Organ-Specific Autoimmunity:
A 1986 Overview

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INTRODUCTION

Organ-specific autoimmunity is nowadays largely synonymous with endocrine autoimmunity. Clinically, these disorders usually affect a single organ or gland in the body, but serological evidence (i.e. autoantibodies) often indicates subclinical disturbances in other related tissues. The target antigens of the immunological response are unique to the affected organ, thus giving rise to the organ-specific and even cell-specific recognition. The precedent for autoimmune disease was set in 1956 with the demonstration that autoimmunity is the basis of Hashimoto's thyroiditis (Roitt et al. 1956). This concept was rapidly extended to atrophic fundal gastritis, pernicious anemia, Addison's disease and ovarian failure (reviewed by Bottazzo & Doniach 1985), reaching full circle with the histological description of lymphocytic hypophysitis (Goudie & Pickerton 1962). It was more than a decade later that the latter findings were substantiated by the description of pituitary antibodies (Bottazzo et al. 1975), which followed the first demonstration of islet cell antibodies (ICA) in insulin-dependent diabetic patients (Bottazzo et al. 1974). Since then the list of organ-specific autoimmune diseases has grown steadily (Table I) and, except for the pineal gland, autoantibodies to other endocrine cells, e.g. to vasopressin cells in the hypothalamus in patients with idiopathic diabetes insipidus (Scherbaum & Bottazzo 1983) or to gastrin cells in the antrum in Type B antral gastritis (Vandelli et al. 1979) have been identified. The presence of organ-specific autoantibodies usually correlates well with the corresponding clinical symptoms or local histology and impairment of hormone secretion. However, we have found this not always to be strictly so as in the case of autoantibodies to glucagon and somatostatin cells in the pancreatic islets (Bottazzo & Lendrum 1976), or to gastric inhibitory peptide cells and secretin

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cells in the gut (Mirakian et al. 1981, Jones et al. 1983). However, all these findings have helped to consolidate the concept of a "polyendocrine autoimmune syndrome" in which different autoimmune conditions co-exist at the clinical or sub-clinical levels in predisposed individuals or families (Valenta et al. 1982), more often than one would expect by chance: an idea which, interestingly, was suggested as long ago as the early 1920s.

Organ-specific autoimmunity can affect target organs through several mechanisms:

a) *A slow destructive process,* by which the normal parenchyma is attacked by lymphocytes (bearing markers of immune activation) and antibodies and is gradually replaced by connective tissue. Loss of physiological function may eventually occur.

b) *A 'stimulating' process,* primarily involving antibodies which react with hormone receptors on the cell surface, mimicking some of the effects of the trophic hormones. An excessive production of the specific peptides/chemical mediators, or stimulation of growth are usually the end result.

c) *A 'blocking' process,* in which non-stimulatory antibodies play a major role by competing with hormones or other mediators for binding to cell-surface receptors (or close by). An impairment of cell function and gradual atrophy of the attacked organ become clinically manifest.

DESTRUCTIVE AUTOIMMUNITY: THE LATEST DEVELOPMENTS

Despite repeated attempts, many investigators have failed to demonstrate specific autoantibodies to enterocytes (EC-Ab) in inflammatory bowel diseases considered to be of autoimmune origin, e.g. Crohn's disease. Autoantibodies which react with colonic mucosal cells were originally described in patients with ulcerative colitis (Broberger & Perlmann 1961), but were shown to crossreact with E. Coli. Recently, however, specific EC-Ab have been demonstrated in about 50% of children with idiophatic protracted diarrhoea (Mirakian et al., in press). Their cytoplasmic reactivity, more pronounced towards the apical border of the villous epithelium, closely resembles the classical pattern of organ-specific autoimmune reactivity. High titres of IgG EC-Ab and their complement-fixing ability indicated a poor prognosis in young patients, despite efforts to ameliorate the disease with aggressive immunosuppressive therapy. Partial or severe atrophy of the jejunal villi with a substantial degree of lymphocytic infiltration was the predominant histological pattern in the biopsy material examined and, in preliminary detailed immunological studies, immunoglobulins and activated T lymphocytes were detected.

