoimmune diseases such as systemic sclerosis and Raynaud's disease [63].

Hormones

An effect of sexual hormones on the development and course of autoimmune diseases is suggested by various clinical evidence. Many autoimmune disorders, for example, display an unbalanced sex ratio prevalence, the majority showing a definite preponderance of women; in SLE the female to male ratio is 9:1, in RA 4:1, and in systemic sclerosis 3:1, although in the younger age group it may reach 7:1. Conversely, ankylosing spondylitis shows a sex predilection for men of 5:1 or higher, while gender does not seem to affect the incidence of Wegener's granulomatosis, a systemic autoimmune vasculitis with a sex ratio of 1:1. Physiological hormonal fluctuations occurring during life also seem to influence the development of some autoimmune disorders (e.g., SLE onset occurs mostly during women's fertile years, relatively sparing prepuberty and postmenopausal periods). Moreover, pregnancy and the postpartum period are the times of highest risk for variations in the disease course (e.g., RA tends to ameliorate during pregnancy and relapse during postpartum), imposing a higher frequency of laboratory and clinical controls on patients and an appropriate adaptation of treatment.

The mechanisms by which sexual hormones affect disease development and activity are not fully understood. In vitro experiments suggest that estrogens stimulate humoral immunity, inducing differentiation and activation of B cells, promoting the production of autoantibodies, and increasing Th2 cytokine production. Moreover, the effects of estrogen administration and androgen deficiency in murine models of SLE confirm the important role of these hormones in modulating disease expression and prognosis. The effect of exogenous estrogens has not been studied in all autoimmune disorders, but recent reports indicate a certain safety of their use in selected patients [64]. The issue is of major impor-

tance when considering its use for oral contraception, particularly important during teratogen treatment and to control cyclic hormonal diseases, and its employment as hormone replacement therapy in the postmenopausal period mainly to preserve bone mineral density and prevent cardiovascular diseases. Most available data come from studies in SLE, where recent studies have not confirmed the early retrospective studies which linked the administration of exogenous estrogens with an increased risk of flares. The issue remains controversial, although the current trend is to allow combination low-dose estrogen-progesterone oral contraceptives in SLE patients in the absence of major organ involvement, such as in active lupus nephritis, and in the absence of anticardiolipin antibodies which carry a higher risk of thromboembolic complications. On the other hand, hormone replacement therapy after menopause, which utilizes naturally conjugated equine estrogens with a much lower estrogenic potency, does not seem to increase lupus activity, and preliminary data from a large multicenter, double-blind, placebo-controlled trial (the Safety of Estrogens in Lupus Erythematosus-National Assessment study) suggest significant health benefits for these patients [65]. Hormone replacement therapy has also been reported to be safe for RA [66].

Drugs used in the treatment of autoimmune diseases

Autoimmune diseases comprise a multitude of conditions involving both disease-specific and non-disease-specific genetic factors and a plethora of disease-triggering and disease-modifying environmental factors, varying from patient to patient and variable during the course of the disease. Further complicating the situation is the fact that response to drugs often varies from patient to patient. The latter complication is due in part to pharmacogenetics and in part to environmental factors, including alcohol consumption and tobacco. Thus, one patient with RA may respond well to methotrexate (MTX) but not to leflunomide (Lef) while another responds to Lef but not to MTX. Faced with such a "protean" clinical pathology which differs from patient to patient and is variable over time, one cannot expect a rigid treatment regimen to be effective in autoimmune diseases. Rather, such conditions require an almost custom-made approach, with treatment tailored according to variations in disease activity. This is possible only with the patient's active participation. Treatment must be a "joint-venture" and will be a long partnership between patient and physician since most autoimmune diseases tend to have a very chronic course. The primary aim of treatment is long-term disease control with a good quality of life and minimum drug toxicity.

Fifty years ago, the range of drugs available to the physician for treating autoimmune diseases was very limited, cortisone being the only efficient drug. The number of immunomodulating compounds has gradually increased and today there are more than 20 different agents. This development now permits the clinician to administer individually tailored treatment, combining drugs with different mechanisms of action in an attempt to increase the overall therapeutic efficacy without concomitant increase in global toxicity.

Corticosteroids

Glucocorticosteroids were long the cornerstone of immunosuppressive treatment. However, side effects, particularly in patients on long-term medication, greatly outweighed the

beneficial effects. These side effects include Cushing's syndrome, osteoporosis and aseptic bone necrosis, cataract formation, increased susceptibility to infections, linear growth arrest in children, and suppression of the hypothalamo-pituitary-adrenal axis leading to corticodependence. To maintain a tolerable level of side effects oral long-acting steroids should be avoided. Short-acting steroids (prednisone, Pred; fluocortolone) given no more than 5 days per week do not suppress the hypothalamo-pituitary-adrenal axis [67]. In children three steroid-free days per week (e.g., Tuesday, Thursday, Sunday) are necessary to permit normal linear growth. In acute situations intravenous pulse therapy has been shown to be more efficacious (125 mg methylprednisolone, MP, or 40 mg dexamethasone, DXM) than daily oral administration of high doses of cortisone. Oral steroids should be administered in a single morning dose.

6-Mercaptopurine

6-Mercaptopurine (6-MP) has now been in use for over 40 years for the treatment of patients suffering from autoimmune diseases. Azathioprine (AZA), an imidazole–6-MP compound, was developed with the aim of only slowly releasing the active part, 6 -MP, in the liver. Some patients tolerate AZA (100 mg/day) better while others tolerate 6-MP (50 mg/day) better, especially in the case of liver involvement. Toxicity, very variable from patient to patient, involves the bone marrow and the gastrointestinal tract. Given daily for long periods, the drug becomes less efficient, probably because of metabolic adjustment. These patients may finally tolerate doses of 6-MP (e.g., 150 mg/day) which would produce bone marrow depression if given as an initial dose in previously untreated patients. Not only the immunosuppressive activity, but also the myelotoxicity diminishes with time. This loss of activity can be avoided if AZA or 6-MP is given during alternate weeks or months.

Methotrexate

In 1963 we investigated the efficacy of the two antimetabolites 6-MP and MTX in treating experimental autoimmune thyroiditis in guinea pigs [68]. The results of these investigations showed that these antimetabolites have a therapeutic effect once the disease is already established. MTX appeared more effective than 6-MP in terms of the toxicity/efficacy ratio. In febrile SLE patients 15 mg MTX given intravenously or intramuscularly produced a rapid normalization of body temperature and clinical improvement for a period of 5–7 days [69]. Oral MTX does not have this dramatic effect. If MTX is given once per week, late toxicity appears after 6–8 weeks (mucosal irritation/soreness). If protected by folic acid (5 mg on 3 consecutive days after MTX injection) and with at least a 1-week pause per month, MTX is well tolerated for long periods by most patients.

Cyclophosphamide

Cyclophosphamide (Cy) is a potent lymphocytotoxic alkylating agent that has been used for many years as an immunosuppressant. It is undoubtedly a very powerful lymphocytotoxic agent, but also entails substantial side effects, particularly when administered orally. After the occurrence of leukemia in 1979 and 1980 in two of our patients on long-term

oral Cy we ceased oral administration of this drug. Since 1980 none of more than 400 patients on intravenous Cy pulse therapy has developed leukemia or any other malignancy. Because of its cancerogenicity Cy is administered only as intravenous pulses, in association with other immunosuppressive drugs, at a maximum dose of 12 pulses of 500 mg per year. Adequate hydration is essential to avoid Cy toxicity during intravenous administration: 1.5–2.0 l tea or water plus 25 mg furosemide to guarantee rapid elimination of Cy and to avoid irritation of the bladder mucosa.

Cyclosporine A

The introduction of the noncytotoxic immunosuppressive agent cyclosporine A (CsA) in 1982 greatly increased the immunomodulating potential of treatment. CsA forms a complex with cyclophyllin in T lymphocytes which binds to calcineurin, leading to inhibition of the production of IL-2 and IL-2 receptor. As a consequence secondary B cell functions are also decreased. Renal toxicity has delayed the general acceptance of CsA which was initially used in monotherapy with nephrotoxic doses (8–12 mg/kg per day). Fortunately, in our patients we limited the dose from the very beginning to less than 5 mg/kg per day. In combination with low doses of cortisone and MTX [70, 71] this dose of CsA showed no or only insignificant signs of toxicity in 192 kidney biopsy specimens. Furthermore, long-term medication (15–20 years) did not lead to impairment of kidney function. Today the daily dose of CsA is kept below 4.5 mg/kg lean bodyweight per day. Abrupt discontinuation of CsA medication is likely to produce a resurgence in disease activity. Accordingly, CsA doses should be reduced only gradually once the patient is in remission. Side effects of CsA include hirsutism, gingival hyperplasia, water retention, hypertension, and increased levels of cholesterol and uric acid. Among these side effects gingival hyperplasia has precluded the use of CsA in 2–3% of our patients, for whom tacrolimus, another calcineurin antagonist, can be used as a substitute for CsA, at a daily dose of 0.1 mg/kg.

Tacrolimus

This macrolide acts as a calcineurin antagonist, similarly to CsA. Its rate-limiting side effect is nephrotoxicity. Hypertension, alopecia, leukopenia, and neurological disturbances are dose dependent. For autoimmune diseases the initial daily dose should not exceed 0.15 mg/kg and that in long-term medication 0.1 mg/kg.

Everolimus

A structural analogue of sirolimus, everolimus binds to the cytosolic immunophyllin FKBP 12. It inhibits growth factor driven cell proliferation at a later stage in the proliferation process than CsA and tacrolimus. Everolimus can therefore be used in association with CsA or tacrolimus at a dose of 1 mg twice daily, increasing the immunosuppressive action without concomitant increase in drug toxicity.

Mycophenolate mofetil

The active metabolite of mycophenolate mofetil (MM), mycophenolic acid, inhibits the de novo pathway of purine synthesis in T and B lymphocytes, which rely almost entirely on this de novo pathway. MM has been reported to be more efficient than 6-MP, but it is also less well tolerated by patients at the recommended daily dose of 2 g. Side reactions involve the gastrointestinal system, myelotoxicity with neutropenia, and occasionally elevation of transaminases.

Hydroxychloroquine

Hydroxychloroquine (HCQ) acts on lysosomal activity which inhibits the function of lymphocytes and macrophages differently from other immunosuppressive agents. Side effects are relatively mild. The development of corneal deposits does not affect vision. Retinopathy, mostly observed with the use of chloroquine, rarely occurs with HCQ (for this reason chloroquine is no longer used as an immunosuppressive drug). Gastrointestinal side effects are dose dependent.

