

# Premature Ovarian Failure and Ovarian Autoimmunity\*

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## I. Introduction

THE most important function of the immune system is to discriminate between 'self' and 'nonself.' The self needs to be protected, whereas the nonself must be destroyed. In some pathological processes the recognition of self is lost and the immune system starts to attack self, leading to a so-called "autoimmune disease."

Currently, the distinction between self and nonself is considered to involve a series of complicated and multistage interactions between various cells of the immune system. There is currently accumulating evidence that some cases of premature ovarian failure (POF)<sup>1</sup> are due to a faulty recognition of self in the ovary by the immune system.

POF or premature menopause is a syndrome clinically defined by failure of the ovary before the age of 40 yr (1). POF is a heterogeneous disorder with a multicausal pathogenesis, and chromosomal (2–9), genetic (4, 10–12), enzymatic (13–14), iatrogenic (15–20), or infectious (21–22) aberrations may all form the basis for the disappearance of ovarian follicles. These aberrations may influence the ovary at any stage of life, including the prepubertal, pubertal, or reproductive stages (23).

This review will primarily focus on the accumulating evidence of an abnormal self-recognition leading to ovarian autoimmunity in a proportion of patients with POF. This places

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<sup>1</sup> The following abbreviations are used: Ag(p), antigen (peptide); APC, antigen presenting cell; APGS, autoimmune polyglandular syndrome; BB, Bio Breeding; CD, cluster of differentiation; Cy-Ad-Abs, adrenal cytoplasmic antibodies; D, diversity; DC, dendritic cell; DTH, delayed type hypersensitivity; EPM1, Unverricht-Lundborg type of progressive epilepsy; HLA, human leukocyte antigen; IDDM, insulin dependent diabetes mellitus; IFN, interferon; Ig, immunoglobulin; IIF, indirect immunofluorescence; IL, interleukin; J, joining; MHC, Major Histocompatibility Complex; MIF, migration inhibiting factor; NK, natural killer; NOD, Non Obese Diabetic; NU, athymic nude; OS, Obese Strain chicken; POF, premature ovarian failure; PTK, protein kinase; ROS, resistant ovary syndrome; SLE, systemic lupus erythematosus; Smlg, surface membrane bound immunoglobulins; SSLP, single sequence length polymorphisms; St-C-Abs, steroid cell antibodies; TCR, T cell receptor; Th, T helper; TNF, tumor necrosis factor; V, variable; ZP, zona pellucida; 17- $\alpha$ -OH, 17-alpha-hydroxylase; 21-OH, 21-hydroxylase.

some cases of POF in the group of autoimmune diseases that affect hormone-producing glands, the so-called "autoimmune endocrinopathies," such as thyroiditis, insulin-dependent diabetes mellitus (IDDM), and Addison's disease.

## II. Definition and Clinical Presentation of Premature Ovarian Failure (POF)

Menopause (cessation of menses on the basis of ovarian failure) is in principle a physiological event. Women in western countries experience menopause at an average age of 50 yr (24–26). The number of primordial follicles decreases with age (27, 28), and the ultimate disappearance of primordial follicles in the ovaries is held responsible for the cessation of ovarian function in menopause. However, morphologically normal oocytes can be found in postmenopausal ovaries using electron microscopy (29), indicating that such disappearance cannot be the sole factor playing a role in the development of the menopausal state. It has, for instance, been hypothesized that the remaining follicles in perimenopausal women are less sensitive to high levels of gonadotropins (30).

POF was defined by de Moraes-Ruehsen and Jones in 1967 (1) as an unphysiological cessation of menses before the age of 40 yr and after puberty (hence, in fact, secondary amenorrhea). Women with POF have a hypergonadotropic-hypoenestrogenic hormone profile. By 1939, the endocrinological profiles of the syndrome had been recognized on the basis of elevated levels of urinary gonadotropins (31). The clinical picture of POF was first described in detail in 1950 by Atria (32). This author reported 20 young women under the age of 35 yr with secondary amenorrhea, hot flushes, infertility, and an atrophic endometrium. In retrospect, these cases presumably were cases of POF, although at that time confirmatory gonadotropin assays were not routinely performed.

Patients with POF are mainly troubled by infertility due to the cessation of ovarian function. They have a typical menstrual history of normal age at menarche (33, 34) followed by regular periods. The disease thereafter presents either with oligomenorrhea or abrupt amenorrhea. Presently, amenorrhea due to POF is also seen after termination of oral contraception (35–37). A family history of POF is incidentally obtained (10–12, 37). Fifty percent of patients with POF experience vasomotor symptoms, such as hot flushes and sweating boosts (37–39) due to the hypoenestrogenic status. Other troubling symptoms are atrophy of the vagina and the urogenital tract, leading to vaginitis, dyspareunia, and cystitis.

The diagnosis of POF rests upon the clinical picture and the demonstration of elevated gonadotropin levels. The level of FSH is disproportionately higher than that of LH (40). Serum levels of FSH greater than 40 IU/liter are the hallmark of the diagnosis. Serum gonadotropin determinations should be repeated at least two or three times to be certain of the diagnosis because serum gonadotropin levels may wax and wane (41–43). POF presents itself not as an all-or-none phenomenon, and the precise timing of onset is often impossible to determine. The disease may have a fluctuating course, with high gonadotropin levels that later return to normal, and a later regain of ovulatory functions and even pregnancy

(44–46). Nelson *et al.* (47) examined 65 POF patients by weekly estradiol sampling and sonography. In 50% of the cases, follicular activity could be demonstrated, and 16% of cases regained ovulatory function. Follicle biopsies were carried out in six patients, and these showed luteinized Graafian follicles (47). Alper *et al.* (44) reported that 7.5% (six of 80) patients were able to conceive after a diagnosis of POF.

The incidence of POF in a population under the age of 40 yr is estimated to be 0.9% (48). The choice of 40 yr as the age that separates premature from normal menopause is arbitrary. If one were to define abnormality as those values less or greater than 2 SD from the mean age of menopause (where  $\pm 95\%$  of the observations of a normally distributed variable are found), then the age of 43 would be the most appropriate lower age limit for the natural cessation of menses.

Kinch *et al.* (49) were the first to identify two histopathological types of POF: the afollicular and the follicular form. In the afollicular form, there is a total depletion of ovarian follicles and hence a permanent loss of ovarian function. Such total depletion of ovarian follicles is mainly due to gonadal dysgenesis, mixed gonadoblastoma, and hermaphroditism [reviewed by Coulam (23)]. Genetic and chromosomal abnormalities are one of the most well known causes of germ cell maldevelopment and disappearance (2). Such an accelerated loss of oocytes is considered to be the cause of POF in individuals with a 47,XXX and 45,XO and 45,XO mosaicism (2, 9). Lack of migration of sex cells or faulty differentiation of the gonadal ridges lead to streak ovaries or, in some cases, to POF, depending on the actual number of primordial follicles that result.

In the follicular form, follicular structures are still preserved and hence a possibility of either spontaneous or induced return of ovarian function exists. The follicular form can be subdivided into: 1) oöphoritis (inflammation of follicles); 2) ovaries with a few follicles present; and 3) ovaries in which numerous primordial follicles are present [the so-called resistant ovary syndrome (ROS)] (50).

Although the histological classification suggests a sharp division between the follicular and afollicular forms, there is evidence that some cases of POF that were originally of the follicular type may progress to an afollicular stage. This is particularly the case in blepharophimosis (51, 52), galactosemia (53), and in the animal models of autoimmune oöphoritis (see below).

Recent research suggests that ovarian autoimmunity is a possible cause of both afollicular and follicular forms of POF. This review will list the arguments, pro and con, to such a view. At first, however, a short introduction into the cells involved in the immune response and immunological principles of self- and non-self-recognition will be given. This information will provide the background necessary to understanding these arguments.

## III. Cells Involved in the Immune Response

### A. Antigen presentation and antigen-presenting cells, in particular dendritic cells

An immune response against nonself- and self-antigens is initiated by presentation of the antigen in a suitable form to

T cells. Antigen can only be presented to T cells in the context of molecules of the major histocompatibility complex (MHC) (54). Hence, practically each nucleated cell of the body is able to present antigen, first by virtue of a constitutive MHC class I expression, and second by a *de novo* expression of MHC class II molecules on the surface of the cell, induced by f.i. interferon- $\gamma$  (IFN- $\gamma$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) exposition (55). Macrophages, B cells, and particularly dendritic cells (DC) are, however, the most efficient (professional) antigen-presenting cells (APC) due to a variety of factors, among which are the constitutive expression of MHC class II molecules, the expression of costimulatory and adhesion molecules, and other characteristics such as motility (56).

The genes encoding for both MHC class I and II molecules are members of the immunoglobulin supergene family. These genes in the human species are arranged on chromosome 6 [reviewed by Weetman (57)]. MHC class I genes encode for the human leukocyte antigens (HLA) A, B, and C. MHC class II genes encode the HLA DP, DQ, and DR antigens. The encoded HLA molecules are dimers and comprise an  $\alpha$ - and  $\beta$ -chain (Fig. 1). The  $\alpha$ -chain of the MHC class I molecules is encoded in the MHC genes; the  $\beta$ -chain is termed  $\beta$ 2-microglobulin and is encoded on a separate chromosome.

The overall structures of the class I and II MHC molecules are comparable (58). The molecular confirmation of the chains forms a groove in which the antigenic peptide is presented. Thus the ability of antigenic peptides to be associated with class I or class II MHC molecules is governed by the actual molecular confirmation (tertiary structure) of the

antigen-binding groove. It is therefore not surprising that organisms with a particular genetic makeup of MHC class I and II molecules have a special capacity to generate immune responses toward specific microbial and self-antigens.

An additional molecule, known as the invariant chain, is intimately involved in the biology of HLA class II molecules. The invariant chain is a membrane glycoprotein, encoded by a non-HLA gene on chromosome 5. The "invariant" designation stems from the observation that, in contrast to the extensive polymorphism of some class II  $\alpha$ - and all class II  $\beta$ -chains, the invariant chain is nonpolymorphic. It forms a trimer with the class II  $\alpha$ - and  $\beta$ -chains in the endoplasmic reticulum during biosynthesis of the MHC class II molecules and directs the trafficking of the trimer through the post-translational machinery of the cell to the endosomal compartment (Fig. 2). Current evidence indicates that the invariant chain also prevents peptides from binding in the class II groove until the class II molecule is delivered to the endosome. The invariant chain then dissociates from the class II molecule, which can consequently bind antigenic peptides processed from exogenous antigens taken up by the APC and degraded in its lysosomal compartment (Fig. 2). The latter fuses with endosomes (59–61). The complex of the class II molecule with its bound peptide is then transported to the cell membrane (Fig. 2); however, the mechanism of transport

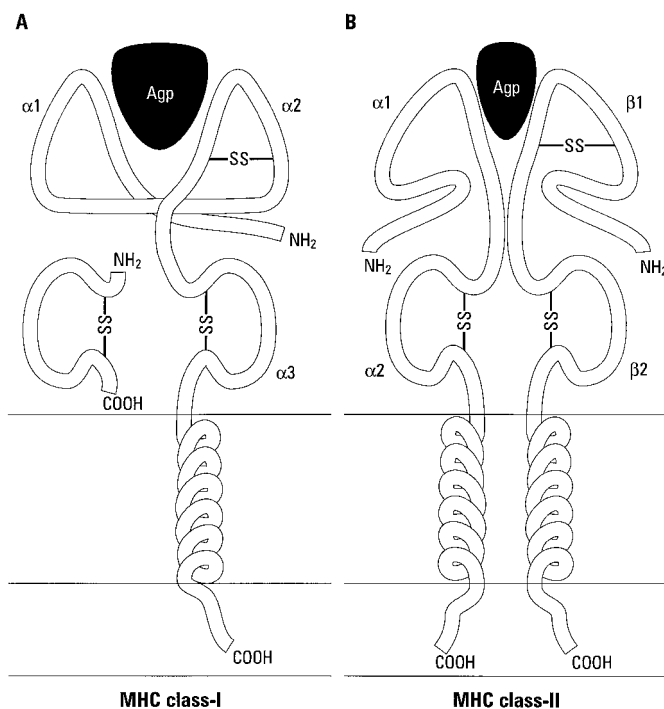


FIG. 1. The structure of MHC-class I (A) and MHC-class II (B) molecules within the binding groove the antigenic peptides (Agp) [Reproduced with permission from the authors from: Benner R, van Dongen JJM, van Ewijk W, Haaijman J (eds) *Medische Immunologie*. Bunge, Utrecht, The Netherlands, 1996].

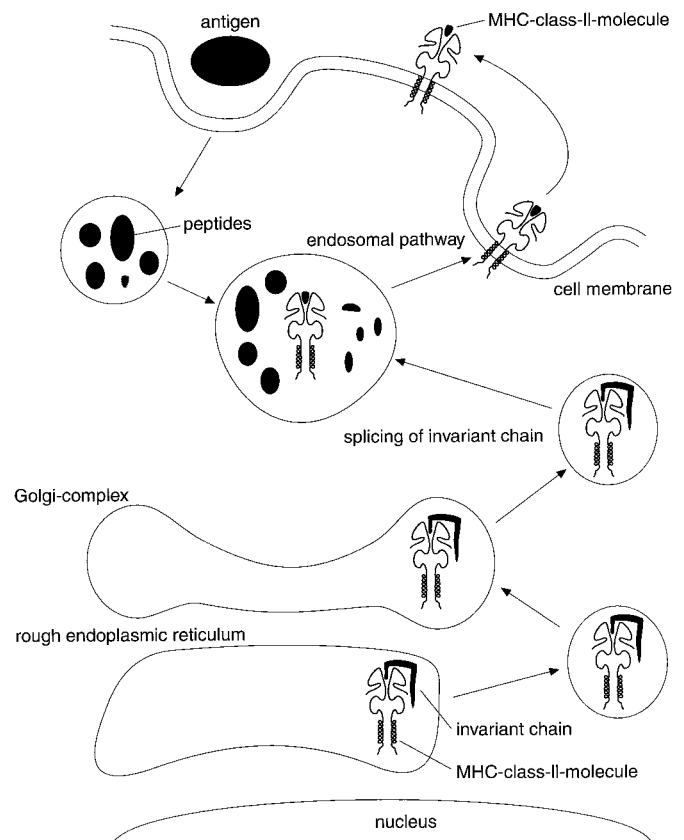


FIG. 2. The processing of exogenous antigens to result in the positioning of antigenic peptides in the groove of the MCH-class II molecule [Reproduced with permission from the authors from: Benner R, van Dongen JJM, van Ewijk W, Haaijman J (eds) *Medische Immunologie*. Bunge, Utrecht, The Netherlands, 1996].



is still unclear. Due to this intracellular pathway, exogenous antigens are mainly presented in association with MHC class II molecules (62). Peptides associated with MHC class II molecules can only be presented to CD4<sup>+</sup> T cells (63), because the CD4 molecule is a special receptor for the MHC class II molecules (Fig. 3).