In contrast to adult organ-specific autoimmunity in which females predominate over males and which are most commonly associated with other organ-specific autoimmune disorders, in childhood autoimmune protracted diarrhoea the sexes

TABLE I
Destructive organ-specific autoimmune diseases

Hashimoto's Thyroiditis
Primary Myxedema
Fundal Gastritis (Type A)
Antral Gastritis (Type B)
Addison's Disease
Gonadal Failure
Idiopathic Hypoparathyroidism
Partial Hypopituitarism
Idiopathic Diabetes Insipidus
Protracted Diarrhea of Infancy
Autoimmune Diabetes Mellitus
Vitiligo
Alopecia Areata/Totalis

tend to be more equally affected and the association with non-organ-specific autoimmune diseases (i.e. connective tissues disorders, liver diseases, etc. [Scherbaum et al. 1986a]) is more pronounced. Although at first sight these findings are somewhat unexpected they are comparable to similar clinical and immunological characteristics described in the Candida-Endocrinopathy syndrome, centred around idiopathic hypoparathyroidisms and again primarily affecting children (Neufeld & Blizzard 1980).

However, the main question remains: how do enterocytes evoke an efficient humoral response against themselves? As mentioned, autoantibody production against enterocytes seems to occur rarely. It is possible that in predisposed young children and infants whose gut mucosal permeability is still generally at a critical stage, the local immune defence mechanisms are still not entirely efficient, thus allowing the entry of external antigens which, in turn, are responsible for the production of harmful cross-reacting antibodies. The existence of these specificities has recenty been revealed in coeliac disease, another complex gut disorder, by the demonstration that anti-gliadin antibodies cross-react with the Erb protein of Adenovirus 12 (Kagnoff et al. 1984).

DESTRUCTIVE AUTOIMMUNITY: THE MAJOR IMPACT IN THE
PATHOGENESIS OF TYPE I (INSULIN-DEPENDENT) DIABETES

Following the first description of islet cell antibodies (ICA) in polyendocrine autoimmune diabetic patients (Bottazzo et al. 1974) and their subsequent identification in uncomplicated juvenile cases (Lendrum et al. 1975), speculation arose as to whether these markers merely represented a secondary phenomenon following a specific attack from a common environmental agent(s), responsible for the initial injury to the pancreatic beta cells. However, although Type I diabetes has

an acute clinical onset, ICA were demonstrated in predisposed individuals years before the onset of the disease: this led to reconsideration of the 'common viral dogma' as the sole cause of diabetes. The new concept of a primary autoimmune attack emerged initially from studies of unaffected members of diabetic families and was subsequently confirmed in retrospective and prospective studies in identical twins discordant for the disease, in polyendocrine non-diabetic patients and even in single sporadic cases (reviewed by Bottazzo et al. 1986). A proportion of these individuals eventually became overtly diabetic. We believe that, because of this new evidence, previously advocated environmental factors should be considered to act more as precipitating rather than initiating factors of beta cell damage. This strongly suggests that 'something' more subtle and definitely more complicated is involved in the pathogenesis of this disease.

Full-blown immunological aggression has been uncovered around and inside islets in an acute diabetic patient who died of the disease close to the time at which overt symptoms began (Bottazzo et al. 1985). Most facets of autoimmune attack were represented in the frozen pancreatic blocks, with abundant evidence of immune complex and complement deposition. CD8+ (?cytotoxic) T cells were a predominant feature of the diseased tissue, with the lymphocytes expressing activation markers. No evidence of Coxsackie, Mumps or other common viruses was detected in the diabetic islets.