Leflunomide

Lef inhibits the synthesis of pyrimidine, leading to apoptosis and cell cycle arrest. It affects the synthesis of cytokines by increasing the mRNA level of IL-10. It also has an anti-inflammatory effect by inhibiting cyclooxygenase-2 activity. Lef is well tolerated by most patients. In the event of intestinal intolerance Lef should be stopped immediately without waiting for laboratory results (transaminases). Lef is teratogenic and remains in the system for a very long time (more than 1 year). Should a patient in remission wish to become pregnant, Lef can be "washed out" of the tissues with cholestiramide (8 g t.i.d. for 11 days).

D-Penicillamine

D-Penicillamine (DP) was introduced for the treatment of RA on account of its capacity to break up S-S bridges, thus degrading IgM molecules. However, this effect requires concentrations of DP which are unattainable in patients. The immunosuppressive effect of DP is presumably a consequence of its action on surface receptor sulfhydryl groups of T lymphocytes and monocytes. DP was originally used at very high doses (900–1200 mg per day) which produced excessive side reactions in patients. With drug-combination therapy a daily dose of 150 mg (at most 300 mg) produces satisfactory results in many patients with few side effects (gastrointestinal intolerance, diminution of taste).

Thalidomide

Thal was introduced in 1956 as a sedative but was withdrawn 5 years later on account of its teratogenicity. However, when used to sedate leprosy patients, Thal was shown to produce a dramatic improvement in erythema nodosum leprosum. In subsequent years Thal

has been reported to improve various immunopathological conditions such as discoid lupus erythematosus, graft-versus-host disease, and erosive lichen planus. The mechanism of action in these conditions is not fully understood. Thal appears to have anti-inflammatory and immunosuppressive properties, which become most evident with large, toxic doses of up to 800 mg/day. More recently Thal has been shown to suppress TNF-α production and to modify the expression of TNF-α induced adhesion molecules on endothelial cells and human leukocytes. Another effect involves inhibition of angiogenesis, which could explain its action on certain tumors. In addition to its sedative and teratogenic effect, another side effect of Thal is peripheral neuropathy, which has become the main rate-limiting factor. Thal is not suitable for monotherapy but is useful in drug-combination therapy at a daily dose of 50–200 mg (maximum).

Sulfasalazine

Sulfasalazine (SSZ) is split in the colon into sulfapyridine and 5-aminosalicylic acid, exerting a local immunomodulatory and anti-inflammatory action.

FTY 720

FTY 720 is a sphingosid analogue which alters lymphocyte recirculation and homing, most probably by modulating glycoprotein-coupled receptors on lymphocytes.

Etanercept

Etanercept is a dimeric fusion protein consisting of the extracellular ligand-binding portion of the human 75-kDa TNF receptor linked to the Fc portion of human IgG1. Administered as a subcutaneous injection, it has a short median half-life of 115 h, which permits a great flexibility in the management of autoimmune disease in which TNF plays a relevant role in the pathogenesis of tissue damage. Etanercept is well tolerated at the recommended dose of 25 mg, given twice weekly as a subcutaneous injection. As with other TNF blocking agents, etanercept increases the risk of infection. A strongly positive Tbc skin test excludes the use of this drug. In this respect the short median half-life is an advantage, leaving enough time for the action of TNF in the event of intercurrent infections.

Infliximab

Infliximab is a chimeric murine/human monoclonal antibody to human TNF-α, given as a slow intravenous infusion. With a median half-life of several weeks, infusions of this drug can be given at intervals of 2–8 weeks (recommended regimen: 3 mg/kg on day 1, a further 3 mg/kg 2 weeks and 6 weeks later, subsequently 3 mg/kg every 8 weeks). The long median half-life of infliximab may be considered as an advantage, but restricts flexibility in the adjustment of treatment to variations in disease activity. The long half-life also increases susceptibility to infections. In particular, if there are signs of tuberculosis activity, infliximab is contraindicated. A Tbc skin test should be done before initiating treatment with infliximab.

Adalimumab

Adalimumab is a recombinant human IgG1 monoclonal antibody specific for human TNF-α. With a median half-life of 2 weeks it is administered subcutaneously at a dose of 40 mg every other week. It entails an increased susceptibility to infections, especially tuberculosis. As with other anti-TNF agents, close supervision of patients for intercurrent infection is necessary. A strongly positive Tbc skin test excludes the use of adalimumab.

Rituximab

Rituximab is a chimeric murine/human monoclonal antibody directed against the CD20 antigen of B lymphocytes. It is administered as a slow intravenous infusion at weekly intervals. Serious side reactions may occur during intravenous infusion. Rituximab may be useful in remission induction of antibody-mediated immunopathology.

Daclizumab

Daclizumab is a chimeric (90% human, 10% murine) IgG1 monoclonal antibody directed against the IL-2 receptor that is expressed on activated lymphocytes. It may be useful in a remission-induction regimen of Th1-mediated autoimmune diseases at a dose of 1 mg/kg at 14-day intervals, for a total of 4 doses.

Efalizumab

Efalizumab is a humanized monoclonal antibody acting on the adhesion molecule lymphocyte function associated antigen 1 (CD11a) which is important for the trafficking of activated lymphocytes from the circulating blood into tissue. It also interferes with the interaction of activated lymphocytes with APC. Intercellular adhesion molecule 1 serves as ligand for lymphocyte function associated antigen 1. This agent has been shown to be effective for the treatment of psoriasis at a dose of 0.3 mg/kg, given as a weekly infusion over a 7-week period. It may be useful in drug-combination therapy for the prevention of graft rejection.

Anakinra

Anakinra is a recombinant, nonglycolysated form of the human IL-1 receptor antagonist. It is well tolerated at daily subcutaneous doses of 1 mg/kg and appears to be efficient in the treatment of Th1-mediated autoimmune diseases, especially in combination with MTX. TNF blocking agents should not be used in conjunction with anakinra.

Glatirameracetate

Glatirameracetate is a synthetic polypeptide salt composed of four amino acids in variable composition (molecular weight: 4.7–10 kDa), simulating degradation products of the basic

myelin protein. Administered subcutaneously, it is thought to act as a tolerogenic agent in the treatment of MS.

Colchicine

Colchicine is an alkaloid that acts on microtubular structures, intracellular in the case of mitotic metaphase, extracellular in cell to cell interaction. It has shown its efficacy in familial Mediterranean fever, gout, BS, and in certain connective tissue diseases with fever. It also delays progression of liver cirrhosis in patients with primary biliary cirrhosis (PBC). Finally, it diminishes the rate of sequestration of autoantibody-coated red cells.

Mitoxantrone

Mitoxantrone is a cytotoxic derivative of anthraquinone. It is occasionally used in the immunosuppressive treatment of MS.

Dapsone

Dapsone (diamino-diphenyl sulfone) has been introduced for the treatment of leprosy. As with Thal, it is active on erythema nodosum leprosum, probably through its immunosuppressive activity.

Levamisol

Levamisol has been shown to increase T cell activity in animals with a diminished function of these cells. Because of this "thymomimetic" activity, levamisol has been used in patients with RA with remarkable results. Not only did the patients feel better after 2–3 weeks of treatment (100 mg orally twice per week), with diminished symptoms and signs of arthritis, but laboratory parameters also improved. Unfortunately, the occurrence of allergic reactions in over 20% of patients has led to the almost total elimination of this drug from standard treatment protocols. Research to find drugs with a similar thymomimetic function has been unsuccessful so far. Levamisol remains a candidate for early intervention in selected patients, well informed of the risk of allergic reactions (the most common being a flulike reaction; agranulocytosis and erythematous skin eruptions occur in fewer than 2% of patients). If a patient does not develop an allergic reaction after 4 months of treatment, the drug may be continued for long periods (years), at a maintenance dose of 100–150 mg as a single weekly dose in the treatment of RA. A complete blood count should be performed every 2 weeks during the first 4 months.

Danazol

Danazol inhibits the synthesis of gonadotrophines and increases the production of C1 esterase inhibitor. It has proven useful in the management of patients with angioneurotic edema and to a lesser extent in autoimmune thrombocytopenia.

Basic strategies of treatment

All autoimmune conditions have in common an ongoing immune response directed towards disease-specific autoantigens, based on a genetic predisposition. They are subject to many disease-triggering and disease-modifying environmental factors. As in all immune responses, specific T and B lymphocytes undergo clonal expansion, which is beneficial in the case of infection, detrimental in autoimmune conditions (see below). APC are equally involved in both the afferent and efferent pathways of the immune response. Accordingly, these cells are targeted in all basic therapeutic approaches to autoimmune disorders.

For many decades cortisone was considered to be the most powerful agent for blocking the pathogenic immune process. However, it gradually became evident that prolonged treatment with cortisone does not provide lasting cure and also produces adverse events which increase with time (e.g., Cushingoid changes, fluid retention, hypertension, osteoporosis, linear growth arrest in children). Furthermore, cortisone does not really halt the progression of the basic disease process. For these reasons steroid monotherapy for very mild forms of autoimmune disease conditions should be limited to no more than 4×5 mg Pred per week.

Today, with more than 20 different immunosuppressive and immunomodulating agents available, the era of cortisone dominance is over. However, it must be stressed that none of these drugs used as monotherapy is capable of controlling relevant disease activity. A combination of drugs, each with a specific target on the immune response but with different side reactions, and each administered at a dose below its toxic level, has thus become the basis of all treatment regimens.

The earlier the pathological immune response is halted, the faster one may expect to see a therapeutic response. With "symptomatic" treatment only, the autoimmune disease process is allowed to continue without being recognized as such. This applies not only to treatment with NSAIDs, but also to steroids. In such situations patients are under the illusion that they are being cured. This is true for all autoimmune disease, but particularly for RA. Patients with RA do not understand why they should not continue with NSAIDs, which give them so much relief. As if "anesthetized," they do not realize that the joint damage is continuing unhindered. When well informed, they readily accept to limit NSAIDs to a minimum. However, they expect and request from the physician an effective intervention with visible results within weeks, not months. Without early intervention the disease process continues, with progressive clonal expansion of autoaggressive lymphocytes rendering immune intervention increasingly difficult.