Endogenous antigens, *e.g.* viral antigens, are degraded by the low molecular mass polypeptide complex present in the cytoplasm (64). Peptides are then delivered to the MHC class I molecules in the lumen of the endoplasmic reticulum. After incorporation of the antigenic peptides into the groove and exposure of the MHC class I molecules on the cell surface, only CD8<sup>+</sup> T cells are able to recognize such peptides, because the CD8 molecule is the special receptor for MHC class I molecules (Fig. 3). After recognition, CD8<sup>+</sup> T cells are able to kill the cell presenting the endogenous antigen (54, 65). Neither class I nor class II MHC molecules can distinguish between self and nonself (66, 67). It must also be noted that the preferential association of exogenous antigens with MHC class II molecules, and endogenous antigens with MHC class I, is not absolute (62, 68).

The complex of MHC molecule-antigenic peptide-T cell receptor (TCR) is insufficient for an adequate activation of

the T cell. For full activation, the interaction of other accessory molecules on APC with their ligands on T cells is needed, such as the interaction of adhesion molecules (69–71). The binding produced by these adhesion molecules predominantly strengthens the interaction between the MHC-antigenic peptide-TCR interaction, but also transduces signals that activate the T cell. Important adhesion molecules are leukocyte function antigen 1 (LFA-1), which interacts with intercellular adhesion molecule 1 (ICAM-1), and leukocyte function antigen 3 (LFA-3), which interacts with CD2 (70, 71) (Fig. 3). Inhibition in this process by monoclonal antibodies to either one of these adhesion molecules inhibits the activation and clonal expansion of T cells (69).

Apart from the adhesion molecule-ligand interaction, the interaction of so-called “costimulatory molecules” on APCs and T cells is essential for further T cell activation and T cell clonal expansion (Fig. 3). If these costimulatory signals are not provided, the result is T cell anergy [a state of specific nonresponsiveness of T cells (72)]. Costimulating signals are predominantly provided by the binding of the B7-1 (CD80) molecule on the APC (73, 74) to the CD28 molecule on the T cell. Additional binding of T-lymphocytic CTLA-4 (cytolytic T lymphocyte-associated antigen) to B7-2 (CD86) molecules on the APC also takes place but it occurs probably later in the process, because CTLA-4 is primarily seen on the T cells after activation (75).

DCs are unique APCs in that they are the only APCs that are able to effectively stimulate naive (CD45RA<sup>+</sup>) T cells (76–78). Recent investigations led to the idea that the DC population is heterogeneous with respect to ontogeny (79). It is certainly heterogeneous with respect to morphology, the expression of adhesion molecules (80–83), and in cytokine production. With regard to ontogeny, part of the DC populations is monocyte-derived and closely associated with macrophages, whereas other DCs may have a separate precursor [this subject was extensively reviewed by Kamperdijk *et al.* (84)]. DCs are found in virtually all tissues and organs of the body. The DC of the epidermis and dermis is known as the Langerhans cell. Langerhans cells contain the peculiar Birbeck granules that are not seen in DC in other organs, apart from the thymus. Langerhans cells of the skin and DC of the gut wall are considered as early (immature) stages in the differentiation of the cell, with a superb capacity to pick up antigens and to degrade these to antigenic peptides and place these peptides in the groove of the MHC molecules. Skin Langerhans cells and gut DCs have been shown to migrate into the afferent lymph as veiled cells to the skin- and gut-draining lymph nodes (Fig. 4). These cells can be seen as interdigitating cells in the T cell areas of these draining lymph nodes, and these stages of the DC are considered as mature stages of the cell with a superb capacity to stimulate T cells (85). It seems likely that DC from other organs, like the heart, kidney, and endocrine organs, may undergo similar migration and maturation.

Whereas macrophages are a clear source of cytokines such as interleukin-1 (IL-1), IL-6, and TNF- $\alpha$ , DC have been shown to produce the mRNAs of these cytokines, without a noteworthy production of the actual products (84, 86, 87). In general, DCs are regarded as poor producers of cytokines, and their excellent APC function lies probably in their mi-

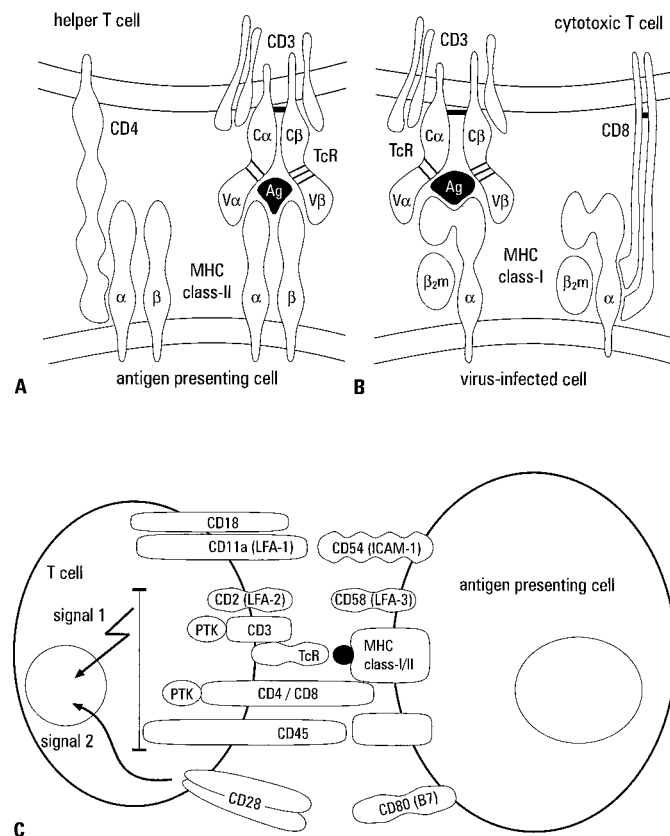


FIG. 3. The interactions between APC (including virus-infected cells) and T cells at the level of antigen-specific interactions, adhesion molecules, and costimulatory molecules. Panels A and B represent interactions between MHC molecules, the TCR, and CD4/CD8 molecules. Panel C depicts interactions between various adhesion molecules (ICAM-1/LFA1, LFA-3/LFA-2, CD45/unknown) providing signal 1 for T cell stimulation, and interactions of the costimulatory molecule CD80(B-7)/CD28, providing second signals (for abbreviations see Table 1).

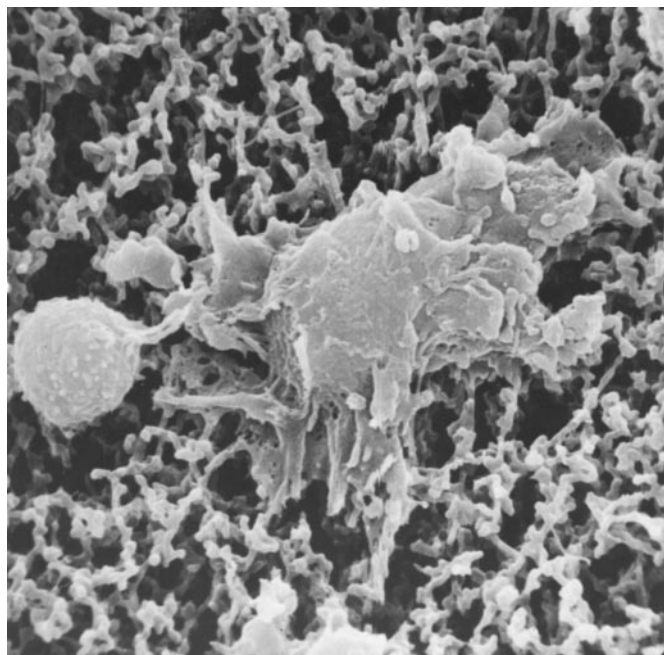


FIG. 4. A scanning electron microscopy photograph of a DC in lymph in contact with a lymphocyte (5000 $\times$ )(pig).

gratory capacity, their capability to form clusters with T cells via adhesion molecules (88, 89), and their high expression of costimulatory molecules such as B7-1 and B7-2 (CD80/CD86) (90).

It is as yet unresolved whether the DC needs other cytokine-producing accessory cells to guide the generated clonal expansion of naive T cells in a certain direction of development. IL-12 is known to be produced by macrophages, and this cytokine is able to push the development of T cells into T cells that predominantly produce IFN- $\gamma$  (91); however, IL-10 (also produced by macrophages) down-regulates such development (92, 93).

### B. T cells

The ability of the immune system to specifically recognize all varieties of possible antigens is based on the enormous diversity of the antigen-specific receptors present on the T cells (TCR) and the enormous diversity of the surface membrane-bound immunoglobulins (smlg-receptors) on the B cell (94–97). When the TCR fits with the antigenic peptide in the groove of the MHC molecule, lymphocyte activation and clonal expansion will be initiated (95–97) provided the sufficient adhesion molecule and costimulating signals are given. A specific TCR can bind only one form of an antigenic peptide, and this will consequently lead to cell division of this type of TCR-specific lymphocytes. This is referred to as clonal expansion.

The TCR is composed in the majority of cases of an  $\alpha$ - and  $\beta$ -chain (96–98) or in a minority of cases of a  $\gamma$ - and  $\delta$ -chain (99–102). The various chains of the TCR are encoded by different gene segments: variable (V), diversity (D), joining (J), and constant (C) gene segments. The V, D, and J gene segments form a large repertoire. The enormous diversity of the TCR is produced by the recombination of the various V,

D, and J genes from this large repertoire during T cell maturation (103,104). Thus, the capacity to react with all possible antigenic peptides is genetically programmed and created by germ line rearrangements and somatic mutations. The TCR is noncovalently linked to a series of transmembrane proteins called the CD3 complex (Fig. 3) (98, 102, 105, 106). Both CD4 and CD8 molecules on the T cells act as coreceptors for the MHC class II and MHC class I molecules, respectively, during the interaction of the TCR with the peptide-MHC complex (107, 108). The entire CD3/TCR/MHC-II/CD4 complex or the entire CD3/TCR/MHC-I/CD8 complex is involved in signal transduction (Fig. 3).

The identification and classification of various T cells (Table 1) is based on the expression of the CD3 complex, the coexpression of either CD4 or CD8 molecules, and the composition of the TCR (genetic makeup of various V, D, and J genes).

Over the past few years it has become clear that the population of CD4<sup>+</sup> T cells can functionally be divided into two subsets based on their profile of cytokine production (109, 110) (Fig. 5). One subset predominantly produces IFN- $\gamma$ , but also IL-2; this is the so-called Th1 subset. The other subset, Th2, produces predominantly IL-4 and IL-5. The functional significance of these different cytokine production profiles is that they represent different T cell-regulatory actions. Th1 cells and their cytokine products stimulate macrophages and hence cell-mediated immunity and macrophage-mediated cellular destruction. Th2 cells and their cytokine products stimulate B cells and hence lead to the humoral immune response. It must be noted, however, that the Th1 and Th2 subtypes represent extremes. There are many CD4<sup>+</sup> T cells clones with a cytokine production profile intermediate between Th1 and Th2 cells. The driving of CD4<sup>+</sup> T cells (Th cells, Fig. 5) into either the direction of Th1 or Th2 is guided in a complicated network by the cytokines IL-1, IL-12, IL-10, IFN- $\gamma$ , IL-4, and products of arachidonic acid metabolism (91–93, 111–115).

### C. B cells

B cells are generally identified by means of the expression of Smlg-receptors on their cell surface or by the expression of B cell-specific molecules (Table 1). When the Smlg-receptor of a B cell recognizes the antigen against which it is directed, and when sufficient additional stimulatory signals are provided (see below), proliferation will occur. The generated B cells will thereafter differentiate into plasma cells that start to secrete immunoglobulins with a specificity similar to that of the earlier membrane-bound form of immunoglobulin.