Regardless of the nature of the initial trigger, the major concern of most investigators and diabetologists is to try to efficiently halt the autoimmune attack against the beta cells once it has been mounted. The initial pilot study with cyclosporin A (Stiller et al. 1984) and the more recent double blind trial in newly diagnosed diabetic patients (Feutren et al. 1986) are, in this context, the best indication that general opinion is coming round to the idea that autoimmunity plays a major role in the attack on the beta cell. If more evidence is needed for the role of autoimmune aggression this has been provided by the unexpected outcome of segmental pancreatic transplantation to diabetic identical twins from their unaffected co-twins who were discordant for the disease for many years (Sutherland et al. 1985). It is generally accepted that discordance for at least 5 yr from the time that the first twin becomes diabetic strongly indicates that the non-diabetic co-twin will remain disease-free, despite the latter still showing certain immunological abnormalities (Alviggi et al. 1984). Hopes for a successful outcome of the transplantations were contradicted by the rapid decompensation of beta cell function in the transplanted diabetic twin in a matter of weeks to a few months. Serial biopsies showed the typical pattern of insulitis with CD8+ (?cytotoxic) lymphocytes being predominant in the infiltrate invading the islets (Sibley et al. 1985). Glucagon and somatostatin cells were spared, thereby resembling the histological pattern of the original disease process (Gepts & De May 1978). It thus appears that over a period of many years (in the case of one of the twins, this was more than 20) the anamnestic autoimmune repertoire against

beta cells has remained intact, especially in its most dangerous and efficient form, i.e. cytotoxic T cells. We believe that it will only be by further dissecting the intricacies of the autoimmune process that, hopefully in the not too distant future, diabetes will be efficiently controlled and prevented (Bottazzo 1984).

DESTRUCTIVE AUTOIMMUNITY: THE CHALLENGE OF IDENTIFYING AND CHARACTERIZING THE RELEVANT AUTOANTIGENS

As mentioned, organ-specific autoimmunity is characterised by a strictly cell-specific response where the patients' autoantibodies are directed against antigens which are unique to the target tissues but are present in all individuals. Classically, these autoantibodies are identified by immunofluorescence (Scherbaum et al. 1986b) and recognise cytoplasmic components which, following conventional separation procedures, are found in the 'microsomal' fraction (Roitt et al. 1964). The precise nature of the thyroid microsomal autoantigen is emerging with the demonstration that it is at least partially identical with thyroid peroxidase (Czarnocka et al. 1985, Portmann et al. 1985). Whether similar enzymes serve as autoantigens in other organs remains to be investigated: however, although the microsomal antigens in different target tissues may share certain biochemical properties, they clearly differ in some structural features. Thus, when sera containing both thyroid and gastric parietal cell antibodies were absorbed with thyroid extract the thyroid reactivity was lost but gastric antibodies were unaffected (Knight et al. 1984). In addition, thyroid antibodies precipitate a 105-107 Kd molecule (Banga et al. 1985, Hamada et al. 1985), ICA a molecule of 64 Kd (Baekkeskov et al. 1982) and melanocyte antibodies a 75 Kd protein (Naughton et al. 1983).

Much discussion has followed the unexpected finding that some hybridomas produced from lymphocytes of diabetic patients (Satoh et al. 1983) and also from normal individuals (Prabhakar et al. 1984) synthesize monoclonal antibodies which cross-react with several tissues, mainly involved in organ-specific autoimmunity. The distinct specificity of these reagents compared with patients' autoantibodies was confirmed by their ability to precipitate a 35 Kd protein from all the organs with which they react (Satoh et al. 1984). Clearly, these fusions have amplified a cryptic autoantibody response not normally detected by conventional assays. The significance of naturally occurring autoantibodies of this type and their relationship to autoimmunity has recently been the subject of lively debate (13th Forum in Immunology of the Annales Institut Pasteur, 1986) in which we participated (Todd et al. 1986d). It has proved very difficult to produce monoclonal antibodies with exactly the same specificities as patients' autoantibodies: the reasons for this are unclear. One possible explanation is the sequestration of the relevant B lymphocytes to the target organ and hence their poor representation in the patients' blood. Furthermore, the differentiation and activation state

of the B lymphocytes may be relevant, since many experimental monoclonal antibodies are IgM, whereas patients' autoantibodies are predominantly of the IgG class.