Adjustment of treatment intensity to disease activity

At diagnosis it is not possible to predict exactly how the disease will evolve. Without early and appropriate therapy a seemingly "mild" disease process may lead to irreparable tissue damage. For this reason and in view of the danger of clonal expansion of autoreactive lymphocytes, early and vigorous immunosuppression is advisable, with subsequent reduction in treatment intensity as disease activity diminishes. With the availability of potent immunosuppressive drugs the approach of the past, i.e., low-dose initial treatment slowly increasing in intensity in the case of insufficient response, is now obsolete. Given the choice,

and recognizing the risk of irreparable damage in the case of insufficiently intense initial treatment, well-informed patients prefer a more vigorous initial treatment.

General rules of treatment regimens

We summarize here our own experience with drug-combination therapy. The main motivation for combining drugs with different mechanisms of action is the devastating effects that have been produced by cortisone monotherapy in patients with autoimmune diseases. This was the reason that we introduced drug-combination therapy for our patients in New York in the early 1960s, with the limited number of drugs available at that time. Today, the abundance of potent agents makes it possible to provide effective treatment with a minimum of drug toxicity. In particular, the risk of cancerogenicity, initially a cause of concern, has proven to be negligible, in contrast to the situation in transplanted patients under intensive immunosuppressive treatment.

Controlled studies are important for the objective assessment of drug efficacy. In autoimmune diseases this approach is problematic since it is almost impossible to set up well-matched homogeneous patient groups with the great variations in disease expression from one patient to another. Despite these difficulties, however, positive results obtained in such studies have been valuable in the assessment of drug efficacy. However, a negative result does not necessarily exclude a drug which has been shown to have immunosuppressive properties.

Once a drug has been well defined with regard to its mechanism of action in the immune response, with positive results in animal models and in clinical studies including the prevention of graft rejection, its clinical application should not be limited to a specific autoimmune disease. It should be realized that a drug which is efficacious for one autoimmune disease is in general also effective in other, clinically very different, autoimmune diseases. In other words, the clinician must recognize that the same basic immunopathological mechanisms are common to more than 40 different autoimmune disease entities. Treatment must be aimed at the underlying autoimmune process.

T and B lymphocytes and APC are similarly involved in all autoimmune conditions. Differences in the response to treatment depend on whether cellular or humoral factors prevail, as well as on the specific organ or tissue involvement of the disease process. Treatment regimens can be oriented on the basis of these differences. However, it is not possible to set up long-term rigid treatment protocols in view of the great variability in disease expression both from one patient to another and during the course of the disease. Thus, physicians should be aware of the need for flexibility and readiness to change treatment should unforeseen events alter the expression of the disease.

Remission-induction regimens and maintenance therapy

Untreated, autoimmune diseases are severe and potentially deadly conditions, and by their nature (genetic predisposition) they tend to be chronic. The enormous progress in diagnosis as well as the great improvement in treatment modalities over the past 50 years has changed the outlook and quality of life for patients affected by an autoimmune disease. Once remission has been achieved, if unaware of the potential chronicity of these conditions, the patient and physician may be tempted to discontinue immunosuppressive treat-

ment altogether. However, such an approach entails a great risk of disease recurrence. For this reason patients must be informed at the beginning that treatment may have to continue for a long time, frequently for years.

The protocols outlined below differentiate between those for remission-induction therapy (R-Th) and those for maintenance therapy (M-Th).

Patients with low initial disease activity

Protocol:

- *R-Th1*
  - The combination of Pred, CsA, MTX, and AZA is well tolerated by most patients and permits rapid control of disease activity. Treatment may be initiated with a single i.v. injection of 15 mg MTX + 80 mg MP, followed by:
  - Pred: 10 mg, 5× per week for 4 weeks, subsequently 4× per week.
  - MTX: 15 mg per week in a single i.m. or i.v. injection for 4 weeks, subsequently every other week.
  - Folic acid: 5 mg per day for 3 days after MTX.
  - AZA: 100 mg per day, 6 days per week during MTX-free weeks.
  - ASA: 100 mg every evening

Once the disease is well controlled, Pred should be gradually reduced until reaching a weekly dose of 20 mg (4×5 mg). MTX may then be given orally at a dose of 10 mg per week during alternate weeks, reaching maintenance therapy M-Th1 (see below). Protocol:

- *M-Th1*
  - Pred: 5 mg, 4× per week.
  - MTX: 10 mg/wk p.o., given on 3 nonconsecutive days, every other week.
  - Folic acid: 5 mg per day on 3 days after MTX.
  - AZA: 100 mg per day, 6 days per week during MTX-free weeks.
  - ASA: 100 mg every evening.

Patients with high initial disease activity and normal creatinine levels

The cortisone effect in these patients can be maximized without significant increase in steroid toxicity by administering intravenous pulses of 125 mg MP or 40 mg DXM. These intravenous pulses may be continued on days 2, 4, and 6. In the case of very severe disease activity additional MP pulses may be necessary (MP produces fewer steroid side effects than DXM because of its short half-life: 3 h vs. 24 h for DXM). The therapeutic cortisone effect is increased during the first week because of the long half-life of DXM. After the initial intravenous steroid pulses Pred is continued orally together with CsA, MTX, and AZA. Protocol:

- *R-Th2*
  - Initiation of treatment with i.v. steroid pulses (see above), followed by:
  - Pred: 10 mg, 5× per week for the first month, subsequently 4× per week.

- CsA: 4.5 mg/kg per day (lean bodyweight) in two daily doses, 6 days per week.
- MTX: 15 mg in weekly i.m. injections for 1 month, subsequently every other week.
- Folic acid: 5 mg per day for 3 days after MTX.
- AZA: 100 mg per day, 6 days per week, during MTX-free weeks.
- ASA: 100 mg every evening
- If disease is not brought under control with the combination of Pred, CsA, MTX, and AZA: Cy 500 mg + MP 125 mg in 15-min perfusion with adequate hydration, once in a 4-week period during an MTX-free week.

Once a patient is in remission, treatment intensity should be gradually reduced. Obviously the drugs with the greatest toxicity potential should be the first to be diminished, particularly Cy: either the number of Cy pulses should be reduced, or Cy completely stopped. Steroid toxicity is always underestimated; 3 steroid-free days should be introduced as soon as possible. CsA doses should be gradually tapered once the disease process has been under control for at least 5 months, either reducing the daily dose or increasing CsA-free days. Rapid discontinuation of CsA entails the risk of relapse ("rebound phenomenon").

Administration of CsA was initially restricted to special cases because of its effect on kidney function, observed with daily doses of 8–12 mg/kg. Today, with 20 years of experience with CsA, this drug has been shown to be well tolerated at daily doses below 5 mg/kg. It is important to adjust the CsA dose to lean bodyweight; otherwise obese patients would receive an excessive dose. Creatinine monitoring is essential to avoid renal toxicity. If the CsA serum level is 1.4 mg/dl, the dose should be reduced; above that level the drug should be discontinued. Individual tolerability of drugs must be considered in the final drug combination used for maintenance therapy. Protocol:

- *M-Th2*
  - Pred: 5 mg, 4× per week.
  - CsA: 4 mg/kg per day, 6 days per week.
  - MTX: 15 mg i.m. during alternate weeks. After 6 weeks without relapse, 10 mg p.o. per week, on 3 nonconsecutive days, every other week.
  - Folic acid: 5 mg per day, on 3 days after MTX.
  - AZA: 100 mg per day, 6 days per week, during MTX-free weeks.
  - ASA: 100 mg every evening.

Pregnancy and maintenance therapy

In patients without signs and symptoms of disease activity for at least 3 months, pregnancy may be considered while taking low doses of Pred (4×5 mg per week), low doses of CsA (3 mg/kg per day) on alternate weeks, and AZA (4×100 mg per week) during the weeks without CsA. HCQ can also be given during pregnancy. Strict obstetric supervision is essential. After the 25th week of pregnancy pre-eclampsia (hypertension, albuminuria, or edema) may occur. Rapid intervention is usually effective: bed-rest with the patient on her left-side, 1 day with a strict low-protein, low-fat diet (just boiled potatoes, salt-free, cooked apples, water or tea) and intestinal antimicrobial medication with paramomycin, 2×250 mg, followed the next day by substitution of the intestinal flora by *Bacillus* acidi lactici). Given at the first symptoms of pre-eclampsia, this therapy gives surprisingly good

results. In the case of high creatinine levels CSA is discontinued and a few steroid pulses (two or three) with 125 mg MP may be necessary. After the 32nd week close obstetric supervision is essential to be prepared for a cesarian section. Breast-feeding is contraindicated. After delivery there is a tendency for autoimmune diseases to be exacerbated. Such relapses can be prevented in most cases by an intravenous injection on day 10 with 15 mg MTX plus 125 mg MP followed by maintenance therapy.

Monitoring of immunosuppressive therapy

Once the patient's condition is clinically well characterized, the following laboratory tests are essential to assess disease activity and possible drug toxicity: complete blood count, erythrocyte sedimentation rate, creatinine, transaminases, γ-glutamyl transpeptidase, and standard urine analysis. At the same time blood pressure and body weight should be recorded. The first control should take place after 2 weeks of treatment, then at monthly intervals for 3 months, thereafter every 3 months.

Specific treatment protocols

Rheumatoid arthritis

Traditionally, patients with RA were given, for a long initial period, symptomatic treatment with nonsteroidal anti-inflammatory drugs (NSAIDs). Finally, however, once the autoimmune nature of RA was established, early intervention with immunomodulatory drugs became mandatory. The difficulty now resides in the assessment of early stages of RA, particularly in patients with an insidious initial disease course without alteration of laboratory parameters. Fortunately, the spleen, an immunocompetent organ which is involved in all collagen diseases, participates very early in the course of RA. Indeed, one of the first signs of RA is a slight enlargement of the spleen, in general not detectable by echographic examination but demonstrable by splenic percussion. The splenic percussion dullness is very constant with a diameter of 5–6 cm diameter in healthy subjects. The strength of percussion must be adjusted to the thickness of the fat-layer and attention paid to the difference between "pulmonary" and "abdominal" air sonority in order to detect the upper and lower poles of the spleen with precision. The dimensions of the spleen can be accurately measured using a linear echographic probe. Mild splenic enlargement (8–10 cm) occurs before any disease-specific laboratory parameters appear. Strong splenic percussion produces a slight to moderate soreness, equal to or stronger than the sensation felt upon liver percussion. In a more acute stage the patient may spontaneously complain of pain in the splenic region. It is interesting to note that steroids, even if given daily for several months, do not correct the splenic enlargement even though clinically the patient appears to be in remission. In patients with joint involvement compatible with RA the finding of an enlarged spleen may thus permit "early intervention" to be initiated if there is no other reason for splenomegaly, for example, thalassemia intermedia and postmalaria splenomegaly.