When antigen-specific B cells and activated T cells recognize the same antigen or a peptide thereof, a so-called "cognate interaction" occurs (Fig. 6). The B cell uses its Smlg receptor for uptake and concentration of the antigen (116). The antigen is processed and the antigenic peptides are presented on the B cell surface in the groove of the MHC class II molecules to the antigen-specific T cell. When the cognate interaction activates the T cell to produce and release the cytokines IL-4 and IL-5 (Th2 pathway), the B cell is stimu-

TABLE 1. CD markers, respectively, for T cells, B cells, monocytes/macrophages, dendritic cells and NK cells

CD	Name	Function
<b>T cells</b>		
CD2	T11 antigen; LFA-2	Receptor for T cell activation; ligand for LFA-3
CD3	T3 antigen	Associated with TCR; signal transduction from TCR to cytoplasm
CD4	T4 antigen	Involved in MHC-class II restricted antigen recognition
CD8	T8 antigen	Involved in MHC-class I restricted antigen recognition
CD11a	LFA-1 antigen	Adhesion molecule binds to ICAM-1 and ICAM-2
CD25	Tac antigen	IL-2 receptor/activation T cells (B cells and macrophages)
CD28	Tp44 antigen	Receptor for B7/BB-1 antigen on activated B cells/T cells proliferation
CD45	LCA; T200 antigen	Function unknown, common leukocyte antigen
CD45RO	Restricted LCA	Activated (memory) T cells
CD45RA	Restricted LCA	Virgin T cells, monocytes
<b>B cells</b>		
CD5	T1 antigen	Function in T cell proliferation, unknown function in B cells
CD19	Pan-B cell antigen	Function in B cell activation
CD20	B cell antigen	Function in B cell activation
CD22	B cell antigen	Function in B cell adhesion and activation
SmIg	Surface membrane immunoglobulin	Binding molecule for antigens
<b>Monocytes/macrophages</b>		
CD11b,c	Adhesion molecule on monocytes/macrophages	MAC-1 antigen, p150-95 antigen; associated with CD18 antigen/adhesion molecule
CD14	Monocytic antigen	LPS receptor
CD68	Macrophage antigen	Function unknown
<b>Dendritic cells</b>		
CD1	T6 antigen	MHC-like protein (antigen-presentation)
CD83	HB15 molecule	Function unknown
S100		Intracellular growth factor
<b>NK cells</b>		
CD16	Fc $\gamma$ R111	Low affinity Fc receptor for IgG (also present on macrophages)
CD56	NCAM	Function unknown
CD57	Human natural killer cell antigen	Function unknown

CD, Cluster of differentiation; CR, complement receptor; ICAM, intercellular adhesion molecule; IgG, immunoglobulin G; LCA, leukocyte common antigen; LFA, leukocyte function antigen; LPS, lipopolysaccharide; MHC, major histocompatibility complex; NCAM, neural adhesion molecule; TCR, T cell receptor. [Derived from Ref. 176.]

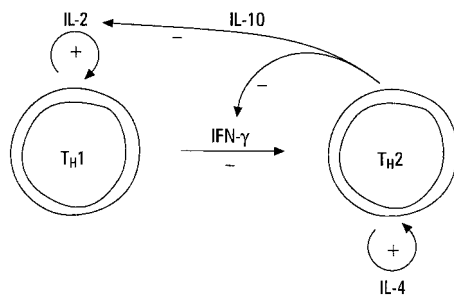


FIG. 5. The dichotomy in the action of CD4<sup>+</sup> T cells in the Th1 and Th2 pathway and the action of various cytokines in the skewing of these pathways (+, activation; -, suppression).

lated to clonally expand and differentiate into a plasma cell to produce specific antibodies (117).

SmIg receptors on B cells show, like the TCR, a specificity toward antigens that is already programmed and created by germline gene rearrangement and somatic mutations (103,104). An enormous variety of antibodies can thus be produced. Antibodies are composed of two Ig heavy chains and two Ig light chains held together by disulfide bonds (118). Antibodies play an important role in the neutralization of bacterial toxins and in the opsonization of microorganisms for phagocytosis.

Antibodies are produced in different isotypes (IgA, IgG,

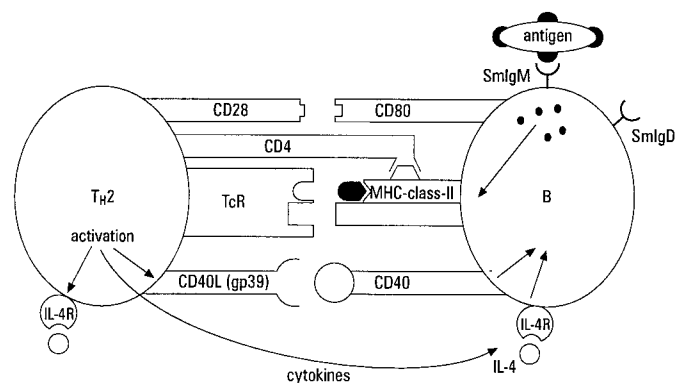


FIG. 6. The cognate interaction between Th2 cells and B cells to result in the specific stimulation of antigen-specific B cells. [Reproduced with permission from the authors from: Benner R, van Dongen JJM, van Ewijk W, Haaijman J (eds) *Medische Immunologie*. Bunge, Utrecht, The Netherlands, 1996.]

IgM, IgE, IgD). The isotype is important in determining whether an antibody will fix complement (118). The majority of B cells in the peripheral blood express IgM and IgD on their cell surface, whereas a few express IgG or IgA. Secreted IgM antibodies are of low affinity and polyspecific. Secreted IgG and IgA antibodies are of high affinity and high specificity and are typical of secondary immune responses. The



switch in immunoglobulin heavy chains from IgM and IgD to IgG or IgA is referred to as the isotype switch (119). This isotype switch is mediated by gene rearrangement in which the V region is coupled to another C region (119, 120). Autoantibodies of the IgG isotype are characteristic of certain pathological autoimmune reactions. To induce such antibodies, the help of autoantigen-specific Th2 cells is a prerequisite.

#### D. Effector cells in immune responses

Effector cells in immune responses are macrophages, natural killer (NK) cells, and cytotoxic CD8<sup>+</sup> T cells. Macrophages form a heterogeneous population. Apart from immune regulation, certain subsets of macrophages act as important scavenging cells. They are able to endocytose and phagocytose microorganisms and cellular debris (121), as well as exerting cytotoxicity against microorganisms or tumor cells (122). The main function of macrophages is considered to be phagocytosis. Phagocytosis of microorganisms and cellular debris is greatly enhanced by opsonization of the material by specific antibodies, as well as the capability of the Th1 cytokine IFN- $\gamma$  to strongly activate the cytotoxic properties of macrophages, increasing their efficiency in killing the microorganism (123). Macrophages also play a role in wound healing (124, 125) and in the regulation of hemo- and lymphopoiesis (126, 127). Histologically classic macrophages are large cells that show lamellapodia and vacuoles and possess an irregular, indented nucleus (128). They stain for nonspecific esterase and acid phosphatase throughout the cytoplasm.

Though precursors of macrophages certainly reside in the monocyte pool the origin of all macrophages is not completely elucidated. A separate precursor in the bone marrow may exist for some subpopulations of macrophages (128). The monoblast and promonocyte remain in the bone marrow very briefly before entering the blood stream as monocytes (129). These latter cells migrate into the tissues where part of the cells mature and differentiate into various lines of macrophages such as the Kupffer cells, the osteoclasts, and the histiocytes. There is not one monoclonal antibody that recognizes all the lines and maturation stages of macrophages and that does not show a cross-reactivity with other hematopoietic cells. The lack of a common marker for all the subpopulations of macrophages is inherent to their functional heterogeneity. In human studies, macrophages are normally identified by specific CD markers (Table 1).

In exerting their various functions, macrophages are able to produce a variety of signaling molecules, such as the cytokines IL-1, IL-6, granulocyte-macrophage-colony-stimulating factor (GM-CSF), and TNF- $\alpha$  (128). Metabolites of arachidonic acid metabolism, nitric oxide and oxygen radicals, are also important products for the regulation of the immune response and the degradation of ingested material.

NK cells and CD8<sup>+</sup> cytotoxic T cells are other important immune cells in the effector arm of the immune system. NK cells do not express conventional antigen receptors, such as the TCR or SmIg-receptors, and the genes for these receptors remain unrearranged (130, 131). They do express the receptor for the Fc part of the IgG molecule, the Fc $\gamma$ RIII (CD16) (132, 133). Other important molecules expressed by NK cells in-

clude CD56, a neural adhesion antigen, and the  $\beta$ -chain of the IL-2 receptor. This allows resting NK cells to respond directly to IL-2 (134). The main function of NK cells is to provide nonspecific cytotoxic activity toward virally infected cells and tumor cells (135, 136). They do so by releasing perforin (pore forming) and serine proteases (137). NK cells, like macrophages, can also kill specifically if provided with an antibody. The process, known as antibody-dependent cellular cytotoxicity, occurs via binding of the antibody to the Fc receptor (CD16). The ontogeny of NK cells is only partially understood. Although NK cells express a number of membrane antigens in common with T cells and share functional properties with some T cell subsets, suggesting a common origin, NK cells are found in the fetus before the development of T cells or of the thymus. In addition, NK cells appear to develop normally in nude, athymic mice (135). Recent studies have indicated that NK cells can arise from triple negative (CD3<sup>-</sup>/CD4<sup>-</sup>/CD8<sup>-</sup>) thymocyte precursors that are CD56<sup>+</sup> but do not express CD34 or CD5 (138). It must also be noted that NK cells are not only considered as effector cells in the immune response, but also as regulator cells. They are sensitive to activation by IL-12, produce  $\gamma$ -IFN that activates the TH1 response, and are polyclonal activators of B cells (139).

Cytotoxic T lymphocytes consist of mature T cells that are usually, but not always, CD8<sup>+</sup>. They exert cell contact-dependent cytotoxic functions through a perforin-dependent pathway (140). The cells also release the cytotoxic cytokine TNF. The perforin-dependent pathway is largely responsible for the T cell-dependent cytotoxic clearance of virus-infected cells and for rejection of tissue grafts and tumors (141).

## IV. Tolerance to Self

The main function of the immune system is to distinguish between self and nonself. In healthy individuals, reactivity toward self is nowadays considered to be a normal event that is controlled by several down-regulating mechanisms. Malfunction of these down-regulating mechanisms may result in an undesired excessive immune reaction toward self, *i.e.* an autoimmune disease. Among the best studied controlling mechanisms are the following: clonal deletion in the thymus, clonal anergy, and active immunosuppression by self-reactive subsets of T and B cells (142).

### A. Clonal deletion

T cells mature in the thymus from prothymocytes to mature T cells. Because TCR rearrangement is random, self-reactive T cells are generated in this process. However, the vast majority of self-reactive T cells are deleted during further maturation in the thymus, the so-called "clonal deletion" (143, 144). DCs occurring in great numbers at the corticomedullary junction of the thymus express self-antigens and are responsible for this deletion. Clonal deletion depends upon recognition of the self-antigenic peptides by not fully matured T cells, which, upon the antigenic recognition signal, do not proliferate but go into apoptosis (145). A similar mechanism of deletion may exist for self-reactive B cells in

the bone marrow (146). This mechanism is, however, not so well studied as clonal T cell deletion in the thymus.

Clonal deletion for T and B cells is, however, incomplete, and T and B cells with a specificity for autoantigenic peptides and autoantigens can easily be found in the circulation (147). This is partly explained by the fact that not all self-antigens are expressed in the thymus and bone marrow. Some self-antigens, such as ocular lens antigens, are sequestered from the immune system. Other self-antigens, such as sperm-antigens, are only expressed during late fetal life or only in adult life. Some autoantigens probably never reach the thymus or bone marrow and are never expressed there. This particularly applies to cryptic epitopes. Cryptic epitopes are *de novo* expressed epitopes on self-antigens that are caused by changes in the antigen after, for instance, an inflammatory process.

### B. Clonal anergy

When autoreactive B and T cells have escaped clonal deletion, a second control mechanism, namely the process of clonal anergy, should come into operation. This process takes place predominantly in the periphery (148–150). The induction of an immunologically anergic state of the T cell is supposed to be due to a lack of provision of sufficient second signals by APCs. Hence, when antigen is presented to T cells by nonprofessional APC, such as MHC class II-positive epithelial cells, clonal anergy will occur. Late in organ-specific autoimmune diseases (*e.g.* in autoimmune thyroiditis and IDDM) there is an aberrant expression of MHC class II molecules on the epithelial cells of the endocrine tissues (151). Initially, this aberrant expression of MHC class II molecules was interpreted as an impetus for the increased self-reactivity (152). However, this aberrant MHC class II expression late in organ-specific autoimmune disease can also be considered as a sign of induction of clonal anergy (151, 153).

### C. Active immunosuppression

When clonal deletion and clonal anergy have failed, yet another down-regulating mechanism should come into operation, namely active immunosuppression exerted by so-called “suppressor” immune cells. These suppressor immune cells do not only include CD4<sup>+</sup> and CD8<sup>+</sup> T cells (154, 155), but also suppressor macrophages. The cells involved in immune suppression may be antigen-specific or non-antigen-specific. They may also operate in an idiotype-antiidiotype network (155). How active immune suppression is regulated remains unclear. Taken together, earlier and recent evidence suggests that in each individual a balance exists between autoreactive effector and suppressor immune cells. In the healthy state this balance tips over in favor of the suppressor forces, whereas in the autoimmune diseased state the balance is in favor of the self-reactive effector forces (see also later animal models of autoimmune oöphoritis).

### D. Balance between Th1 and Th2 pathways

A recently developed theory approaches the problem of the control of self-reactivity from yet another angle. Endocrine autoimmune diseases with an ultimate failure of the

target gland, such as IDDM, are predominantly caused by Th1-mediated pathways in which the endocrine cells are destroyed by  $\gamma$ -IFN-activated scavenger macrophages. The recently developed theory emphasizes the reciprocal relation between the Th1 and Th2 pathways (109, 156) and suggests that if the Th1 pathway is diverted into the Th2 pathway that the Th1-mediated autoimmune reactivity is dampened. In essence, tolerance to self is not restored, but the harmful autoimmune reaction is diverted to a less harmful one. There are indeed reports on cytokine treatments that are able to induce such a switch from Th1 to Th2 pathways, resulting in an amelioration of the endocrine autoimmune disease. Circulating antibodies, whose production is switched on by the stimulation of the Th2 cells, apparently contribute little to the damage of the target cells. It is known that endocrine autoantibodies may exist for years in the circulation before endocrine autoimmune disease develops (157, 158).

## V. Autoimmune Endocrine Disease: Developmental Stages and Genetic Predisposition

Before presenting the pros and cons for considering POF as a possible endocrine autoimmune disease, it must be noted that there are similar failures of endocrine organs that are currently classified as autoimmune destructive diseases. These endocrine failures include hypothyroidism (thyroid failure), IDDM (failure of the islets of Langerhans), and Addison's disease (failure of the adrenal cortex). The pathogenesis of the failure of these endocrine tissues has mainly been studied in animal models of the spontaneous forms of these autoimmune endocrinopathies. The obvious reason for this approach is that in the patients, tissues, cells, and sera are difficult to obtain and only then in the progressed stages of the disease.