Cytoplasmic antibodies still remain an invaluable clinical tool for diagnostic and prognostic purposes but the pathogenic role of any autoantibody must lie in its ability to bind autoantigens expressed on the cell surface. This is a prerequisite for activating the complement cascade and killer lymphocytes. Surface reactive antibodies have been demonstrated in several organ-specific systems but there are substantial differences in the nature of their reactivity patterns in various tissues. In the thyroid, for example, it was initially demonstrated that virtually all sera containing thyroid microsomal antibodies also recognised surface antigens of viable thyroid cells in culture and in suspensions (reviewed by Pinchera et al. 1984), and the addition of complement led to lysis of the thyrocytes (Khoury et al. 1981b). This indicated that the cytoplasmic microsomal antigen was also expressed on the cell surface, suggesting that the specific autoantibodies could play a direct pathogenic role. However, ths situation was complicated by the subsequent demonstration that the surface expression of this autoantigen was restricted to the microvillar apical border facing the colloid space on the interior of the thyroid follicles (Khoury et al. 1984). These results unexpectedly demonstrated the existence of another sequestered antigen, which, like the eye and the sperm, is apparently inaccessible to the immune system. However, further work indicated that some thyroid follicles isolated from the glands of patients with autoimmune thyroid disease showed a spontaneous reversal of the cellular polarity with the microvillar border exposed at the vascular pole (Hanafusa et al. 1984). The precise stimuli which induce this phenomenon *in vivo* in predisposed individuals is unknown, but it is interesting to note that a similar 'inside-out' effect can be obtained *in vitro* by culturing follicles in media with high protein content (Hanafusa et al. 1984), a phenomenon originally described with rat thyroid follicular cells (Nitsch & Wollman 1980).

The second autoantigen relevant to destructive thyroid autoimmunity is thyroglobulin (Tg). Surface binding of anti-Tg autoantibodies could be demonstrated by some workers (Fenzi et al. 1982) but not by others (Khoury et al. 1981). However, asialoagalacto-Tg (but not native Tg) binds to the surface of cultured thyrocyte monolayers at sites distinct from thyroid microsomal antibody where it can bind the appropriate autoantibodies (Roitt et al. 1984). The anti-Tg autoantibodies may therefore also play a role in the autoimmune destruction. However, it remains to be established whether this incomplete Tg molecule can be expressed at the vascular pole of thyroid follicles since all the experiments so far have been performed on monolayer cultures in which only the apical/microvillar pole of the cells is exposed.

Surface expression of cytoplasmic antigens has also been demonstrated in adrenal (Khoury et al. 1981a) and gastric parietal cells (Masala et al. 1980),

although in the latter system autoantigens unique to the plasma membrane have been identified by testing some sera of patients with pernicious anemia but which lack cytoplasmic reactivity (De Aizpurua et al. 1983a, b). Whether the problem of cell-polarization also applies to these cells *in vivo* is presently unknown.

The situation in pancreatic autoimmunity is more complicated and remains unsettled. It was initially claimed that sera of Type I diabetic patients contained islet-cell surface antibodies (ICSA) which reacted with viable rodent islets (Lernmark et al. 1978) and these findings were confirmed on human fetal islets (Pujol-Borrell et al. 1982). The fact that there was no direct correlation with the presence of cytoplasmic ICA in the serum led to the conclusion that islet cells have surface autoantigens distinct from those of the cytoplasm (Freedman et al. 1979). However, the existence of ICSA was strongly questioned when it was shown that this specificity could be mainly due to antibodies reacting with bovine serum albumin (BSA), which is normally present in the medium used for culturing islets (Colman et al. 1985). Diabetic sera were also unable to precipitate radioactive material from islets surface-labelled with ^{125}I, indicating that they lack autoantibodies specific for surface components which can be labelled in this way. However, the same sera were able to precipitates a 64 Kd protein when the islets were, as previously, metabolically labelled with ^{35}S-methionine (Baekkeskov et al. 1982). This suggests that the organ-specific antigen so far identified in the islets may be a cytoplasmic component rather than being expressed on the surface (Colman et al., submitted). It is only by repeating immunofluorescence studies with patients' and control sera preincubated with BSA that the existence of ICSA can be finally clarified. Other work has suggested that gangliosides may, together with proteins, form an integral part of the cytoplasmic islet autoantigens (Nayak et al. 1985).