The following protocols describe treatment regimes for RA according to clinical expression of disease.

Mild forms of RA: moderate arthralgia with joint distribution typical for RA, without joint effusion

Protocol:

- *RA-Th1*
  - Pred: 10 mg 4× per week.
  - CsA: 4.5 mg/kg per day in two equal doses, 6 days per week.
  - MTX: 15 mg i.m. or i.v. per week for 1 month, subsequently every other week.
  - Folic acid: 5 mg per day on 3 days after MTX.
  - HCQ: 200 mg per day during MTX-free weeks.

Severe forms of RA

Severe forms: fever, swollen joints without effusion Protocol:

- *RA-Th2*
  - Initiate treatment with 15 mg MTX + 40 mg DXM i.v. followed by:
  - Pred: 15 mg 4× per week.
  - CsA: 4.5 mg/kg per day in two equal doses, 6 days per week.
  - MTX: 15 mg + 125 mg MP, i.v., 3 weeks out of 4; upon remission, MTX without MP.
  - Folic acid: 5 mg per day on 3 days after MTX.
  - HCQ: 400 mg per day during MTX-free week.

Severe forms: swollen and warm joints, effusion in one or both knees Protocol:

- *RA-Th3*
  - Arthrocentesis with aspiration of joint fluid and injection of 40 mg Depot Medrol (MP) + 5 mg MTX in each knee.
  - Initiation of treatment with 500 mg Cy + 125 mg MP in a 15- to 20-min perfusion under adequate hydration.
  - Continue with treatment regimen RA-Th2.

Severe forms: as in RA-Th2, with the possibility of using anti-TNF agents Protocol:

- *RA-Th4*
  - Treatment regimen RA-Th2 + either etanercept or infliximab.

The efficacy of anti-TNF-α (infliximab and etanercept) has been well documented. Infliximab has a long half-life and thus can be administered with long intervals between injections (4–8 weeks) but limits treatment flexibility. Etanercept with its short half-life allows greater treatment flexibility and thus is to be preferred. Etanercept can be given as subcutaneous injections at increasingly long intervals and discontinued when a stable clinical remission is obtained.

Not all cases of RA are well-controlled with this regimen. This is especially so in patients who for long periods have received only symptomatic or insufficient immunosup-

pressive treatment. In these cases several immunomodulatory drugs are available which can be given in addition to the baseline therapy with Pred, CsA, MTX and HCQ:

- Lef, 20 mg per day. If well tolerated and efficacious after a 3-week trial, it may be continued for a long period.
- DP, 150 mg per day during the first week; if well tolerated, 300 mg per day for longer periods.
- Thal, 100 mg (maximum 200 mg) per day. After 4 weeks Thal should be given every other week. Prolonged treatment with Thal produces drug-resistance (after 4–10 months). The addition of vitamin B_1 and B_6 may diminish the risk of peripheral neuropathy. Alcohol should be strictly avoided.
- Anakinra, 1 mg s.c. per day (anakinra precludes the use of anti-TNF agents).
- SSZ, 500 mg two or three times per day.
- Dapsone, 25 mg per day for 1 week, subsequently 50 mg per day.
- Colchicine, 0.5 mg per day for 1 week; subsequently, if well tolerated, 1 mg per day.

With these seven drugs it is not yet possible to direct treatment according to the genetic subtype of RA. For the time being we orient treatment empirically with drugs known to interfere with the immune response. Lef is an effective immunomodulating agent but is not tolerated by all patients. Low-dose DP has given surprisingly good results in many patients with few side reactions. The dose of 300 mg should not be exceeded. If there is no improvement after 4 weeks, DP should be replaced by another immunosuppressive agent. Low-dose Thal has been of great help in many cases. Initial results with anakinra, which blocks the action of IL-1, are promising but experience is still limited with this drug. SSZ, dapsone, and colchicine have proven useful in patients not responding well to the baseline treatment.

Systemic lupus erythematosus

SLE is another autoimmune disease entity with a very wide spectrum of clinical expression, a variability which probably depends on genetic background. In general the individual pattern at onset of SLE tends to persist throughout the course of disease. Below are seven treatment protocols for SLE, each intended for a specific clinical expression of the disease.

Mild forms of SLE: patients with arthralgia, butterfly rash, discrete kidney involvement, and normal serum creatinine values

Protocol:

- *SLE-Th1*
  - Pred: 10 mg per day, with 2 nonconsecutive Pred-free days per week.
  - MTX: 15 mg i.m. per week for 4 weeks, subsequently every other week.
  - Folic acid: 5 mg per day on 3 days after MTX.
  - CsA: 4.5 mg/kg per day, 6 days per week.
  - HCQ: 400 mg per day for 4 weeks, subsequently during MTX-free weeks.
  - ASA: 100 mg every evening.

ASA is given for the prevention of vascular accidents in all SLE patients, regardless of whether antiphospholipid antibodies are present. After 2–3 months the Pred dose should be reduced and MTX changed from intramuscular to oral administration, 10 mg per week during alternate weeks on 3 nonconsecutive days.

Patients with nephrotic syndrome and normal creatinine values (≤1.3 mg/dl)

Protocol:

- *SLE-Th2*
  - DXM: 40 mg i.v. on days 1, 3, and 5.
  - MP: 125 mg i.v. on days 6, 8, 10, and 12.
  - *After day 12:*
  - Pred: 20 mg per day orally with 2 Pred-free days per week.
  - CsA: 4.5 mg/kg per day, 6 days per week.
  - MTX: 15 mg i.v. or i.m. per week, 3 weeks out of 4.
  - Folic acid: 5 mg per day on 3 days after MTX.
  - Cy: 500 mg + MP 125 mg in MTX-free weeks (as a slow infusion over 15–20 min) until proteinuria falls below 1 g/l, subsequently every 8 weeks.
  - ASA: 100 mg every evening.

Lupus nephritis with elevated serum creatinine (>1.5 mg/dl)

Protocol:

- *SLE-Th3*
  - DXM: 40 mg i.v. on days 1, 3, 5.
  - MP: 125 mg i.v. on days 6, 8, 10, and 12
  - *After day 12:*
  - Pred: 20 mg per day 5 days per week.
  - CsA: after reduction of creatinine to 1.2 mg/dl, CsA 2.5 mg/kg per day for 1 week, increasing to 3.5 mg/kg per day for 2–3 weeks, subsequently 4 mg/kg per day.
  - Cy: when creatinine ≥2.5 mg/dl, 300 mg Cy + 125 mg MP i.v. When creatinine=2.0 mg/dl, 500 mg Cy + 125 mg MP alternating on a weekly basis with MTX.
  - MTX: 10 mg i.m. or i.v. when creatinine <2.5 mg/dl, 15 mg i.m. or i.v. when creatinine <1.5 mg/dl, in alternation with Cy. After 4–6 weeks, MTX should be given 3 out of 4 weeks, Cy-MP i.v. infusion only during MTX-free weeks.
  - Folic acid: 5 mg per day on 3 days after MTX.
  - ASA: 100 mg every evening.

Adjustment of treatment according to clinical response. Cy-MP infusions diminished as a priority (every 6–8 weeks, before complete discontinuation).

SLE with cutaneous vasculitis, not responding to SLE-Th3

Protocol:

- *SLE-Th4*
  - Addition of Thal, 100 mg every evening; after 4 weeks, on an alternate week basis.
  - Dapsone: 25 mg b.i.d. during Thal-free weeks.

SLE with cardiac involvement (peri- or myocarditis) with or without pleural effusion

Protocol:

- *SLE-Th5*
  - MP: 125 mg i.v. on alternate days during first week.
  - Pred: (after 1st week) 20 mg per day with 2 nonconsecutive Pred-free days per week.
  - MTX: 15 mg + 125 mg MP i.v. 3 out of 4 weeks. After 6–8 weeks, MTX without MP.
  - Folic acid: 5 mg per day on 3 days after MTX.
  - AZA: 100 mg per day during MTX-free weeks.
  - CsA: 4.5 mg/kg per day, 6 days per week.
  - ASA: 100 mg every evening.

SLE with "anti-phospholipid syndrome," either deep vein thrombosis or cerebral involvement

Protocol:

- *SLE-Th6*
  - SLE-Th1 or SLE-Th5 protocol + oral anticoagulation ad vitam, maintaining an international normalized ratio level of 3–4, after 3 months between 2.5 and 3.5.

Special forms of neuro-lupus

Seizures. Convulsive seizures can antedate SLE or occur in the course of the disease. In addition to basic SLE treatment, anticonvulsive medication is indicated for a prolonged period of time, and especially for as long as EEG abnormalities are present.

Landry's ascendant paralysis and acute transverse myelitis. These are rare but very serious complications in the course of SLE with the risk of permanent damage if not treated properly [72]. Intrathecal treatment is very efficacious, if given immediately. Protocol:

- *SLE-Th7*
  - DXM: 20 mg + 10 mg MTX, via lumbar puncture on days 1 and 8.

– Cy: 500 mg + 125 mg MP as i.v. perfusion over 15–20 min on the day of intrathecal treatment (days 1 and 8)

In addition, protocol SLE-Th5. In the case of relapse, intrathecal treatment must be resumed.

Encephalopathy with hallucinations and/or comatose state. Same treatment as for Landry's syndrome. Recurrences are common in this form of SLE encephalopathy (one patient relapsed 8 times, another patient 18 times; both were treated with intrathecal treatment for each relapse, and both recovered after these series of relapses).

Chorea. Protocol SLE-Th5 with intrathecal treatment on days 1 and 8, each time in association with Cy i.v. (see SLE-Th7).

Multiple sclerosis

MS is now generally accepted as a condition belonging to the autoimmune diseases. On the one hand, these involve a multigenetically based spectrum of different forms of MS; on the other, no other autoimmune disease is so strongly affected by environmental factors. Psychological events rank particularly high among the disease-triggering and disease-modifying factors, which renders the assessment of treatment efficacy in controlled studies very difficult.

With these difficulties in setting up rational therapies, animal models of experimental autoimmune encephalitis have been of great help. In experimental models CNS lesions have been shown to be produced by specific T lymphocytes which first must penetrate the blood-brain barrier in order to reach their target. This process is probably very similar in MS patients. In contrast to all "collagen diseases," none of the peripheral laboratory parameters is affected by the MS disease process. Normal values are found with regard to inflammatory indicators; patients are not anemic and never show spleen enlargement.