The animal models for autoimmune disease of the thyroid are the Obese Strain of chicken (OS chicken) (159), the Bio Breeding (BB) rat (160), and certain strains of the Non Obese Diabetic (NOD) mouse. The BB rat and NOD mouse also suffer from an autoimmune insulinitis leading to IDDM. Animal models for spontaneous autoimmune adrenalitis and oöphoritis are lacking. Only manipulations of normal mice (immunization with crude adrenal and ovarian extracts, and thymectomy plus cyclosporin A treatment) will lead to autoimmune adrenalitis and/or oöphoritis (161). A word of caution is necessary when trying to extrapolate data obtained in the animal models to the human situation: the animal models clearly show exaggerated and extreme forms of thyroiditis and insulinitis, which already differ between the models themselves (let alone from patients), indicating a heterogeneity of the disease process. Hence, general conclusions drawn on the basis of studies in one animal model should always be verified in other animal models and certainly in human patients.

The animal models of insulinitis and thyroiditis indicate that the pathogenesis of the autoimmune failure of an endocrine gland is a multistep process, requiring several genetic and environmental abnormalities to come together before full-blown autoimmune thyroiditis and/or insulinitis develops. The following phases in the disease process can be discerned



(Fig. 7): 1) An initial phase of early accumulation of APC and accessory cells (DCs, subclasses of macrophages) in the endocrine tissue; 2) A later phase of an apparently uncontrolled production of autoreactive CD4<sup>+</sup> and CD8<sup>+</sup> T cells and of autoantibodies of the IgG class in the draining lymph nodes; 3) A last phase where the target endocrine tissue becomes susceptible for the autoimmune attack by the generated autoreactive T cells and autoantibodies; this finally results in the destruction of the glandular tissue.

In the thyroids of patients with Graves' disease or Hashimoto goiter, and in the thyroids and islets of the above described animal models, increased numbers of specific subsets of macrophages and MHC class II-positive DC have been described (162). In the animal models, an increase in the number of these cells in the future target glands and a local clustering of these cells with T cells are the first signs of the developing autoimmune reaction (151, 163). This local enhanced accumulation precedes the clonal expansion of T cells and B cells in the draining lymph nodes, the production of autoantibodies by these lymph nodes, and further signs and symptoms of the later autoimmune disease. Foreign antigens of viral or bacterial origin (164), or self-antigens altered by toxins and drugs (165), or an excessive metabolic activity of the endocrine tissue (166) have all been described as separate possible causes of the attraction of the DCs to the endocrine tissue, already indicating a heterogeneity in causal factors at the level of the initiation of an endocrine autoimmune disease.

The initial phase of glandular accumulation of macro-

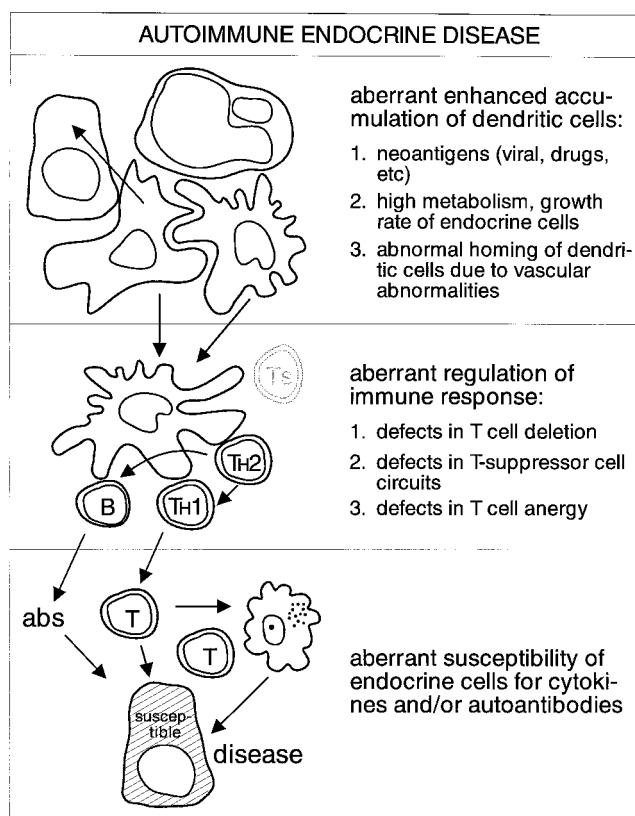


FIG. 7. The three developmental stages in an autoimmune endocrine disease.

phages and APCs is followed by a phase of an apparently uncontrolled clonal expansion of autoreactive T cells and B cells and the production of autoantibodies in the draining lymph nodes. In both the BB rat and the NOD mouse, there are strong indications for a genetically linked systemic immunodysregulation leading to the local exaggerated production of T cells, B cells, and IgG antibodies to various self-antigens. This systemic immune abnormality is partly associated with the presence of particular MHC class I and class II haplotypes (see below) and apparently leads to abnormalities in the stimulation and differentiation of cells involved in tolerance induction, such as the APCs, macrophages, and/or T cells (169). Indeed, APCs of NOD mice (169) and BB rats (our unpublished observations) have defects in their capability to generate T suppressor cells. With regard to such abnormal maturation of immunoregulatory T cells, the BB rat is special in that it lacks a regulator population of T cells (the RT6 cells). BB rats also show a rapid thymic involution (170). The OS strain of chickens has inborn defects in its suppressor cell system (159). Whether there are similar inborn defects in immunoregulatory cells in the human that lead to an endocrine autoimmune disease needs to be established. There are, however, numerous reports on both numerical and functional deficits in the suppressor cell system of patients with thyroiditis and IDDM (171).

Deficits in immunoregulatory cells do not only exist on an inheritable, genetic basis. They can also be acquired by (fetal) viral infections. In chickens, Avian Leucosis Virus has proven to exert a detrimental effect on thymus and bursa development, which disturbs delicate immune regulatory systems, leading to thyroid autoimmunity (172). Whether similar viruses or retroviruses with an affinity for immune cells are operative in human endocrine autoimmune diseases has been speculated upon but has not yet been proven. Experiments to detect virions and/or retroviral antigens have not been conclusive in showing the involvement of infectious viruses in human endocrine autoimmune disease (172).

After the stage of the excessive generation of autoreactive T cells and IgG autoantibodies, yet another factor or factors, at least in the OS chicken, determine whether or not a full blown autoimmune disease will develop (173). A prerequisite for clinical thyroid failure in this bird is a susceptibility of the target, the thyrocyte, for an autoimmune attack by the generated autoreactive T cells and IgG autoantibodies. Experiments have shown that this susceptibility factor is genetically determined, and it has been speculated that this factor might be an abnormal susceptibility of the thyrocytes for the cytokines produced by the autoreactive immune cells after infiltration. Whether such susceptibility factors are also important in the other animal models and in human disease needs further investigation, although in the BB rat a high susceptibility of pancreatic islet cells for IL-1 has been established.

Population, family, and twin studies have clearly shown that genetic factors exert a significant influence on the predisposition for an autoimmune endocrine disease. It is also clear that environmental factors (diet, infections, etc) contribute to disease expression because concordance rates in monozygotic twins and inbred animals are often imperfect. Since endocrine autoimmunity can be transferred by lym-

phocytes and bone marrow precursor cells into recipients, genes associated with the immune system have received prime attention. At present, convincing evidence does not exist for a relationship between the predisposition to an endocrine autoimmune disease and particular TCR haplotypes or polymorphisms, immunoglobulin allotypes and idiotypes, or cytokine genes (174). However, there is a clearly established genetic association with genes encoding for the MHC. In the BB rat this is the Rt<sup>1</sup> haplotype (167), and in the NOD mouse the H2 g<sup>7</sup> haplotype (168). The human thyroid, islet, and adrenal autoimmune diseases are predominantly associated with HLA-DR3, DR4, and DR5 haplotypes (57). The MHC may affect predisposition to endocrine autoimmune disease by several mechanisms that are not mutually exclusive. Autoantigenic peptides of glandular autoantigens may combine more easily with these particular MHC molecules than with others. It is, however, also possible that the disease association with these MHC haplotypes is due to a specific MHC-controlled shaping of the T cell repertoire.

Regardless of the mechanisms, it is apparent that the MHC haplotype *per se* is insufficient for the development of an endocrine autoimmune disease, as shown by the fact that autoimmunity-associated HLA-DR haplotypes are also found in perfectly normal individuals. Also, the H2 g<sup>7</sup> haplotype of NOD mice in congenic strains does not lead to IDDM in these animals. The genetic analysis of endocrine autoimmune diseases evidently requires an approach other than detailed typing of the MHC encoding genes. Such an approach has been found in the study on microsatellites.

Microsatellites or single-sequence length polymorphisms (SSLPs) are repeat sequences [usually dinucleotides, *e.g.* (CA)<sub>n</sub>] that exhibit high degrees of polymorphism both between individuals and in the number of repeats at a given chromosomal site. SSLPs are abundant (>100,000) and are randomly dispersed throughout the mammalian genome,

thereby providing an enormous pool from which to derive markers (175). Several thousand microsatellite markers have thus far been identified and mapped to the mouse/rat and human genomes, respectively. Specific SSLP loci can easily be defined by PCR using oligonucleotide primers specific for conserved sequences flanking the individual repeats, and length polymorphisms among individuals are identified by electrophoresis of the amplified products on agarose or polyacrylamide gels. Todd *et al.* (168) pioneered the use of microsatellites and other informative markers to define broadly the genes associated with diabetes in NOD mice. Scanning the entire genome of the NOD mouse, they obtained evidence of linkage with ten distinct loci, termed *Idd-1* to *-10*, distributed on at least nine different chromosomes and affecting different immunopathological features (Table 2). With the exception of *Idd-1*, which is linked with the MHC locus on chromosome 17, no individual locus appears to be absolutely essential for disease onset.

In addition to susceptibility loci, microsatellite mapping studies in F<sub>2</sub> crosses between NOD and a diabetes-free strain, as well as between NOD congenic strains expressing normal background alleles at specific *Idd* loci, have permitted the identification of several protective alleles that confer various degrees of resistance to diabetes. It can be predicted that similar diabetes resistance genes exist in diabetes-free humans with diabetogenic MHC haplotypes.

In human IDDM, previous intrafamilial association studies and limited chromosomal marker analyses have shown linkage to the MHC (IDDM1) on chromosome 6, and the insulin locus (IDDM2) on chromosome 11. Two recent studies using dense microsatellite maps (~300 markers at an average spacing of ~11 centimorgans), reconfirmed the major importance of IDDM1, but provided limited, if any, support for IDDM2. Both studies also identified new susceptibility loci (Table 2).

TABLE 2. Susceptibility loci and candidate genes for IDDM

Chromosomal location	Locus designation	Candidate genes	Remarks
NOD mouse			
1	<i>Idd-5</i>	<i>Bcg/Lch/Lty (Nramp)</i>	Linked to Orch-5
3	<i>Idd-3</i>	<i>IL-2</i>	
3	<i>Idd-10</i>	<i>Fcgr1, Csfm, Cd53</i>	
4	<i>Idd-9</i>		
6	<i>Idd-6</i>	<i>Bphs</i>	
7	<i>Idd-7</i>		
9	<i>Idd-2</i>		
11	<i>Idd-4</i>	<i>Nosi</i>	Linked to Orch-3
14	<i>Idd-8</i>		
17	<i>Idd-1</i>	<i>MHC</i>	Linked to Orch-1
Human IDDM			
2q		<i>GAD1</i>	
6p21	<i>IDDM1</i>	<i>MHC</i>	
6q	<i>IDDM5</i>	<i>SOD2</i>	
8p			
8q			
10q		<i>GAD2</i>	
11q13	<i>IDDM4</i>		
11p15	<i>IDDM2</i>	<i>INS</i>	
18q			

Abbreviations: *Bphs*, *Bordetella pertussis*-induced histamine sensitization; *Csfm*, colony stimulating factor, macrophage; *GAD1*, glutamic acid decarboxylase 1; *GAD2*, glutamic acid decarboxylase 2; *INS*, insulin; *Nosi*, nitric oxide synthase; *Nramp*, natural resistance-associated macrophage protein; *SOD2*, superoxide dismutase 2. [Derived from Ref. 174.]

## VI. POF in Association with Adrenal Autoimmunity and/or Addison's Disease

One of the first signs that autoimmunity could be responsible for a failure of ovarian function came from the observation that ovarian failure could precede the onset of Addison's disease by 8–14 yr (177).

Addison's disease is an uncommon disorder (10–20 per million) caused by a deficiency of adrenocortical hormones. The prevalence is highest in the fourth decade of life, and there is a marked female preponderance (2.5:1). The nature of idiopathic Addison's disease in the majority of patients in developed countries is now regarded as autoimmune (178), in contrast to the nature of the disease in developing countries, which is still mainly due to tuberculosis (179). Autoimmune Addison's disease seldom develops in isolation, and several other endocrine glands and organs are generally affected (180), leading to an autoimmune polyglandular syndrome (APGS). Two main forms of APGS can be clinically discerned. APGS type I mainly affects children and is characterized by the association of mucocutaneous candidiasis, hypoparathyroidism, and Addison's disease. Ovarian failure is often part of the syndrome (in approximately 60% of cases). APGS type 1 is also termed APECED (autoimmune polyendocrinopathy-candidosis-ectodermal dystrophy). APGS type II is characterized by adrenal failure in association with hypothyroidism. The latter mainly occurs in the fourth decade of life and has a female preponderance. In this syndrome only 25% of women have amenorrhea and 10% have a classic POF (181, 182).

With regard to POF, the literature indicates that 2–10% is associated with Addison's disease and/or adrenal autoimmunity (183).

### A. Antibodies in POF patients with adrenal autoimmunity and/or Addison's disease

The discovery in the 1970s of autoantibodies to the adrenal cortex (adrenal cytoplasmic antibodies, Cy-Ad-Abs) formed an important impetus for the studies on the autoimmune nature of idiopathic Addison's disease. Two varieties of adrenal antibodies were subsequently recognized in the sera of patients with Addison's disease using indirect immunofluorescence (IIF) and cryostat sections of human or monkey adrenal glands. One variety demonstrated reactivity with the three layers of the adrenal cortex only, whereas the other variety also reacted with cytoplasmic antigens of other steroid-producing cells present in the ovary, testis, and placenta (184, 185). This latter subvariety of adrenal cytoplasmic antibodies was called steroid-cell antibodies (St-C-Abs), and its reactivity could be absorbed by adrenal homogenates, thus confirming the cross-reactivity with the adrenal cytoplasmic antibodies (186). There is an absolute association between the presence of St-C-Abs and that of Cy-Ad-Ab, the former being detectable only when the latter is also present. St-C-Abs are of the IgG type and bind within the ovary to the hilar cells, the cells of a developing follicle, such as theca and granulosa cells, and to the corpus luteum cells.

Almost all patients with a primary amenorrhea and Addison's disease have a detectable serum titer of St-C-Abs; 60%

of patients with a secondary amenorrhea and Addison's disease show these antibodies (Table 3). In the absence of clinically overt gonadal failure, St-C-Abs have been described in about 15–20% of patients with clinical or latent Addison's disease (181). In the follow-up of the St-C-Abs-positive Addisonian patients, about 40% of females developed ovarian failure in a period of 10–15 yr, whereas in males the St-C-Abs did not herald gonadal failure (however, numbers of studied patients were small).

Heterogeneity exists between type I and type II APGS in relation to St-C-Abs (Table 3): 60–80% of patients with hypoparathyroidism and Addison's disease (type I APGS) and 25–40% of patients with type II APGS show these antibodies. In type I APGS without Addison's disease, 10% of patients show St-C-Abs. The high prevalence of St-C-Abs in patients with APGS type I probably explains the common association with gonadal failure seen in this group, and the appearance of the St-C-Abs in a female patient with APGS type I without adrenocortical or ovarian failure signals a high risk of their development (185, 187, 188). The sensitivities/specificities/predictive values for St-C-Abs in females with type I APGS who initially had normal adrenocortical and ovarian function were 1.0/0.56/0.50 in predicting ovarian failure and 0.86/0.83/0.86 for St-C-Abs in predicting adrenocortical failure (188).

The mere presence of an autoantibody in the serum of a patient is certainly not evidence for the pathogenic significance of the antibody; the autoantibody may also be the consequence of cellular destruction, such as is seen after the destruction of cardiac muscle cells in myocardial infarction, giving rise to anti-heart cell antibodies. It has been shown, however, that sera of patients with APGS type I and Addison's disease, positive for Cy-Ad-Ab and St-C-Abs, are cytotoxic for cultured granulosa cells in the presence of complement, when high titers of these antibodies were demonstrated in nine of 23 cases (189). Complement-dependent cytotoxicity of the St-C-Abs might indeed be one of the mechanisms leading to destruction of steroid-producing cells *in vivo* and thus to ovarian failure.

In recent years, considerable progress has been made with regard to the identification of the target antigens of Cy-Ad-Abs and possibly of St-C-Abs (190). It has been found that the adrenal cytochrome p450 enzyme 21 hydroxylase (which converts 17- $\alpha$ -progesterone and progesterone into 11-deoxy-

TABLE 3. Prevalence of steroid-cell antibodies (St-C-Abs) in patients and controls

Ovarian failure	
Unselected infertility/amenorrhea	<1%
With autoimmune thyroid disease or, type 1 diabetes	5–10%
With Addison's disease - primary amenorrhea	100%
- secondary amenorrhea	60%
Addison's disease (without ovarian failure)	
Isolated cases	10–20%
With hypoparathyroidism/candidiasis (type I APGS)	60–80%
With autoimmune thyroid disease (type II APGS)	25–40%
Type I APGS, mucocutaneous candidiasis and hypoparathyroidism without Addison's disease	10%
Autoimmune thyroid disease or type 1 diabetes	<1%
Healthy controls	<1%

[Derived from Refs. 181, 186, 188, and own data.]



cortisol and deoxycorticosterone), is the major autoantigen recognized by autoantibodies present in patients with Addison's disease (191, 192), either in the form of isolated adrenal failure or associated with hypothyroidism (type II APGS).

In type I APGS it is thought that autoantibodies are directed to other members of the cytochrome p450 enzyme family, namely to the p450 side-chain cleavage enzyme (p450-scc) and to 17- $\alpha$ -hydroxylase (17- $\alpha$ -OH) (192–196), and to an ill-defined 51-kDa protein (197). However, there is some confusion on this subject, and not all investigators could confirm the presence of these autoantibodies in type 1 APGS [negative results: p450-scc (198); 17- $\alpha$ -OH (198, 199)]. Of the steroidogenic p450 enzymes 21-hydroxylase is adrenal-specific, 17- $\alpha$ -OH is expressed in both adrenals and gonads, whereas p450-scc is present in adrenal, gonads, and placenta. The 51-kDa protein is present in islets, granulosa cells, and placenta.

Possible targets of the St-C-Abs in POF patients not be-

longing to the groups of APGS I or II are thus 17- $\alpha$ -OH and the p450-scc enzyme. However, in the one such patient with St-C-Abs, 17- $\alpha$ -OH was not recognized (191). To the authors' knowledge, studies have not been published on correlations between the presence and activity of St-C-Abs and autoantibodies to either 17- $\alpha$ -OH or p450-scc in patients without APGS type 1. Also, studies in which St-C-Ab activity would be adsorbed with the enzymes 17- $\alpha$ -OH or p450-scc would further illuminate the subject.

Apart from the autoantibodies, another strong argument for considering St-C-Ab-positive ovarian failure as an autoimmune disease is the histology of the ovarian lesions.

#### B. Histology of the ovaries in patients with POF in combination with adrenal autoimmunity and/or Addison's disease

Table 4 gives an overview of the reported histology of POF, including the reported cases of histologically confirmed oö-

TABLE 4. Histology of ovaries in relation to the antibody profile (adrenal/steroid cell antibody positive or negative) of the POF patients

Reference	Year	Cases	Antibodies		Other antibodies/diseases	Histology of cases				
			Ovary	Adrenal		Oöphoritis	No follicles	Few follicles	Numerous follicles	
<b>A. With adrenal/steroid cell antibodies</b>										
Irvine <i>et al.</i> (185)	1968	1	+	+	Testis	+				
Irvine (293)	1980	5	+	+	Addison's disease 5×	+				
Coulam <i>et al.</i> (294)	1981	1	+	+	Thyroid abs <sup>+</sup> , gastric abs <sup>+</sup>	+				
Gloor and Horlimann (202)	1984	1	-	+	-	+				
Rabinowe <i>et al.</i> (295)	1986	1	+	+	Addison's disease 3 yr later	+				
Sedmak <i>et al.</i> (201)	1987	1	+	+	Thyroid abs <sup>+</sup>	+				
Anonymous (272)	1987	1	-	+	Thyroid abs <sup>+</sup>	+				
Wolfe and Stirling (296)	1988	1	+	+	Testis abs <sup>+</sup>	+				
Biscotti <i>et al.</i> (297)	1989	1	+	+	Testis abs <sup>+</sup> , gastric abs <sup>+</sup>	+				
Bannatyne <i>et al.</i> (200)	1990	1	-	+	Thyroid abs <sup>+</sup> , Hashimoto's disease	+				
		1	-	+	Gastric abs <sup>+</sup> , ANA <sup>+</sup>	+				
		1	+	+	-	+				
Lonsdale (298)	1991	1	+	+	-	+				
		1	+	+	Thyroid abs <sup>+</sup>	+				
<b>B. Without adrenal/steroid cell antibodies</b>										
Russell <i>et al.</i> (299)	1982	1	-	-	Granulomatous oöphoritis	+				
		1	n.a.	n.a.	n.a.	+				
Friedman <i>et al.</i> (300)	1987	1	-	-	Thyroid abs <sup>+</sup>	+				
Bannatyne <i>et al.</i> (200)	1990	1	-	n.a.	n.a.	+				
		1	n.a.	n.a.	n.a.	+				
		1	-	-	-	+				
Kinch <i>et al.</i> (49)	1965	9	n.a.	n.a.	n.a.	-	6	1	2	
Emperaire <i>et al.</i> (301)	1970	7	n.a.	n.a.	n.a.	-	5	2	0	
Zarate <i>et al.</i> (302)	1970	7	n.a.	n.a.	n.a.	-	4	3	0	
Sharf <i>et al.</i> (303)	1972	10	n.a.	n.a.	n.a.	-	8	2	0	
Starup and Sele (33)	1973	15	n.a.	n.a.	n.a.	-	7	6	2	
Falk (304)	1977	3	n.a.	n.a.	n.a.	-	2	1	0	
Duignan <i>et al.</i> (305)	1978	8	n.a.	n.a.	n.a.	-	3	3	2	
Board <i>et al.</i> (306)	1979	8	-	-	-	-	2	4	2	
Rebar <i>et al.</i> (41)	1982	9	n.a.	n.a.	Thyroiditis 3×	-	5	4	n.a.	
Russell <i>et al.</i> (299)	1982	19	-	-	-	-	14	0	3	
Menon <i>et al.</i> (307)	1984	43	n.a.	n.a.	n.a.	-	27	16	n.a.	
Aiman and Smentek (308)	1985	14	-	-	Crohn's disease 1×, SLE 1×, Graves' 1×	-	9	2	5	
Miyake <i>et al.</i> (244)	1987	10	-	-	IDDM 1×, Thyroiditis 3×	-	10	0	0	
Rebar and Connolly (309)	1990	12	-	-	n.a.	-	5	7	n.a.	
Muechler <i>et al.</i> (219)	1991	17	-	-	ANA <sup>+</sup> 5×, thyroid abs <sup>+</sup> 2×, testis abs <sup>+</sup> 1×	-	12	2	3	
Total		215				24 11%	119 55%	53 25%	19 9%	

abs, Antibodies; ANA, antinuclear antibody; IDDM, insulin-dependent diabetes mellitus; n.a., data not available; SLE, systemic lupus erythematosus.

phoritis. All St-C-Ab-positive cases had lymphocytic oöphoritis, and of all lymphocytic oöphoritis cases reported, 78% had St-C-Abs.

The macroscopic appearance of ovaries with lymphocytic oöphoritis was either cystic (50% of the cases), with smaller and larger cysts, or normal. The cyst formation is hypothesized to be due to the elevated levels of gonadotropins seen in these patients.

Concerning the pattern of microscopical infiltration, there is a marked similarity in the reported cases of lymphocytic oöphoritis in the different compartments of the ovary. In most cases the primordial follicles are unaffected, as well as the cortex of the ovary. It is the developing follicle that is predominantly infiltrated by mononuclear inflammatory cells. There is a clear pattern of increasing density of the infiltration with more mature follicles. Preantral follicles are surrounded by small rims of lymphocytes and plasma cells, whereas larger follicles have a progressively more dense infiltrate usually in the external and internal theca. The granulosa layer is usually spared in this process until luteinization of the degenerating follicle occurs. When cysts are present, they are luteinized with a marked leukocytic infiltration in the cyst wall and destruction of the lining cells. Atretic follicles and, when present, corpora lutea or corpora albicantia are infiltrated as well. This pattern of infiltration confirms that steroid-producing cells are a main target for the autoimmune attack. Mild infiltration might be seen in the medulla and hilar region of the ovaries. There is a perivascular and, surprisingly, a perineural infiltration in the hilus of the ovary (200).

Immunohistochemical analysis of the lymphocytic oöphoritis reveals that the inflammatory cells are mainly formed by T lymphocytes (CD4<sup>+</sup> and CD8<sup>+</sup>) with a few B cells, together with large numbers of plasma cells. Macrophages and NK cells can also be found. The plasma cells mainly secrete IgG, but also IgA or IgM (201, 202), which probably indicates the local production of ovarian autoantibodies. That T cells are important in the ovarian destructive autoimmune reaction is mainly supported by data generated in the animal models of autoimmune lymphocytic oöphoritis (see below). The involvement of T cells also in human oöphoritis is suggested by a case report on a patient with autoimmune thyroiditis, adrenalitis, and POF in whom migration-inhibiting factor (MIF) production by peripheral T cells toward ovarian as well as testicular antigens was found (203). The MIF test is a sensitive antigen-specific test for the production of a cytokine, MIF, by peripheral blood T-lymphocytes when cultured in the presence of specific antigens. It must also be noted in this respect that granulosa cells of POF patients are more sensitive to  $\gamma$ -IFN, another T cell cytokine, than normal granulosa cells (55).

#### *C. Immunogenetic aspects of POF in association with adrenal autoimmunity and/or Addison's disease*

POF in association with adrenal autoimmunity and/or Addison's disease has not been analyzed for any separate immunogenetic susceptibility for the ovarian component. Autoimmune Addison's disease itself is associated with the haplotype HLA-B8/DR3, and in particular with the DR B1\* 0301 allele (204).

Ovarian failure in the context of the APGS type I syndrome has been studied in more detail, and APGS type I does not display an HLA-B8/DR3 association. The only association of APGS type I and HLA haplotypes reported so far has been with HLA-A28 (205). Positive associations were found between the presence of HLA-A28 and hypoparathyroidism, adrenocortical failure, and IDDM within the APGS type I syndrome, but not with ovarian failure (205), which is important for this review. Interestingly, in the APGS type I patients with ovarian failure, HLA-A3 was more frequent while HLA-A9 was less frequent than in those with normal ovarian function (205). Using the microsatellite approach, the responsible gene for APGS type I has recently been mapped to the long arm of chromosome 21 (206). The Unverricht-Lundborg type of progressive epilepsy EPM1 has been mapped to the same locus, viz 21q22.3, and a candidate gene (EHOC-1) for APGS type I, but in particular for EPM1, has been identified as a gene coding for a protein with partial homologies to transmembrane proteins including sodium channel proteins (207).

#### *D. Conclusions*

If the above observations are correct, POF in the presence of Addison's disease and/or adrenal autoimmunity (only 2–10% of cases) is almost certainly an endocrine autoimmune disorder. This view is supported by: 1) the presence of autoantibodies to steroid-producing cells in the patients, 2) the characterization of shared autoantigens between adrenal and ovarian steroid-producing cells, and 3) the histological picture of ovaries of such cases (lymphoplasmacellular infiltrate particularly around steroid-producing cells).