The following clinical example illustrates this aspect. A 52-year-old woman developed within a few months a very severe form of MS with diplopia, slurred speech, and great difficulty in swallowing. She lost 8 kg in weight in 2 months. Steroid pulses with 3×1 g MP were unsuccessful, as were plasmapheresis and high immunoglobulin pulses and high-dose oral steroids. This patient consulted us in January 1992. Confronted with this desperate situation, we hospitalized the patient for intrathecal treatment (20 mg DXM + 10 mg MTX). The clinical response was dramatic, and the patient was able to leave the hospital 2 days later on mild maintenance therapy (low-dose steroids + MTX). Five weeks later she relapsed but again responded to intrathecal treatment. After a further 6 weeks she relapsed again. This time, an intravenous infusion of 500 mg Cy was added to the intrathecal regimen. Furthermore, we added Cy-MP pulses to the maintenance therapy (500 mg Cy + 125 mg MP, every month). After 6 months without further relapse, pulses were given less frequently, every 6 weeks, finally every 8 weeks, leaving a treatment of low-dose Pred (4×5 mg per week) and MTX (15 mg intramuscularly in alternate weeks). Since then the patient has remained in complete remission (last medical examination, 2003). This experience corroborates the situation in experimental autoimmune encephalitis. We postulate that the role of Cy was to prevent MS-specific T and B cells from entering the CNS territory, and we now always give Cy pulses with an intrathecal treatment.

As in all autoimmune diseases, early treatment is essential, particularly in conditions in which there is the risk that irreparable damage may compromise the patient's future. Early diagnosis of MS is a prerequisite for the initiation of immunosuppressive therapy. Magnetic resonance imaging (MRI) has become an essential tool for establishing a diagnosis of MS at an early stage, thus permitting early initiation of therapy. However, in a small number of cases MRI may not reveal any demyelinated foci during the first months or even years of the disease. In one of our patients with a typical clinical MS pattern the diagnosis of MS was not accepted by the neurologists until, after 3 years and with progressive loss of sight in one eye, the MRI finally showed some demyelinated foci at typical sites.

The distinction between "recurrent-remittent" vs. "primary progressive" forms of MS is essential in the management of MS. In the recurrent-remittent form of the disease, response to treatment is far better than in patients with the primary progressive form. Accordingly, different levels of treatment intensity are necessary for these two types of MS. If untreated, recurrent-remittent MS may gradually transform into a primary progressive course of disease, requiring a highly intensive treatment.

MS with a recurrent-remittent course of disease

In the majority of these patients the disease is characterized by self-limiting episodes followed by remissions which may last months or years. If the first disease episode is followed by a complete remission with normalization of all neurological findings observed in the active phase of the disease, no preventive maintenance therapy is needed, but the patient must be aware of the possibility of future attacks, so that remission-induction treatment can be given in the first days of a disease recurrence.

Remission induction in recurrent-remittent MS Protocol:

- *MS-Th1*
  - DXM: 40 mg i.v. on days 1, 3, 5, 8, and 16
  - Cy: 500 mg on days 1, 8, and 16 in an i.v. infusion over 15–20 min with adequate hydration (1.5–2 l) + 25 mg furosemide to guarantee rapid elimination of Cy.

Maintenance treatment for recurrent-remittent MS, mild forms

- *MS-Th2*
  - IFN-β_{1a}: 3×6 10^6 U s.c. per week.

Treatment with IFN-β has yielded positive results, with diminution of the number of relapses and diminution of progression as shown by MRI. IFN treatment is generally limited to 2 years. Monotherapy with IFN only acts on APC, leaving T and B lymphocytes unaffected. In the case of relapse or signs of persistent disease activity, treatment should be adjusted according to the severity of symptoms (MS-Th1 and MS-Th4).

- *MS-Th3*
  - Glatiramer (acetate): 20 mg s.c. per day for long periods.

This treatment has been shown to reduce the frequency of relapses.

Drug-combination maintenance therapy for mild to moderately severe forms of MS Protocol:

- *MS-Th4*
  - Pred: 4×5-10 mg per week.
  - MTX: 10 mg per week, given p.o. over 3 nonconsecutive days per week, every other week.
  - Folic acid: 5 mg per day on 3 days after MTX days.
  - AZA: 100 mg per day during MTX-free weeks.

The MS-Th4 approach permits more flexibility than MS-Th2 and MS-Th3. Depending on the course of disease, the intensity of maintenance therapy can be increased by changing from oral to intramuscular administration of MTX (15 mg every other week) and by adding CsA, 4 mg/kg per day, 6 days per week.

MS with a primary or secondary progressive course of disease

Treatment is initiated with remission-induction protocol.

Remission induction in primary progressive MS. Depending on the severity of disease at the start of immunomodulating therapy, remission induction may be carried out according to MS-Th1, or by intrathecal protocol MS-Th5. For patients with severe disease activity, or progression of disease despite MS-Th6 maintenance therapy, see below. Protocol:

- *MS-Th5*
  - Day 1:
    20 mg DXM + 10 mg MTX intrathecal (by lumbar puncture)
    Cy 500 mg + MP 125 mg i.v. infusion over 15–20 min.
    Furosemide 25 mg
  - Days 3, 4, 5:
    40 mg DXM i.v.
  - Day 8:
    Intrathecal injection of 20 mg DXM + 10 mg MTX
    500 mg Cy + 125 mg MP i.v. infusion over 15–20 min.
    Furosemide 25 mg
  - Day 16:
    40 mg DXM i.v.

Maintenance therapy in primary progressive MS

Protocol:

- *MS-Th6*
  - Pred: 4×5-10 mg per week.
  - MTX: 15 mg i.m, 3 weeks out of 4.
  - Folic acid: 5 mg per day, on 3 days after MTX.

- CsA: 4 mg/kg per day, 6 days per week.
- Cy: 500 mg + MP 125 mg, i.v. infusion, + 25 mg furosemide, once every 4 weeks.

Depending on the course of disease, the intensity of maintenance therapy can either be slowly reduced by increasing the intervals between Cy-MP pulses or increased by adding 100 mg Thal every evening during alternate weeks. In the event of disease exacerbation the remission protocol MS-Th1 or MS-Th5 should be applied immediately because of the risk of irreversible damage.

Autoimmune diseases with splenomegaly

In the following autoimmune conditions, mild splenomegaly reflects the systemic nature of the disease: juvenile idiopathic arthritis, Still's disease, uveitis, dermatomyositis, polymyalgia rheumatica, primary Sjögren's syndrome, progressive systemic sclerosis, Wegener's granulomatosis, Churg-Strauss syndrome, Crohn's disease, ulcerative colitis, ankylosing spondylarthritis, chronic active hepatitis, primary biliary cirrhosis, recurrent-remittent pericarditis, interstitial pneumopathy, peripheral motor neuropathy, Behçet's syndrome, and sarcoidosis.

Juvenile idiopathic arthritis

Among the different forms of juvenile idiopathic arthritis the most frequent is juvenile oligoarthritis (mono- or pauciarticular), with a 20% incidence of iritis. Less frequent are polyarticular and enthetic arthritis, the latter corresponding to adult AS with a 5% incidence of iritis. In children suffering from these conditions cortisone has produced the most devastating effects, which are completely avoidable with drug combination therapy. Indeed, the response to drug combination is particularly good in children, with an excellent long-term prognosis on maintenance therapy generally limited to 5 years. Furthermore, with this type of treatment, no child in the oligoarthritis group developed uveitis. The spleen is enlarged on percussion in all children with active disease, palpable in only 25% of affected children. The youngest patients are under 2 years of age. Protocol:

- *JIA-Th*
  - Pred: 0.15 mg/kg per day, 4 days per week.
  - CsA: 4.5 mg/kg per day, 6 days per week.
  - MTX: 10 mg/m^2 i.m. per week during alternate weeks.
  - Folic acid: 0.05 mg/kg per day on 3 days after MTX.
  - AZA: 1.5 mg/kg per day, 6 days per week during MTX-free weeks.
  - Depending on the degree of disease activity, Etanercept may be added: 1 mg/kg (max. 25 mg) s.c. twice per week for 4 weeks, subsequently increasing the interval between injections from 4 to 7 days until complete remission.

Still's disease

In 1897 Still described a systemic disease with high fever, lymphadenopathy and splenomegaly (80%), PC, and/or pleurisy (35%), iritis (10%) and arthritis of the larger joints. White blood cell values can reach 20,000–30,000. This serious condition occurs mostly in children but occasionally also in young adults.

Treatment protocol R-Th2 followed by M-Th2 for maintenance is generally effective and produces minimal side effects. Two-thirds of patients experience permanent complete remission.

Idiopathic autoimmune uveitis

Anterior uveitis (iritis and iridocyclitis) as well as posterior uveitis (choroiditis and chorioretinitis) can occur in patients with systemic autoimmune diseases but can also occur alone as "idiopathic" autoimmune uveitis (UV). The presence of a mild splenomegaly in this idiopathic form reflects its systemic character. Treatment of uveitis must take into account the systemic nature of the disease. Local therapy is certainly not sufficient to bring uveitis under control. Patients with anterior uveitis have ocular and periocular pain with lacrimation as well as photophobia with circumcorneal injection. In the case of posterior uveitis, blurred vision and metamorphopsia with reduction in visual acuity are the main symptoms.

Both forms of uveitis respond to immunosuppressive therapy according to protocol R-Th2. Maintenance therapy (M-Th2) is necessary for a period of 1–5 years. Relapses are not infrequent and require an immediate remission-induction regimen.

Dermatomyositis

Dermatomyositis (DM) is a systemic connective tissue disease which affects mostly children in the 5- to 15-year age group and less frequently adults between the ages of 40 and 60 years. It is characterized by inflammatory changes in muscles (polymyositis) and, particularly in children, in the skin. The cutaneous eruption may resemble the lupus butterfly rash. Periorbital edema with a heliotrope hue are typical for DM, as is erythema localized in the V of the neck, shoulders, forearms, legs, and dorsum of proximal interphalangeal and metacarpophalangeal joints. Transition from DM to progressive systemic sclerosis (PSS) and SLE can occur. The skin lesions fade under immunosuppressive therapy. Subcutaneous calcifications may develop at typical sites (elbows and knees), particularly in childhood dermatomyositis. In adults the disease may be limited to the muscles with proximal muscle weakness and polymyalgia, always with elevation in muscle enzymes, especially creatinine phosphokinase. Tumor screening should be performed in patients aged over 50 years since DM can represent a paraneoplastic (usually carcinoma) condition.