The existence of an animal model for the autoimmune syndrome of adrenalitis/oöphoritis (see below) lends additional support to this view. It is clear that further genetic studies need to be performed to analyze whether there is a separate (immuno)genetic susceptibility for the ovarian component within the syndrome oöphoritis/adrenalitis.

### **VII. Signs of Ovarian Autoimmunity in Patients with Idiopathic POF in the Absence of Adrenal Autoimmunity and/or Addison's Disease**

#### *A. Histology of the ovaries in patients with idiopathic POF in the absence of adrenal autoimmunity and/or Addison's disease*

The histological picture of ovaries of POF patients without adrenal autoimmune disease is also summarized in Table 4. Approximately 60% of such cases of POF lack ovarian follicles, and in these cases fibrotic ovaries are found. In 40% of the cases, ovarian follicles are detectable and numbers vary from few to numerous. About 10% of such follicular cases have numerous follicles, and these latter cases probably belong to the ROS (208).

In 1969, Jones and de Moraes-Ruehsen (50) were the first to report on three patients with ROS; they called it the "Savage" syndrome after the name of their first patient. The syndrome is defined by the presence of numerous primordial follicles in the ovaries, a hypergonadotropic hypogonadism

hormone profile, and a hyporeceptivity for high dosages of exogenous gonadotropins given for ovulation induction in patients with either primary (49, 50) or secondary amenorrhea (51, 209–216). ROS patients with a secondary amenorrhea clinically present as POF patients. The etiology of the syndrome is unknown, although several hypotheses have been put forward. These range from a lack of gonadotropin or estrogen receptors, postreceptor pathway disturbances, gonadotropins with inadequate bioactivity (217), serum factors modulating the action of FSH (218), and immune factors such as antibodies to gonadotropin receptors and thymus pathology, which is relevant for this review (216).

It is important to note that cases of lymphocytic oöphoritis can hardly be found in POF patients in the absence of adrenal autoimmunity/Addison's disease [six of 215 cases (Table 4)]. Muechler *et al.* (219), however, showed the presence of immunoglobulins in such non-oöphoritis-affected ovaries using direct immunofluorescence: in 50% of his cases he found vascular wall staining (IgA, IgM, or IgG), and in 30% the stroma and follicular cells were positive for immunoglobulins. Hypothetically, autoantibodies to the ovary may have

been present in the ovary without reaching detectable levels in the serum or inducing a local inflammation. It must also be noted that Muechler's data have not been confirmed by others, and in fact the histology of POF in the absence of adrenal autoimmunity/Addison's disease is not helpful in supporting an immune pathogenesis of the disease. This also applies to the atrophy found in the majority of cases. This phenomenon may represent the endstage of an autoimmune process directed against ovarian structures (as is seen in animal models, see below), but it may also represent a final depletion of oocytes due to genetic or environmental factors.

More positive evidence of isolated POF representing an endocrine autoimmune disease is the reported higher than normal frequency of some other endocrine and neurological autoimmune diseases in POF patients (Table 5).

### B. Autoantibodies in patients with idiopathic POF in the absence of adrenal autoimmunity and/or Addison's disease

1. Autoantibodies to endocrine organs (Table 5). Thyroid autoimmunity is the most prevalent (14%) associated endocrine

TABLE 5. Antibody profile of patients with idiopathic POF without adrenal autoimmunity

Reference	Year	POF cases	Thyroid abs/disease	Parietal cell abs/perniciososa	ICA/IDDM	ACH-R-abs/MG	Non-organ-specific abs/Systemic autoimmune disease
Behrman (310)	1964	16	0	0	1	0	
Lundberg and Persson (291)	1969	1	0	0	0	1	
Vallotton and Forbes (311)	1969	2	0	2	0	0	
de Moraes-Ruehsen <i>et al.</i> (221)	1972	16	6	4	0	0	Idiopathic thrombocytopenic purpura 1×
Ayala <i>et al.</i> (312)	1979	2	2	0	0	0	Sicca syndrome 2×
Board <i>et al.</i> (306)	1979	7	0	0	0	1	
Collen <i>et al.</i> (313)	1979	1	0	0	0	0	Vitiligo 1×, rheumatoid arthritis 1×
Williamson <i>et al.</i> (314)	1980	2	1	0	0	2	
Coulam and Luffkin (315)	1981	1	0	0	1	0	Anti-ovarian abs (RIA) 1×
Kuki <i>et al.</i> (212)	1981	1	0	0	0	1	
Chiauzzi <i>et al.</i> (233)	1982	2	0	0	0	2	FSH-receptor abs 2×
Russell <i>et al.</i> (299)	1982	19	1	0	0	0	2× oöphoritis (Table 4)
Rebar <i>et al.</i> (41)	1982	26	3	0	0	0	
Bateman <i>et al.</i> (292)	1983	1	0	0	0	1	
Coulam and Ryan (227)	1985	71	5	0	0	1	
Tang and Faiman (234)	1983	9	1	0	0	0	Rheumatoid arthritis 1×, vitiligo 1×, Hashimoto 1×
Aiman and Smentek (308)	1985	32	3	0	1	0	Crohn's disease 1×, SLE 1×
Alper and Garner (34)	1985	32	11	0	0	0	Vitiligo 1×
Anonymous (271)	1986	1	0	0	0	0	SLE 1×
Pekonen <i>et al.</i> (224)	1986	10	0	0	0	0	SLE 1×
Friedman (300)	1987	1	1	0	0	0	1× oöphoritis (Table 4)
Miyake <i>et al.</i> (244)	1987	20	7	6	0	1	ANA <sup>+</sup> 8×, thyroiditis 1×
Ho <i>et al.</i> (245)	1988	44	2	0	0	0	ANA <sup>+</sup> 1×, Rheuma factor <sup>+</sup> 1×
Mignot <i>et al.</i> (316)	1989	21	4	4	4	0	ANA <sup>+</sup> 10×, nDNA <sup>+</sup> 6×, Rheuma factor <sup>+</sup> 9×, SM <sup>+</sup> 8×
Wolffenbittel <i>et al.</i> (317)	1987	1	1	0	0	0	
Rebar and Connolly (309)	1990	115	12	0	0	0	
Luborsky <i>et al.</i> (229)	1990	43	5	n.a.	1	n.a.	Rheumatoid arthritis 1×, SLE 1×
van Weissenbruch <i>et al.</i> (235)	1991	22	2	1	1	1	Anti-pituitary abs <sup>+</sup> 1×, ANA <sup>+</sup> 1×, SM 2×
Muechler <i>et al.</i> (219)	1991	12	2	0	0	0	ANA <sup>+</sup> 5×, testis ab <sup>+</sup> 1×
Belvisi <i>et al.</i> (318)	1993	44	9	1	1	0	ANA <sup>+</sup> 4×, SM <sup>+</sup> 4×
Nelson <i>et al.</i> (247)	1992	23	5	4	0	0	Rheumatoid arthritis 1×, ANA <sup>+</sup> 9×
Tsirigotis and Craft (216)	1994	1	n.a.	n.a.	n.a.	1	
Hoek <i>et al.</i> (248)	1995	30	3	1	1	2	ANA <sup>+</sup> 2×, SM <sup>+</sup> 8×
Total		629	86 13.8%	23 3.7%	11 1.7%	14 2.2%	

abs, Antibodies; ANA, antinuclear antibodies; ACH-R-abs, acetylcholine receptor antibodies; ICA, islet cell antibodies; IDDM, insulin-dependent diabetes mellitus; MG, myasthenia gravis; n.a., data not available; SLE, systemic lupus erythematosus; SM, smooth muscle. [Derived from Ref. 183.]



autoimmune abnormality reported in POF patients without an adrenal autoimmune involvement, followed by the presence of parietal cell antibodies (4%), IDDM (2%), and myasthenia gravis or positivity for acetylcholine receptor antibodies (2%) (Table 5). However, the general prevalence of positivity for thyroid antibodies and gastric parietal cell antibodies is only slightly above the prevalence found in normal populations. It is, however, remarkable that IDDM and myasthenia gravis, both relatively uncommon autoimmune diseases ( $\ll 1\%$ ) are found relatively frequently in POF patients (2–4%). Whether this high frequency is due to publication bias or to shared underlying immune dysregulating factors remains to be established. Systemic lupus erythematosus (SLE), antinuclear antibodies, and rheumatoid factors have also been reported with a higher frequency than normal in POF patients (Table 5). A relationship of POF with SLE is further strengthened by the finding of the presence of anti-ovarian antibodies in 84% of the cases of female SLE patients by Moncayo-Naveda *et al.* (220).

**2. Ovarian autoantibodies.** Strong support for an autoimmune character of isolated POF would be the presence of antibodies to ovarian structures in the serum of these patients. However, the major conclusion drawn from several investigations using IIF on gonadal tissue (animal or human) is that patients are negative for St-C-Abs (186–188, 221–225). It is worthwhile to note that positive results regarding anti-ovarian antibodies have been obtained using assay methods other than routine IIF (see Table 6), but control subjects without idiopathic ovarian failure, postmenopausal women, and patients with iatrogenic ovarian failure were also found positive in these assays. It has become gradually clear from these studies that the presence and clinical activity of POF does not correlate with the presence of these antibodies in serum. Moreover, the results indicate that although antibodies to ovarian antigens are common in POF, their pathogenic role remains questionable. They may be the consequence rather than the cause of the disease.

**3. Receptor autoantibodies.** Conflicting results have also been obtained in investigations on so-called “receptor antibodies.” Receptor antibodies are directed to membrane receptors for hormones, and these antibodies can mimic the action of the hormone if they have a similar specificity and affinity for the receptor. Stimulating antibodies to the TSH receptor are the cause of the hyperthyroidism and goiter formation in patients with Graves’ disease (230). On the other hand, receptor antibodies may also block the action of the corresponding hormone when they lack a stimulatory action but still bind

to the receptor. Blocking receptor antibodies have been described as causal for myasthenia gravis (blocking antibodies to the acetylcholine receptor), some forms of insulin-resistant diabetes (blocking antibodies to the insulin receptor), and primary hypothyroidism [blocking antibodies to the TSH receptor (231)].

Thus, it is easily understood that receptors such as the LH and FSH receptors might become targets for blocking antibodies (Fig. 8), and such hypothetical antibodies could be a cause of ovarian failure. Experiments showing an interaction of antibodies of POF patients with FSH and LH receptor (function) have been described by various authors (Table 7). However, inconsistent data were generated with regard to the prevalence and the exact target of these antibodies; moreover, receptor antibodies were also found in patients with iatrogenic ovarian failure.

Recent data using cloned human LH and FSH receptors indicate that the human gonadotropin receptors are highly selective for their human ligands (240, 241), and this selectivity may also apply for the receptor antibodies. Therefore, Anasti *et al.* (237) used recombinant human gonadotropin receptors to detect a putative presence of immunoglobulins directed against the gonadotropins or their receptors in sera of patients with POF. The authors were unable to demonstrate the presence of blocking antibodies to LH or FSH receptors in any of the 38 POF patients studied.

In conclusion, the data on receptor antibodies in POF are not conclusive; antibodies to the LH and FSH receptors may exist, but their precise role and prevalence require further studies.

**4. Antibodies to zona pellucida (ZP).** Yet another specific set of ovarian antibodies playing a role in POF might be the antibodies to the ZP. The ZP is the acellular matrix that surrounds developing and ovulated oocytes and is also detectable in atretic follicles. Autoantibodies to ZP have been described as a cause of infertility in women. In women with unexplained infertility, these antibodies were seen in 5.6% of the cases, whereas in the normal controls positivity was seen in only 1.7% (242). ZP antibodies were thought to interfere with the sperm-oocyte interaction, thus inducing infertility. Animal models have demonstrated, however, that the ZP antibodies interfere with follicular development, and the presence of these antibodies in the experimental animals leads to follicular depletion and amenorrhea (see below). Grootenhuis *et al.* (unpublished observations), using an enzyme-linked immunosorbent assay, recently found three of 34 POF patients positive for IgG antibodies toward human recombinant ZP3. However, three of six postmenopausal

TABLE 6. Ovarian antibodies in patients with idiopathic POF

Reference	Year	Assay system	Substrate	POF	Controls	Other diseases
Coulam and Ryan (226)	1979	RIA	Human ovary homogenate	14/15	1/10 3 /12 p.m.	n.a.
Coulam and Ryan (227)	1985	RIA	Human ovary homogenate	30/110	n.a.	n.a.
Damewood <i>et al.</i> (228)	1986	IIF	Human ovarian sections	14/27	0/24 1 /22 p.m.	5/17 other AID
Cameron <i>et al.</i> (30)	1988	IIF	Monkey ovarian sections	n.a.	1/13	4 /10 IOF
Luborsky <i>et al.</i> (229)	1990	ELISA	Human ovary/oocytes	25/36	0/20	7/9 POF after pelvic surgery

IIF, Indirect immunofluorescence; ELISA, enzyme-linked immunosorbent assay; AID, autoimmune disease; p.m., postmenopausal women; n.a., not available; IOF, imminent ovarian failure.

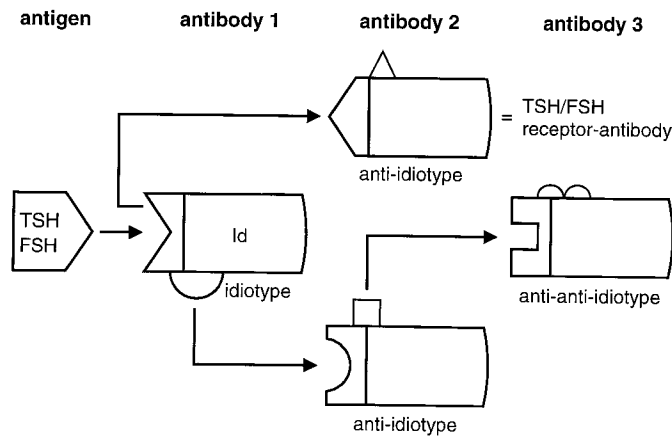


FIG. 8. An idiotype-antiidiotype cascade driven by a TSH or FSH molecule. As can be seen the second anti-idiotypic antibody group in the cascade is an antibody group that may contain antibodies reacting with the TSH or FSH receptor.

women were also positive, and it is thus likely that the antibodies toward ZP3 are the result of ovarian follicle damage, rather than their cause (in analogy to the other anti-ovarian antibodies described earlier).

C. Cellular immune abnormalities in patients with idiopathic POF in the absence of adrenal autoimmunity and/or Addison's disease

Recent literature (243) in the field of thyroid autoimmunity and IDDM indicates that immune cells, such as CD4<sup>+</sup> Th1 lymphocytes, macrophages, and CD8<sup>+</sup> T cytotoxic cells, are more important in the destruction of endocrine cells in endocrine autoimmunity as compared with the autoantibodies. So what is the evidence of such immune cell involvement in POF in the absence of adrenal autoimmunity and/or Addison's disease?

Table 8 gives an overview of studies on the numerical presence of various lymphocyte subsets present in the peripheral blood of patients with idiopathic POF. Although the data on the numbers of CD3<sup>+</sup>, CD4<sup>+</sup>, and CD8<sup>+</sup> T cells vary between the reported studies, a consistent pattern of an increased number of activated T cells (as defined by MHC-class II<sup>+</sup> or IL-2R<sup>+</sup>) is evident in the majority of the studies. Similar increased numbers of activated peripheral blood T cells have been described in other autoimmune endocrinopathies, such as recent onset Graves' disease (249), IDDM (250), and Addison's disease (251). A word of caution is needed, however, because we recently observed that postmenopausal women may also show raised numbers of activated peripheral T cells (248). Estrogen substitution lowered the number of activated peripheral T cells in women with POF, although not to completely normal levels. Ho *et al.* (252) also demonstrated the importance of the estrogen status for the number of peripheral blood lymphocyte subsets. We therefore consider the hypergonadotropic hypogonadism present in POF patients and postmenopausal women as partly responsible for the raised numbers of activated blood T cells. Another more direct indication of the involvement of the T cell system in the pathogenesis of POF is given in the ex-

TABLE 7. Antibodies blocking the action of FSH or LH/HCG receptors in patients with idiopathic POF

Reference	Year	Assay system	Substrate	POF with adrenal autoimmunity	POF without adrenal autoimmunity	Controls/other diseases
Austin <i>et al.</i> (232)	1979	LH/HCG receptor binding assay	Rat/human corpus luteum	n.a.	0/14	0/14
Chiauzzi <i>et al.</i> (233)	1982	FSH receptor binding assay	Rat testicular membrane	n.a.	2/11	0/5
Tang and Faiman (234)	1983	FSH/LH receptor binding assay	Bovine testicular membrane	n.a.	1/9	0/18
Moncayo and co-workers (220,238,239)	1990	LH/HCG receptor binding assay	Bovine corpus	n.a.	n.a.	11/35 (IVF, endometriosis, sterility)
van Weissenbruch <i>et al.</i> (235)	1991	Cytochemical bioassay for FSH induced granulosa cell DNA synthesis	Rat ovarian follicles	4/4	17/22	0/13
Wheatcroft <i>et al.</i> (236)	1994	LH/HCG receptor binding assay	Bovine	2/2	7/33	4/8 (Turner syndrome, iatrogenic POF)
Anasti <i>et al.</i> (237)	1995	LH/FSH receptor binding assay	Human recombinant	0/1	0/37	4/44 (healthy controls)

n.a., Data not available.

periments of Pekonen *et al.* (224). They detected in several cases of POF a positive MIF test toward gonadal antigens.

Taken together, the data on T cells in the literature may thus provide some support of the existence of a T cellular autoimmune response toward gonadal antigens in POF. However, again the question of consequence or cause must be addressed.

With regard to the peripheral B cell numbers, two of three studies reported an increase in the number of peripheral blood B cells (245, 248). One (245) correlated the raised numbers of B cells to the presence of various auto-antibodies; we were unable to confirm this correlation (248). A similar increase in the number of peripheral B cells has been observed in other autoimmune endocrinopathies. It is therefore not unreasonable to interpret the raised numbers of peripheral blood B cells as a sign of activation of the humoral immune system crucial for autoantibody production, especially because estrogen substitution in POF women did not lower the raised number of peripheral B cells (248).

With regard to the number and activity of peripheral NK cells in POF, two reports have been published. We showed a decrease in the number of peripheral CD56<sup>+</sup>/CD16<sup>+</sup>/CD3<sup>-</sup> NK cells (248). Pekonen (224) showed decreased activity (lysis of K562 cells) of normal numbers of peripheral blood NK cells in 30% of POF women. A lowered activity of NK cells has also been described in patients with Graves' disease (253). Because NK cells play a role in immunoregulation, it has been hypothesized that these lowered numbers of NK cells or the lowered activity of the cells might influence B and T cells, resulting in the production of autoantibodies. On the other hand, it has been hypothesized that a decreased activity of the peripheral blood NK cells indicates a susceptibility for viral infection, thus increasing the chance for a viral oöphoritis. However, there is little clinical or histopathological evidence for a viral infection in POF.

An interesting new avenue is the study on the number and functions of monocytes and monocyte-derived DCs in endocrine autoimmune disease. In IDDM (254) and Graves' disease (255), an abnormal function of monocytes and monocyte-derived DCs (abnormal polarization, abnormal interaction with T cells) has been found by our group. Recently, studies were extended to POF, and similar disturbances were found that were not correctable by estrogen substitution (256).

The abnormalities in the function of peripheral monocytes, monocyte-derived DCs, T cells, and B cells in patients with POF seem to be part of a more complex cell-mediated immune abnormality, including defects in the delayed type hypersensitivity (DTH) reactivity to *Candida* antigen (256) and the MIF production of peripheral T cells toward this commensal antigen (246). Although we do not understand the clinical significance of these general defects and abnormalities in cell-mediated immunity in POF patients (patients did not show recurrent infections), they might be related to an immunodysregulation leading to endocrine autoimmunity. It must be noted in this respect that patients with chronic mucocutaneous candidiasis (part of the APGS type I) and patients with recurrent vaginal candidiasis (who do show DTH abnormalities to *Candida*) also show a raised incidence of autoantibodies toward ovarian antigens (257). Whether

the APGS type 1 syndrome (where there is a connection between candidiasis and oöphoritis) represents the extreme of a spectrum of disorders combining T cellular deficiencies with ovarian autoimmunity requires further investigation.

#### D. Conclusions

In conclusion, there is some, albeit debatable, evidence that some cases of idiopathic POF in the absence of Addison's disease/adrenal autoimmunity may belong to the group of endocrine autoimmune diseases.

The positive evidence is formed by the fact that these cases of POF show similar cellular immune abnormalities to other endocrine autoimmune diseases such as IDDM, Graves' disease, and Addison's disease. These cellular immune abnormalities include abnormalities in the numbers and/or function of peripheral monocytes, monocyte-derived DC, and subsets of T cells and B cells. Another point of positive evidence might be the more than normal association of POF with IDDM and myasthenia gravis. However, data need to be confirmed and their relevance investigated. Moreover, data on anti-ovarian antibodies and anti-receptor antibodies are not conclusive, because these antibodies, although found by the majority of authors, might be the consequence rather than the cause of the disease. Another point of doubt is that the histology of POF in the absence of Addison's disease/adrenal autoimmunity hardly shows oöphoritis (<3%).

With regard to immunogenetic studies, one report (258) gives support for a concept that POF belongs to the endocrine autoimmune disorders: POF was associated with HLA-DR3 (258). This study, however, involved only 22 POF patients without adrenal autoimmunity. It must be noted that others were unable to confirm the association in later studies, using larger groups of POF patients of whom only a neglectable number had associated Addison's disease (259, 260).

### VIII. Animal Models of Autoimmune Oöphoritis

Animal models can be helpful in elucidating the question: "Which form of POF is autoimmune in character?" Various models have been developed, and ovarian failure due to autoimmune destruction of the ovaries can be induced in animals using the following approaches:

- A. Immunization with crude ovarian antigens.
- B. Immunization with well defined ovarian antigens, such as ZP3, or peptides thereof.
- C. Neonatal thymectomy in certain strains of mice.
- D. Transfer of normal T cells into syngeneic athymic nude (nu/nu) mice.

These four approaches will be described in detail in this review. Other approaches (variants of C. and D.) are the neonatal treatment of mice with cyclosporin A (261), engrafting of fetal rat thymic grafts to nu/nu mice (262), transplantation of neonatal thymic grafts to nu/nu mice (263), and transfer of normal neonatal spleen cells, neonatal thymocytes, and adult thymocytes to syngeneic nu/nu recipients (264).



TABLE 8. Peripheral blood lymphocyte subsets in patients with idiopathic POF

Reference	Year	Total POF cases	POF + adrenal abs	CD3/CD2	CD4	CD8	ratio CD4/CD8	HLA-DR <sup>+</sup> T-cells	B-cells	NK-cells	Other
Pekonen <i>et al.</i> (224)	1986	18	2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	N	NK cell activity < MIF production ovarian antigen > Leu-12 <sup>+</sup> B cells >
Miyake <i>et al.</i> (244)	1987	19	0	N	N	N	>	>	n.a.	n.a.	
Ho <i>et al.</i> (245)	1988	45	1	>	>	>>	<	n.a.	>	n.a.	
Rabinow <i>et al.</i> (223)	1989	23	2	N	N	N	N	>	n.a.	n.a.	CD5 <sup>+</sup> T cells >
Mignot <i>et al.</i> (246)	1989	23	3	N	>	N	>	n.a.	N	n.a.	MIF response microbial antigens < IL-2 receptor <sup>+</sup> T cells >
Nelson <i>et al.</i> (247)	1992	24	1	n.a.	n.a.	n.a.	n.a.	>	n.a.	n.a.	DTH candida <
Hoek <i>et al.</i> (248)	1995	30	0	N	N	N	N	>	>	<	

abs, Autoantibodies; DTH, delayed hypersensitivity skin test; MIF, migration inhibiting factor; N, normal; n.a., data not available; NK, natural killer; >, increased; <, decreased.

### A. Immunization with crude ovarian antigens

Experimental autoimmune oöphoritis can be induced in animals, such as the rat and the BALB/c mouse, using immunization with bovine or rat ovarian extracts in complete Freund's adjuvant (265–267). The immunization establishes an autoimmune allergic oöphoritis as early as day 14 after immunization, with infiltration of the ovaries by immune cells. The autoimmune nature of the oöphoritis is underlined by a positive DTH reaction toward the injected ovarian antigens by day 14, illustrating the existence of a cell-mediated immune reaction toward the ovarian antigens. The appearance of germinal centers in the thymus and increased T cell activity and B cell stimulation in the spleen indicates that this experimental oöphoritis involves both T and B cells (267). The experimental autoimmune oöphoritis could also be induced by passive transfer of peripheral blood lymphocytes, spleen cells, and enriched T and B cell suspensions from ovarian antigen-immunized rats to naive recipients, indicating that T cells and B cells are important in the pathogenesis of the disease.

Anti-ovarian antibodies in the serum of the affected animals were not detectable before day 28 (265). The reproductive capacity of the rats, measured by litter size, could be correlated to the titer of the anti-ovarian antibodies. Moreover, passive immunization of rats with rabbit anti-rat ovarian serum resulted in a temporary dose-dependent reduction in litter size (268), indicating a role of the antibodies in the pathogenesis of the disorder. The antibodies produced in this experimental oöphoritis animal model are thought to interfere with ZP antigens inhibiting fertilization, and/or to disturb ovulation (269).

Histological examination of the ovarian tissue at day 14 after immunization showed characteristic perivenous accumulations of lymphocytes and macrophages as well as plasma cells (265, 266). The infiltrate was found beneath the tunica albuginea and in the interfollicular tissue, as well as in the granulosa layer of follicles. Occasionally, cell infiltrates were found in the external theca. The large secondary follicles and corpora lutea seemed unaffected, in contrast to the primordial and small secondary follicles. While the number of follicles and corpora lutea were decreased, the number of atretic follicles was increased. It is evident, in comparing the histology of this experimental oöphoritis rat model to the known cases of human autoimmune oöphoritis, that there

are major differences. In human autoimmune oöphoritis, the main targets are the steroid-producing cells of the theca of maturing follicles and the corpus luteum, and not the interfollicular space and the secondary and primordial follicles such as seen in this animal model. This implies that the model may have only limited value in the study of human autoimmune oöphoritis and POF.

### B. Immunization with heterologous ZP antigens or purified ZP3 antigens

Immunization of New Zealand white rabbits with heterologous ZP antigens shows an induction of ovarian failure due to follicle depletion. Immunization experiments with porcine ZP in rabbits showed the development of ZP antibodies in the immunized animals. It was demonstrated that rabbits actively immunized with ZP proteins ceased to ovulate in response to hCG administration (269). The immunization of the rabbits induced a marked reduction in follicles and an atretic appearance of primary follicles. Growing follicles disappeared completely by 30 weeks post immunization. The reduction in number of normal follicles was accompanied by a striking increase in the number of oocyte-free cell clusters. An oöphoritis such as that seen in the immunization experiments with crude ovarian extracts was not detected (270).

The alteration in ovarian function and histology in the rabbits could be correlated with the presence of serum antibodies to ZP glycoproteins. These studies and the histological pictures indicate first that the antibodies to ZP alter ovarian function and histology by interfering with cells during the stage of follicle differentiation at which ZP proteins are being synthesized (270), and second that the model might be of relevance in the study of human POF in the absence of adrenal autoimmunity. It has been hypothesized that ROS or premature depletion of ovarian follicles might represent the human counterpart of this animal model. However, Starup and Sele (33) showed that in the ovaries of ROS patients there was a normal ultrastructural appearance of the early follicles and no "oocyt-empty" follicle remnants, such as described by Skinner *et al.* (270) in the rabbit model. On the other hand, two cases of ROS have been reported in whom hyalinization of preantral follicles was described (271, 272).