Treatment: early intervention with remission-induction protocol R-Th2. Maintenance therapy is necessary for a long period of time, usually several years (M-Th1 or M-Th2).

Polymyalgia rheumatica

This chronic condition is known to respond readily to steroids. However, continuous disease activity requires a prolonged treatment entailing a high risk of cortisone side effects, especially in patients over 50 years of age. Thus, "traditional" steroid monotherapy has to be abandoned. The disease process can be brought under control with minimum side effects by drug combination therapy of polymyalgia rheumatica using protocol R-Th1 or R-Th2.

Maintenance treatment is necessary for a long time, usually several years, with protocol M-Th1 or M-Th2 depending on the degree of disease expression.

Primary Sjögren's syndrome

Mild splenomegaly is present even in the early stages of this condition, which involves lacrimal, salivary gland, and vaginal secretion. Interstitial nephritis rarely affects renal function. The lacrimal glands are the most vulnerable target of the disease since their number is very limited, augmenting the danger of irreparable loss of lacrimation with corneal lesions.

Early intervention by protocol R-Th2 is usually effective. Under CsA medication ocular bruising may subside after only 10 days of treatment, although prolonged treatment is necessary in most cases. Maintenance therapy: M-Th2.

Progressive systemic sclerosis

The scleroderma spectrum encompasses multiorgan disease processes with alteration in the microcirculation. The disease may be limited to the skin or affect simultaneously or separately the gastrointestinal tract, pulmonary system (interstitial pneumopathy, IP), heart, and kidney (microangiopathy with malignant hypertension). More common in women, rare in children, mortality in PSS is almost ten times higher than in SLE.

PSS limited to skin involvement. Protocol:

- *PSS-Th1*
  - Pred: 4×5 mg per week.
  - CsA: 4.5 mg/kg per day (contraindicated in the case of hypertension).
  - MTX: 15 mg i.m. every other week.
  - Folic acid: 5 mg per day on 3 consecutive days after MTX.
  - DP: 1 or 2×150 mg per day.
  - ASA: 100 mg every evening.

Local treatment of affected skin: massage with cream containing ASA and heparin or heparinoid to prevent ulcers on finger-tips. For extensive and severe skin involvement symptomatic treatment with infusions of prostacyclin analogues produces temporary improvement in the microcirculation. Maintenance therapy: M-Th2.

PSS with IP and/or esophago-gastrointestinal involvement. IP is a very serious condition which fortunately may respond to early intervention (characteristic signs of pulmonary auscultation!). Esophago-gastrointestinal involvement can be most troublesome, not only for mechanical reasons but also because of the consequences of "malabsorption." For this reason, oral antibiotics may be ineffective in cases of severe infection. These patients often feel an improvement upon parenteral administration of vitamins B_1, B_2, B_6, and B_{12}. Protocol:

- *PSS-Th2*
  - Pred: 10 mg, 5 days per week.
  - CsA: 4.5 mg/kg per day, 6 days per week (with strict control of blood pressure which, if necessary, must be corrected with a thiazide diuretic and an antiangiotensin receptor agent).
  - MTX: 15 mg i.m. per week for 4 weeks, subsequently every other week.
  - Folic acid: 5 mg per day for 3 days after MTX.
  - Cy: 500 mg + 125 mg MP in a 15- to 20-min infusion with adequate hydration, during MTX-free weeks, once per month until remission is reached.
  - DP: 150 mg b.i.d.
  - ASA: 100 mg every evening.
  - Vitamin B complex: i.m. 2× per week for 2 months, subsequently once per week.

Gradual adjustment of treatment intensity. Life-long maintenance treatment (M-Th2) is necessary for this severe form of PSS.

PSS with malignant hypertension and symptoms of left-ventricular failure. Protocol:

- *PSS-Th3*
  - PSS-Th2 protocol without CsA.

Antihypertension treatment is essential to rectify blood pressure. Maintenance therapy: M-Th1.

Wegener's granulomatosis

This granulomatous inflammatory disease is initially localized in the upper respiratory tract, gradually extending to become a systemic vasculitis. Kidney involvement may lead to renal failure in the absence of early immunosuppressive treatment which is essential to prevent this detrimental course of disease. Oral administration of Cy has been used with some success for the treatment of this condition, but toxicity is high and benefit limited. Cy is nevertheless the most effective drug if administered in intravenous pulses in combination with other immunomodulatory drugs. Protocol:

- *WEG-Th*
  - Pred: 10 mg, 4 days per week.
  - CsA: 4.5 mg/kg per day, 6 days per week.
  - Cy: 500 mg + MP 125 mg, in an i.v. infusion over 15–20 min with adequate hydration. Initially every other week for 6 weeks, subsequently every 4 weeks.

- MTX: 15 mg i.m. during Cy-free weeks.
- Folic acid: 5 mg per day on 3 days after MTX.
- ASA: 100 mg every evening.

Close surveillance of the respiratory tract is necessary. In the case of infection antibiotic therapy must be started immediately. Maintenance treatment: M-Th2.

Churg-Strauss eosinophilic granulomatous syndrome

Initially this condition involves the respiratory tract long before the appearance of systemic disease manifestations such as patchy pulmonary infiltrates. Other systemic symptoms such as peri- and/or myocarditis, attacks of intestinal involvement, and peripheral neuropathy may occur. At the onset patients complain of nasal respiratory difficulty (presence of nasal polyps), asthma attacks, and chronic cough. Without proper treatment there is a high mortality rate. The degree of eosinophilia usually reflects disease activity. The spleen is slightly enlarged and tender on percussion. Protocol:

- *CHURG-Th*
  - Pred: 15 mg, 5 days per week for 1 month, subsequently 4 days per week.
  - CsA: 4.5 mg/kg per day, 6 days per week.
  - MTX: 15 mg + 125 mg MP i.v. once per week for 1 month; subsequently every other week, i.m. without MP.
  - Folic acid: 5 mg per day on 3 days after MTX.
  - AZA: 100 mg per day during MTX-free weeks.
  - ASA: 100 mg every evening.

Otorhinolaryngological intervention is advisable to remove polyps, usually located around the ostia of the maxillary sinuses.

Maintenance therapy: M-Th2.

Crohn's disease

This granulomatous inflammatory condition primarily involves the terminal part of the ileum with possible extension in both directions. Its systemic nature is reflected in the enlargement of the spleen and in extraintestinal manifestations including arthritis, episcleritis and erythema nodosum. If untreated, the disease process may produce a stenosis of the terminal ileum, severe enough to require surgery. Early intervention is thus essential. Since TNF-α is relevant in this inflammatory process, anti-TNF agents are potentially useful for remission induction. Protocol:

- *Crohn-Th*
  - Pred: 4×10 mg per week.
  - CsA: 4.5 mg/kg per day, 6 days per week.
  - MTX: 15 mg i.m. every other week.
  - Folic acid: 5 mg per day on 3 days after MTX.
  - AZA: 100 mg per day, 6 days per week during MTX-free weeks.

- SSZ: 1 g t.i.d.
- ASA: 100 mg every evening.
- Etanercept: for remission induction in the event of acute attacks, 25 mg s.c. twice per week.

Since the immune conflict takes place in a microbe-rich environment, with microbes as potential disease-triggering and disease-modifying factors, intermittent local antimicrobial treatment is advisable: 250 mg metronidazole t.i.d. during episodes of disease activity, and/or, 250 mg paromomycin twice on 1 day only, with a light protein-free diet (e.g., steamed potatoes and steamed apples) with administration of *Bacillus* acidi lactici on the following day. In the event of a positive result, this procedure may be repeated twice per month.

Maintenance therapy (M-Th2) is necessary for a long period, in general 3–5 years. In the case of relapse, treatment protocol Crohn-Th should be resumed immediately.

Ulcerative colitis

The target of this systemic granulomatous autoimmune disease is the distal colon and the rectum. The spleen is always slightly enlarged in active stages of the disease. Extracolonic involvement includes peripheral arthritis, sacroileitis, erythema nodosum, uveitis, and spondylitis. Both ankylosing spondylitis and uveitis have a strong association with the HLA antigen B-27. As in Crohn's disease, TNF-α is implicated in the inflammatory process, justifying the use of anti-TNF agents. Crohn-Th protocol is equally applicable for UC, including the use of paromomycin.

Ankylosing spondyloarthritis

The primary target of AS is the spine, in particular the apophyseal vertebral joints, paravertebral soft tissues, and sacroiliac joints. In later stages peripheral joints may be affected. Iritis occurs in about 10% of cases, especially in patients with the histocompatibility antigen HLA-B27. Both Crohn's disease and UC may be associated with AS. Response to immunosuppressive therapy is weak; nevertheless the Crohn-Th protocol is worth trying. Since TNF-α is an important mediator of inflammation in AS, anti-TNF therapy should be considered in patients with severe clinical activity.

Chronic active hepatitis

In this autoimmune disease, environmental factors are of primary importance. "Idiopathic" HCV- and HBV-negative chronic active hepatitis (CAH) is infrequent. Indeed, most patients with CAH are HCV positive and, to a lesser degree, HBV positive. Treatment of the viral condition must be undertaken (pegylated IFN plus ribavirin in hepatitis C, lamivudin for hepatitis B), together with immunosuppressive treatment of CAH. It should be borne in mind that IFN, necessary for the treatment of hepatitis C, has a negative effect on autoimmune hepatitis (increase in transaminases and hypergammaglobulinemia). In patients failing to respond to antiviral treatment, long-term immunosuppressive therapy of CAH is

necessary in view of the possible progression into hepatic cirrhosis. Since the hepatic inflammatory process of chronic hepatitis C is mediated by the ongoing, albeit unsuccessful, antiviral immune response, and not by a direct viral action, immunosuppressive therapy is not contraindicated in patients carrying HCV. In fact, it diminishes the antiviral phlogistic process as well as the autoimmune activity of CAH.

In patients with persistence of HCV-RNA, prevention of viral expansion under immunosuppressive therapy may be attempted with antiviral medication (200 mg ribavirin t.i.d. + 100 mg amantadine b.i.d.). Protocol:

- *CAH-Th*
  - If transminase levels are elevated to more than 60 U/l alanine aminotransferase, treatment is initiated by i.v. pulses of 125 mg MP, every other day, followed by oral therapy:
  - Pred: 15 mg, 4× per week. After 1 month, 10 mg 4× per week.
  - CsA: 4 mg/kg per day, 6 days per week for 2 months; subsequently on an alternate week schedule.
  - 6-MP: 50 mg per day, 6 days per week for 2 months; subsequently during the CsA-free weeks.
  - ASA: 100 mg every evening.

  Maintenance therapy: M-Th2.

Primary biliary cirrhosis

Initially, this autoimmune disorder involves lymphocytic infiltration of medium-size bile ducts and portal tracts. Untreated, patients with primary biliary cirrhosis (PBC) develop slowly progressive fibrosis, finally leading to liver cirrhosis. The skin is pigmented and tanned as a result of melanocytic stimulation. Elevation in alkaline phosphatase and bile acids cause pruritus. The spleen is slightly enlarged in untreated patients. Immunosuppressive therapy has given controversial results, but none of our patients lost liver function under drug-combination therapy, including two patients who were already on the waiting-list for a liver transplant. Protocol:

- *PBS-Th*
  - Pred: 5 mg, 4× per week.
  - CsA: 4 mg/kg per day, 6 days per week.
  - DP: 150 mg o.d., after 1 week b.i.d. or t.i.d., 6 days per week.
  - Colchicine: 0.5 mg per day for 1 week, subsequently 1 mg per day.
  - MTX: 15 mg i.m. per week for 1 month, subsequently every other week.
  - Folic acid: 5 mg per day on 3 days after MTX.
  - 6-MP: After 1 month of treatment with Pred, CsA, DP, MTX, folic acid, start 6-MP 50 mg per day during MTX-free weeks.
  - Ursodeoxycholic acid: 300 mg b.i.d. (for relief from pruritus).
  - ASA: 100 mg every evening.

Upon remission, treatment should be gradually adjusted according to clinical results. Treatment is necessary for at least 4 years.

Recurrent-remitting pericarditis

The presence of slight splenomegaly permits to integrate this syndrome into the group of autoimmune conditions. Remission induction therapy protocol:

- *PC-Th*
  - MP: 125 mg on days 1, 2, 4 and 6, followed by:
  - Pred: 5 mg 4× per week.
  - CsA: 4 mg/kg per day, 6 days per week.
  - MTX: 15 mg i.m. every other week. After 6 months without relapse, stop MTX.
  - Folic acid: 5 mg per day on 3 days after MTX.
  - ASA: 100 mg every evening.

  Long-term maintenance therapy with M-Th1 or M-Th2 is necessary.

Interstitial pneumopathy

This autoimmune condition can occur in isolation, without any other organ involvement except mild splenomegaly. Long-term immunosuppressive treatment is indicated. Protocol:

- *IP-Th*
  - Pred: 5 mg 4× per week.
  - CsA: 4 mg/kg per day, 6 days per week.
  - MTX: 15 mg i.m. every other week.
  - Folic acid: 5 mg per day on 3 days after MTX.
  - DP: 150 mg per day for 1 week; subsequently b.i.d., 6 days per week.
  - ASA: 100 mg every evening.

In the event of pharyngobronchitis, immediate antibiotic treatment is indicated (e.g., 400 mg moxifloxacin per day for 5 consecutive days).

Peripheral motor neuropathy

In contrast to amyotropic lateral sclerosis, which is primarily a motor neuron disease with gradual loss of spinal motor neurons, peripheral motor neuropathy (PMN) affects the motor nerve axon or its myelin sheath. Sensory neuropathy may accompany PMN. Unlike in amyotropic lateral sclerosis, slight splenomegaly is present in PMN patients. Early immunosuppressive intervention is necessary to prevent irreparable damage. Protocol:

- *PMN-Th*
  - Pred: 10 mg 4× per week.
  - CsA: 4.5 mg/kg per day, 6 days per week.
  - Cy: 500 mg + 125 mg MP i.v. in a 15–20 min infusion with adequate hydration: once per month.
  - MTX: 15 mg i.m. per week, for 3 consecutive weeks out of 4, for 2 months; subsequently every other week.

- Folic acid: 5 mg per day on 3 days after MTX.
- ASA: 100 mg every evening.

Behçet's syndrome

BS is a multisystem inflammatory disorder with mucocutaneous manifestations (painful mouth and genital ulcers, erythema-nodosum-like lesions) and variable involvement of the eyes, joints, CNS, and gastrointestinal tract. With regard to ocular involvement the most common lesion is relapsing iridocyclitis, but the posterior segment may also be involved with choroiditis, retinal vasculitis, and papillitis. There is a 2:1 male:female ratio in BS. Response to immunosuppressive therapy is variable. Treatment regimens must be flexible and adjusted according to the severity of the disease. Protocol:

- *BS-Th*
  - Pred: 10 mg 5 times per week for 1 month; subsequently 4× per week.
  - CsA: 4.5 mg/kg per day, 6 days per week.
  - Colchicine: 1 mg per day, 6 days per week.
  - MTX: 15 mg i.m. per week for 1 month; subsequently every other week.
  - Folic acid: 5 mg per day on 3 days after MTX.
  - AZA: 100 mg per day, 6 days per week during MTX-free weeks.
  - Cy: 500 mg + MP 125 mg in a 15- to 20-min infusion with adequate hydration, once per month during MTX-free week. Cy is only added for patients not responding well to the combination of Pred, CsA, colchicine, MTX and AZA.
  - ASA: 100 mg every evening.

  Long-term maintenance treatment: protocol M-Th2 plus colchicine.

Sarcoidosis

Sarcoidosis is a multisystem granulomatous disorder characterized histologically by epitheloid tubercles without necrotic foci. The lungs may show diffuse infiltrates and mediastinal adenopathy. The spleen is always enlarged on percussion. Additional disease manifestations include granulomatous uveitis, arthritis, CNS (especially facial paralysis), and asymptomatic hepatic granulomas.

Immunohistologically sarcoidosis is characterized by granulomatous lesions involving APC ("epitheloid cells") and T and B lymphocytes. Drug-combination therapy must therefore be aimed at these cell types. Cortisone monotherapy has been shown to be useful, but ineffective in severe cases, particularly in patients with granulomatous uveitis. Furthermore, long-term cortisone monotherapy produces unacceptable side effects.

Remission induction: protocol R-Th2. Maintenance treatment: protocol M-Th2 for 1–5 years.

Antibody-mediated autoimmune diseases

The following conditions are mediated by a direct and selective antibody attack on the respective targets: autoimmune hemolytic anemia, autoimmune thrombocytopenia, pemphi-

gus (PEM), bullous pemphigoid (BPEM), and MY. The severity of the disease depends on quantity and quality of antibodies. Treatment is directed primarily towards the antibody-producing B cells but also towards the regulation of B cell activity via APC and T lymphocytes. Early intervention is again of utmost importance to prevent clonal expansion of antigen-driven autoreactive B cells. Clonal expansion not only increases the number of antibody-forming B cells but also modifies the nature of the antibodies. Ongoing somatic mutations lead to the selection of B cells with increased binding strength of antibodies to respective targets, thus augmenting the pathogenicity of the autoimmune process.

In very severe clinical conditions, rapid remission induction can be achieved by mechanical removal of antibodies and simultaneous inhibition of antibody production (protocol AB-Th1) Protocols:

- *AB-Th1*
  - Apheresis with removal of 3 l of plasma on days 1, 3, and 5.
  - Cy: 500 mg + 125 mg MP (or 40 mg DXM) on days 4 and 11, in a 15- to 20-min infusion with appropriate hydration.
  - Ig: 1 g/kg in slow i.v. infusion on day 5 or 6.

  Maintenance treatment as of day 12 is with protocol A5-Th2.

- *AB-Th2*
  - Pred: 20 mg, 5× per week. After 2–3 weeks, gradual diminution of doses to 10 mg, 5 times per week.
  - CsA: 4.5 mg/kg per day, 6 days per week.
  - MTX: 15 mg i.m. every other week.
  - Folic acid: 5 mg per day on 3 days after MTX.
  - AZA: 100 mg per day, 6 days per week during MTX-free weeks.
  - Cy: 500 mg + 125 mg MP in a 15- to 20-min infusion with adequate hydration, once per month during MTX-free week (only if above drug combination yields insufficient result).

Autoimmune hemolytic anemia

In Coombs' positive AIHA, antibody production may occur as part of a systemic autoimmune disease such as SLE, in the course of chronic lymphocytic leukemia, or as an "isolated" autoimmune condition. AIHA may be the first disease manifestation of SLE, sometimes years before the systemic development of SLE. The presence of hypergammaglobulinemia and/or of antinuclear antibodies is predictive of such a course. The antibody-coated red cells are eliminated in the spleen, which becomes enlarged as a consequence of massive phagocytosis of red cells. The degree of splenomegaly in idiopathic AIHA reflects the duration and importance of red cell sequestration.

Remission induction: protocol AB-Th1 is generally not necessary in early intervention in AIHA. The following remission-induction regimen may be sufficient:

- *AIHA-Th1*
  - DXM: 40 mg i.v. on days 1, 2, 4 and 6.
  - MP: After day 6, 125 mg i.v. every other day for 1–2 weeks.

  - Cy: 500 mg in a 15-min infusion on day 1 together with DXM, and on day 8 with MP.

Maintenance treatment protocol: AIHA-Th2 after day 6:

- *AIHA-Th2*
  - Pred: When i.v. MP has been stopped, oral administration of 20 mg, 5 days per week.
  - CsA: 4.5 mg/kg per day, 6 days per week.
  - Colchicine: 1 mg per day, 6 days per week.
  - MTX: initiation 1 week after second Cy infusion, 15 mg i.m. every other week.
  - Folic acid: 5 mg per day on 3 days after MTX.
  - ASA: 100 mg every evening.

Maintenance treatment is adjusted according to clinical response. Colchicine, if well supported, should be given for a long period of time, at least as long as the spleen is enlarged, and the number of reticulocytes is above 1.7% of red blood cells. Relapses may occur after years in remission.

Idiopathic thrombocytopenic purpura

Thrombocytopenic purpura without any apparent exogenous cause is relatively common, particularly in children with self-limiting thrombocytopenia. In fewer than one-third of children, but in the majority of adults, thrombocytopenia has a chronic course. ITP may be combined with AIHA or be part of a systemic autoimmune disease, especially SLE. It can actually be the first sign of SLE, years before it is diagnosed. Thrombocytopenia can also be part of the antiphospholipid syndrome. Splenomegaly, which is absent in ITP, indicates in all these systemic conditions the presence of an underlying connective tissue disorder.