The proteins of the ZP are conserved among mammals

(273). ZP3 is a major ZP glycoprotein that functions as a sperm receptor (274), and mouse and human ZP3 proteins are 67% identical. It has been shown that a 15-amino-acid peptide of ZP3 was able to induce oöphoritis in (C57BL/6×A/J)F1(B6AF1) mice after immunization in Freund's complete or incomplete adjuvant. The histology of the lesion was reminiscent of the picture of human oöphoritis. ZP3-specific T cell responses and antibodies directed to ZP3 were detectable in these ZP3-immunized animals (273). In an adoptive transfer experiment to naive mice, ZP3-specific CD4<sup>+</sup> T cells were sufficient for induction of the oöphoritis without observable antibody production to the ZP. The ZP3-specific CD4<sup>+</sup> T cells mainly produced IL-2, IFN- $\gamma$ , and TNF, but not IL-4, indicating that the disease-specific T cells belonged to the TH1 subset of CD4<sup>+</sup> T cells.

Subsequently, Lou and Tung (275) very elegantly showed that a transfer of T cells that were directed to the small T cell epitope of ZP3 (15-amino acid peptide) alone differed from the adoptive transfer of T cells to whole ZP3. The former transfer could already result in a full-blown autoimmune oöphoritis, and, apart from a T cell response to the self-peptide and histomorphologically confirmed oöphoritis, serum antibodies to native ZP3 and preferential binding of the antibody to the ZP *in vivo* were found. Crucial in the experiments was the presence of the ovaries during the antigen-specific CD4<sup>+</sup> T cell transfer. The phenomenon shows that B cells autoreactive to ovarian antigens can be generated after a T cell transfer, and that these cells can be activated by ZP3-specific CD4<sup>+</sup> T cells to produce antibodies that are directed to and bind ZP3 *in vivo*. It is thought that the ovarian antigen required for antibody production in this model is provided by the normal ovaries, since the ZP antigens may be generated through a process of follicular atresia (epitope spreading).

Another mechanism by which the ZP autoantibodies can be induced is by idiotype mimicry of autoantigens in the absence of the antigen itself. Tung and colleagues (276) also investigated whether a nonovarian peptide could be recognized by ZP3-specific T cells. The author detected, by searching the protein sequence library, nonovarian peptides sharing sufficient residues with ZP3. Interestingly, the  $\delta$ -chain of the murine acetylcholine receptor and the ZP3 peptide had certain homology. The ZP3 peptide derivative and the  $\delta$ -chain of the acetylcholine receptor both elicited severe oöphoritis and also stimulated the ZP3-specific T cell clone to proliferate. Through the mechanism of T cell peptide mimicry, using a  $\delta$ -chain of the murine acetylcholine receptor, autoimmune oöphoritis could be elicited by clonal activation of ZP3-specific pathogenic T cells. Hence, T cell epitope mimicry as autoimmune disease mechanism was detected in the murine model, and this mimicry may explain the clinical association between POF and myasthenia gravis. Unfortunately, however, in the human there does not exist a homology between ZP3 and the acetylcholine receptor (277). Still, it remains remarkable that there is a marked coincidence between POF and myasthenia gravis (Table 5). The fact that in human oöphoritis a clear perineural infiltration of the ovarian hilus nerves is seen might also suggest a shared pathogenic mechanism between ovarian and neuronal diseases.

### C. Neonatal thymectomy models

Neonatal thymectomy in BALB/c mice (and also some other strains, see below) at day 3 after birth results in oöphoritis, among other organ-specific autoimmune manifestations such as thyroiditis, gastritis, and parotitis (278–280). The inflammations are characterized by the presence of T cell infiltrates in the affected organs and the development of organ-specific antibodies in the serum. There is a strict temporal relationship between the development of the autoimmune syndrome and the day of thymectomy, which has to occur between the second and the fifth day after birth (280, 281). An explanation for the phenomenon has been proposed and is based on the premise that self-reactive CD4<sup>+</sup> T cells are generated in the thymus throughout life and exported to the periphery. In euthymic animals, autoimmune disease is not observed because these autoreactive CD4<sup>+</sup> T cells are controlled by CD4<sup>+</sup> T cells with regulatory or suppressor activity. These cells are also generated in the thymus, but only after the first week of life. Hence thymectomy at day 3, restricting the T cell repertoire to only effector autoimmune CD4<sup>+</sup> T cells, explains the spontaneously occurring autoimmune diseases, because the balance between self-reactive T cells and regulatory T cells tips over to the former.

That animal oöphoritis is directly due to autoimmune T cells is shown by transfer experiments of CD4<sup>+</sup> T cells of thymectomized donors to young recipients, which causes an oöphoritis in these recipients (282, 283). This transfer of oöphoritis could be prevented by infusion of CD4<sup>+</sup> CD5<sup>+</sup> T cells from normal adult mice in an early stage after the transfer of the CD4<sup>+</sup> cells of the thymectomized donors.

The histopathological events of the oöphoritis in the thymectomized mice occur in an orderly manner. Initially the oöphoritis is evident as a patchy or diffuse infiltration of lymphocytes; later, developing follicles are clearly affected and monocytes, macrophages, neutrophils, and plasma cells are found between and within ovarian follicles. The onset of puberty markedly potentiates the oöphoritis, indicating that probably a change in antigen profile due to the gonadotropin stimulation is important. The oöphoritis is most severe between 4–14 weeks after thymectomy. This is accompanied by loss of ova and collapse of ovarian follicles. Autoantibodies are detected in the circulation by week 4, with a peak between weeks 7–9. Autoantibodies are directed toward oocytes, ZP, and in lower titers also to steroid-producing cells such as the granulosa cells, the theca cells, and the luteal cells. The inflammation subsides after 14 weeks, and the ovary becomes atrophic (278–280). IgG-producing plasma cells are found, but not frequently. The overall picture of the oöphoritis is one of a cell-mediated autoimmune reaction.

With regard to the genetics of this ovarian autoimmunity model, certain strains of mice are susceptible, such as the BALB/c and A/J mice, whereas other strains (C57bl/6J, DBA/2 mice) are resistant. Since susceptibility and resistance are not associated with the MHC haplotype (H2) of the mice, these molecules are apparently of minor importance. Using the susceptible and resistant mice strains and backcrosses of these strains in combination with a microsatellite approach, a locus has been found on chromosome 16, controlling the abrogation of the tolerance due to neonatal

thymectomy day 3 (284). This so-called Aod1 locus was associated with the presence of oöphoritis in the mice. Interestingly, the markers on chromosome 16 failed to exhibit a significant linkage to the concomitant ovarian atrophy in this mouse oöphoritis model. Rather, this atrophy exhibited an association with markers on mouse chromosome 3 (284).

With regard to another experimental mouse model of gonadal autoimmunity, viz the male counterpart of allergic oöphoritis, experimental allergic orchitis, studies have shown a similar complexity of gene involvement and the recognition of various susceptibility and resistance loci (the Orch genes) (285). In this model the H2 locus is of importance as a susceptibility locus, and the so-called Orch 1 gene has been mapped to the Hsp 70.3/G7 interval within the H-2S/H-2D region. Genes controlling resistance have also been identified: Orch 3 maps centrally on chromosome 11, while Orch 4 maps on chromosome 1. Orch 5, also on chromosome 1, is probably a gene governing the extent of the inflammatory lesions seen in susceptible mice. Most significant is the linkage of Orch 3 to *Idd-4* and Orch 5 to *Idd-5*, two susceptibility genes that play a role in IDDM of the NOD mouse model (see above and Table 2).

The histological and serological manifestations of the murine autoimmune oöphoritis are comparable to the histological and serological picture of human autoimmune oöphoritis in association with Addison's disease. It is remarkable, however, that the adrenal glands are unaffected in the neonatally thymectomized mice, even in the presence of antibodies to steroid-producing cells. However, modifications of the model, viz immunomodulation using Cyclosporin A after birth, does affect the adrenals (261).

As the inflammation of the ovaries subsides, serum anti-ovocyte and anti-zona antibodies also decrease to sometimes undetectable levels at day 150–360, when oocytes have completely disappeared from the atrophic ovary (278–280). Therefore, the absence of serum autoantibodies does not exclude an autoimmune etiology of the ovarian disease. This finding may be of relevance in patients with adrenalitis and/or amenorrhea; detection of St-C-Abs may not always be expected unless they are looked for in an early stage of the disease.

#### D. Transfer of normal T cells to athymic (*nu/nu*) mice

Yet another mechanism for the induction of an autoimmune oöphoritis is the transfer of T cells to athymic nude mice.

The nude mouse model is characterized by a deficient T cell function because the most important function of the thymus, education of T cells to properly recognize self and nonself, cannot take place. When CD4+CD8-thymocytes from normal neonatal or adult BALB/c mice are transferred to athymic mice, approximately 50–75% of the recipients develop an autoimmune oöphoritis and/or gastritis (264). Neonatal CD4+ splenocytes are also able to transfer the autoimmune diseases, whereas T cells from adult spleen do not (286). However, a fraction of adult spleen CD4+ with a low expression of CD5+ can induce oöphoritis in athymic recipients. The disease-generating CD4+ T cells are of the Th1 type. Regulatory T cells that down-regulate self-reactive T

cells in this animal model are also present, as studied by the combined infusion of neonatal spleen cells that enhance autoimmune oöphoritis and adult spleen cells that inhibit this process (264). The exact nature of these regulatory cells in this animal model is not yet elucidated; however, it is hypothesized that these belong to the Th2 subset of the CD4+ T cells.

The ovarian histopathology of day 3 thymectomized animals and nude mice that develop an oöphoritis after an adoptive transfer experiment are indistinguishable; hence it might be a good model for POF in the presence of adrenal autoimmunity/Addison's disease.

In the human situation, experiments of nature show us that athymic girls show evidence of dysgenetic, atrophic ovaries, devoid of follicles (287). Whether this is the ultimate consequence of an autoimmune process that began very early on remains hypothetical.

#### E. Conclusions

The murine animal oöphoritis models of neonatal thymectomy or T cell transfer in the nude animal clearly show that pathogenic self-reactive T cells exist in the normal neonatal and adult repertoire of at least the mouse, and that these autoreactive effector T cells are controlled by regulatory T cells also existing in the normal adult repertoire. There evidently exists a genetically controlled balance between these two T cell populations that ensures tolerance to ovarian and other self-antigens. When the balance tips over in favor of effector T cell activity, autoimmune oöphoritis develops (often in association with thyroiditis and other endocrine autoimmune disorders). This type of murine autoimmune oöphoritis is histologically and serologically similar to the human autoimmune oöphoritis occurring in association with adrenal autoimmunity and Addison's disease (~2-10% of POF cases). The autoantibodies and the histology of this type of POF shows that steroid-producing cells are one of the main targets of the autoimmune response, and autoantigens of these steroid-producing cells are gradually identified (important enzymes in steroid synthesis). When all the data are considered together, POF in association with adrenal autoimmunity and/or Addison's disease and positive for St-C-Abs can now with certainty be considered as an endocrine autoimmune disease.

The animal models of immunization with crude and purified ZP antigens demonstrate that autoimmune ovarian failure can also be reached via other histological pictures and mechanisms. First, there is the follicular infiltration by various immune cells in the ZP3-peptide immunization model, similar to the thymectomy model and the T cell transfer model in athymic nude mice. This picture is again reminiscent of the autoimmune oöphoritis seen in POF patients with St-C-Abs and associated with Addison's disease. The infiltration ultimately leads to follicular depletion and fibrosis. Second, there is the simple depletion of follicles after immunization with crude ZP antigens in the absence of a clear lymphocytic infiltration, but with the production of antibodies. This model might be most relevant for POF cases without adrenal involvement and with fibrotic ovaries. Also in the human, ZP autoantibodies have been detected and there is some, though weak, evidence that such POF cases belong to



the group of autoimmune diseases (associations with other autoimmune diseases, abnormal numbers and functions of peripheral lymphocytes and monocytes; see earlier discussions in this review). However, further experiments are required to establish or refute such a view.

### IX. Summary

Premature ovarian failure (POF) is defined as a syndrome characterized by menopause before the age of 40 yr. The patients suffer from anovulation and hypogonadism. Approximately 1% of women will experience menopause before the age of 40 yr. POF is a heterogeneous disorder with a multicausal pathogenesis involving chromosomal, genetic, enzymatic, infectious, and iatrogenic causes. There remains, however, a group of POF patients without a known etiology, the so-called "idiopathic" form. An autoimmune etiology is hypothesized for the POF cases with a concomitant Addison's disease and/or oophoritis.

It is concluded in this review that POF in association with adrenal autoimmunity and/or Addison's disease (2–10% of the idiopathic POF patients) is indeed an autoimmune disease. The following evidence warrants this view: 1) The presence of autoantibodies to steroid-producing cells in these patients; 2) The characterization of shared autoantigens between adrenal and ovarian steroid-producing cells; 3) The histological picture of the ovaries of such cases (lymphoplasmacellular infiltrate around steroid-producing cells); 4) The existence of various autoimmune animal models for this syndrome, which underlines the autoimmune nature of the disease.

There is some circumstantial evidence for an autoimmune pathogenesis in idiopathic POF patients in the absence of adrenal autoimmunity or Addison's disease. Arguments in support of this are: 1) The presence of cellular immune abnormalities in this POF patient group reminiscent of endocrine autoimmune diseases such as IDDM, Graves' disease, and Addison's disease; 2) The more than normal association with IDDM and myasthenia gravis. Data on the presence of various ovarian autoantibodies and anti-receptor antibodies in these patients are, however, inconclusive and need further evaluation.

A strong argument against an autoimmune pathogenesis of POF in these patients is the nearly absent histological confirmation (the presence of an oophoritis) in these cases (<3%). However, in animal models using ZP immunization, similar follicular depletion and fibrosis (as in the POF women) can be detected.

Accepting the concept that POF is a heterogeneous disorder in which some of the idiopathic forms are based on an abnormal self-recognition by the immune system will lead to new approaches in the treatment of infertility of these patients. There are already a few reports on a successful ovulation-inducing treatment of selected POF patients (those with other autoimmune phenomena) with immunomodulating therapies, such as high dosages of corticosteroids (288–292).

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