Remission induction: protocol AB-Th1 or AIHA-Th1 may be used, depending on the severity of the condition. In severe childhood thrombocytopenia, high-dose immunoglobulin has been shown to yield positive results (400 mg/kg per day for 3–4 days, or 1 g/kg as a single infusion). In the past, splenectomy was frequently performed in the management of ITP. With the improvement in therapeutic means, splenectomy is now performed only in patients not responding to drug combination therapy. It is contraindicated in patients under 20 years of age.

Maintenance treatment: protocol AIHA-Th2 is also suitable for the management of ITP but without colchicine (this drug has not shown any therapeutic activity in the treatment of ITP).

Pemphigus

PEM is a potentially fatal skin disorder, which also affects (sometimes exclusively) the buccal mucosa. Autoantibodies are directed against desmogleine 3, which is responsible for the interconnection of epithelial cells in the skin and in the epithelium of the mouth. These antibodies can be revealed by an indirect diagnostic immunofluorescence test on skin sections; positive results can also be obtained with serum from patients with PEM

limited to buccal lesions. Today PEM can be brought under control with drug combination therapy, provided treatment is initiated at a very early stage of the disease. If the skin lesions are permitted to spread without treatment, the danger of infection increases, especially in patients on cortisone medication.

Remission induction: in severe cases with rapidly progressive skin lesions, protocol AB-Th1 represents a very effective approach. Protocol PEM-Th1 is less invasive. Protocol:

- *PEM-Th1*
  - MP: 250 mg i.v. on days 1 and 2, subsequently 125 mg on days 4, 6 and 8.
  - After day 8, maintenance treatment PEM-Th2.

MM, shown to be particularly effective in some PEM patients, should be included in long-term treatment regimens at doses tolerated by individual patients. Thal has also been useful in controlling disease activity. Protocol:

- *PEM-Th2*
  - Pred: 20 mg, 5 days per week for 2 weeks, subsequently 4 days per week, with gradual diminution of the daily dose from 20 to 10 mg.
  - CsA: 4.5 mg/kg per day, 6 days per week.
  - MTX: 15 mg i.m. every other week.
  - Folic acid: 5 mg per day on 3 days after MTX.
  - Cy: 500 mg + 125 mg MP in a 15- to 20-min infusion with appropriate hydration, once per month during an MTX-free week.
  - MM: 500 mg b.i.d., if well tolerated, t.i.d.
  - Thal: 100 mg 6 times per week for 1 month; subsequently every other week.
  - ASA: 100 mg every evening.

  Adjustment of treatment intensity according to diminution of disease activity.

Bullous pemphigoid

BPEM is a chronic bullous eruption seen mainly in the elderly. In this condition autoantibodies are directed towards the basement membrane of the epidermis. The mouth is never involved. BPEM is a much less severe disease than PEM. No special remission-induction procedure is generally necessary. Long-term treatment protocol:

- *BPEM-Th*
  - Pred: 15 mg 5× per week for 4 weeks, subsequently 4× per week with gradual diminution of daily dose from 15 to 10 mg.
  - CsA: 4 mg/kg per day, 6 days per week.
  - MTX: 15 mg i.m. every other week.
  - Folic acid: 5 mg per day on 3 days after MTX.
  - MM: 500 mg b.i.d., 6 days per week for 4 weeks, subsequently every other week.
  - AZA: 100 mg per day, 6 days per week, every other week.
  - Thal: 100 mg per day, 6 days per week for 4 weeks, subsequently every other week (weeks without AZA).
  - ASA: 100 mg every evening.

Upon remission, treatment should be gradually diminished. Thal should be the first drug to be stopped, subsequently according to individual drug tolerability.

Myasthenia gravis

MY is caused by autoantibodies against acetylcholine receptors of the neuromuscular junction. The disease may affect patients of any age, with two peaks, one in adolescents and young adults, the other in adults over the age of 40 years. The condition shows a strong relationship with tumors of the thymus, sometimes too small to be visualized on MRI. Today thymectomy is performed in most patients under the age of 50 years, also without detectable enlargement of the thymus. The results of thymectomy are unpredictable; in some patients it leads to improvement, in a few to permanent cure. In general, MY is a disease limited to the action of antibodies against the acetylcholine receptor. However, in a small proportion of patients, MY is part of a systemic autoimmune disease with arthralgia, splenomegaly, and presence of anti-nuclear antibodies. Clinical expression of MY chiefly concerns muscles innervated by cranial nerves with a variable disease intensity, from very mild to very severe.

In very mild cases treatment is in general limited to cholinesterase inhibitory drugs. In very seriously affected patients, with life-threatening signs of respiratory distress, remission-induction protocol AB-Th1 is indicated, possibly requiring more than three plasmapheresis sessions. Subsequently, protocol AB-Th2 may be applied with gradual diminution of medication according to regression of disease symptoms.

Maintenance treatment: protocol M-Th1 or M-Th2 may be sufficient. Simultaneous administration of cholinesterase-inhibiting drugs may be necessary (neostigmine or pyridostigmine).

Conclusions

During the past 50 years much has been accomplished in the definition and understanding of autoimmune diseases in terms of genetics, mechanisms of disease, and the role of environmental factors. The conditions for a rational treatment of autoimmune diseases thus seem to be at hand with the ever-increasing number of drugs acting at different levels of the immunopathological process. What then renders the clinical application of this scientific progress so difficult?

1. Generally in today's medical practice one expects strict treatment protocols. However, such protocols are not suited to the treatment of chronic autoimmune diseases, variable from patient to patient and even within the same patient during the course of disease.

2. Compartmentalization between the numerous fields of medicine has created great barriers between the various clinical specialties, rendering difficult the exchange of treatment experience between fields.

3. Consideration must be given to the fact that, for pharmacogenetic and/or environmental reasons, a given drug (with well-defined activity and toxicity) is not equally well accepted by all patients. Hence there is the need to use alternative drugs in cases of intolerance.

4. Diagnosis and treatment is commonly based on immunological parameters which represent humoral (B cell) markers. This is justified in autoimmune diseases such as AIHA,

where damage is caused by these antibodies. However, in the majority of autoimmune diseases there is a prevalence of T cell pathology for which no diagnostic T cell laboratory tests exist. In T cell dependent pathologies such as RA, systemic sclerosis, Sjögren's syndrome, and Wegener's granulomatosis, lack of B cell markers does not, as commonly thought, necessarily indicate absence of disease activity. With this misinterpretation, the danger exists that the opportunity for early therapeutic intervention will be missed. Conversely, persistence of these B cell markers does not necessarily indicate disease activity.

What can be done to improve the clinician's therapeutic approach to autoimmune pathologies? He must first abandon his belief in rigid treatment protocols. The principle of "flexible treatment regimens" must be understood and applied as the logical consequence of the great variability in autoimmune disease expression.

The creation of centers for the treatment of autoimmune diseases would permit a broad therapeutic approach to patients suffering from a multitude of autoimmune conditions. However, the compartmentalization of medicine in university and hospital structures makes such an undertaking impossible.

Transversal study groups could provide the infrastructure for the transfer of treatment results from one sector to another. As the most frequent autoimmune disorders are located within the territories of rheumatology and neurology, these two fields could become the core of such a transversal autoimmune group. Success of treatment depends to a great extent on active collaboration between patient and physician. In addition, an interdepartmental autoimmune study group could create an information center for patients, as has been done successfully for diabetics and hypertensives. Indeed, patients suffering from chronic diseases should be well informed about their illness and the action of the drugs prescribed for them.

Appendix

List of 43 autoimmune diseases

- Alopecia totalis
- Ankylosing spondyloarthritis
- Anticardiolipin syndrome
- Aplastic anemia
- Autoimmune hemolytic anemia
- Basedow's disease
- Behçet's syndrome
- Bullous pemphigoid
- Chronic active hepatitis
- Churg Strauss syndrome
- Cogan's syndrome
- Crohn's disease
- Cryoglobulinemia
- Dermatomyositis
- Discoid lupus erythematosus
- Hashimoto thyroiditis
- Idiopathic interstitial pneumopathy

- Idiopathic thrombocytopenic purpura
- Juvenile idiopathic arthritis
- Lambert-Eaton myasthenic syndrome
- Lichen planus
- Motor neuron disease with splenomegaly
- Multiple sclerosis
- Myasthenia gravis
- Nephrotic syndrome with splenomegaly
- Pemphigus
- Pericarditis, idiopathic with splenomegaly
- Polyarteritis nodosa
- Polymyalgia rheumatica
- Polymyositis
- Primary biliary cirrhosis
- Psoriasis
- Rheumatoid arthritis
- Sarcoidosis
- Sjögren's disease
- Still's disease
- Systemic lupus erythematosus
- Systemic sclerosis
- Takaiasu arteritis
- Ulcerative colitis
- Uveitis, idiopathic with splenomegaly
- Weber-Christian disease
- Wegener's granulomatosis

Abbreviations used in the text

AIH	autoimmune hepatitis
AIHA	autoimmune hemolytic anemia
APC	antigen-presenting cells
AS	ankylosing spondyloarthritis
ASA	acetylsalicylic acid
AZA	azathioprine
BPEM	bullous pemphigoid
BS	Behçet's syndrome
CAH	chronic active hepatitis
CD	cluster of differentiation
CsA	cyclosporine A
Cy	cyclophosphamide
DC	dendritic cells
DM	dermatomyositis
DP	D-penicillamine
DXM	dexamethasone
EBV	Epstein-Barr virus
HCQ	hydroxychloroquine

HCV	hepatitis C virus
HLA	human leukocyte antigen
IFN	interferon
IL	interleukin
IP	interstitial pneumopathy
ITP	idiopathic thrombocytopenic purpura
JIA	juvenile idiopathic arthritis
Lef	leflunomide
LKM	liver-kidney microsomal
MHC	major histocompatibility complex
MM	mycophenolate mofetil
MP	methylprednisolone
6-MP	6-mercaptopurine
MRI	magnetic resonance imaging
MS	multiple sclerosis
MTX	methotrexate
MY	myasthenia gravis
NSAID	nonsteroidal anti-inflammatory drug
PBC	primary biliary cirrhosis
PC	recurrent-remitting pericarditis
PEM	pemphigus
PMN	peripheral motor neuropathy
Pred	prednisone
PSS	progressive systemic sclerosis
RA	rheumatoid arthritis
RF	rheumatoid factor
SLE	systemic lupus erythematosus
SSZ	sulfasalazine
TCR	T cell receptor
Th	T helper
Thal	thalidomide
TNF	tumor necrosis factor
UC	ulcerative colitis
UV	idiopathic autoimmune uveitis

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