One clear-cut example, which has been documented, of a disease having surface antibodies in the absence of cytoplasmic reactivity was in patients with uncomplicated vitiligo, when their sera were tested on viable melanocyte preparations (Naughton et al. 1983). Interestingly, the 9 cases described which did possess cytoplasmic melanocyte antibodies were all polyendocrine cases centred on idiopathic hypoparathyroidism (reviewed by Betterle et al. 1984). Although idiopathic hypoparathyroidism is conventionally included in the list of organ-specific autoimmune diseases, it has proved difficult to consistently demonstrate specific autoantibodies in this disorder. The original description of cytoplasmic antibodies in these patients (Blizzard et al. 1966) has not been confirmed in larger series which, however, identified antibodies to oxyphil cells (Swana et al. 1977, Betterle et al. 1985), although these actually reacted with mitochondrial antigens, and were not disease-specific. The possibility that the humoral response against parathyroid chief cells is confined to their surface (by analogy with vitiligo) has recently been suggested (Posillico et al. 1986). However, a word of caution should be added here since viable parathyroid cells from adenomas show atypical antigen expression, so

that false positive reactions can easily occur (Mirakian, unpublished observation). Furthermore, expression of Fc receptors, as on viable pituitary cells (particularly the ACTH-producing cells) can mask organ-specific surface reactions (Pouplard et al. 1976).

It is thus apparent that the precise identification and characterization of relevant autoantibodies and the autoantigens which they recognize is still a major challenge in organ-specific autoimmunity. The data produced so far clearly indicate the antigenic heterogeneity from one organ to another, and also between patients affected by apparently similar clinical conditions.

STIMULATING ANTIBODIES: A GROWING FAMILY

As for classical anti-thyroglobulin antibodies, 1986 is the 30th anniversary of the discovery of the Long Acting Thyroid Stimulators (LATS) in Graves' disease (Adams 1956). It is now well-established that thyroid stimulating antibodies (TSAb) are directed against the TSH receptor (TSH-R), mimicking the action of TSH by stimulating thyroid hormone production. However, it is postulated that there is more than one binding site on the TSH-R itself and the multiplicity of methods devised over the years to detect TSAb in affected patients illustrates the diversity of their antibodies (reviewed by Todd & Bottazzo 1985a). The production of monoclonal antibodies by fusing lymphocytes from Graves' patients with suitable immortal cell lines should further help to clarify this important issue (reviewed by Kohn et al. 1984). In comparing assays for TSH-R antibodies it is worth emphasizing that binding does not necessarily correlate with ability to stimulate. Mixtures of 'stimulating' and 'blocking' antibodies in the same serum complicate the matter still further, but if the problem is approached correctly, and assays of greater specificity devised, this combination of antibodies could provide a plausible explanation for the heterogeneous clinical picture of thyrotoxicosis, which includes rapid and prolonged remission in certain patients and sudden relapse in others (Bottazzo & Doniach 1986).

Until recently, non-toxic simple and nodular goitres were not considered to have an autoimmune etiology. However, they are known to occur more frequently than expected by chance in families with autoimmune thyroid diseases and 45% of these patients have low titres of autoantibodies to thyroid microsomal antigen and/or thyroglobulin in their sera. The discrepancy between toxicity and goitre size in Graves' disease and the flat TRH responses in some cases of non-toxic goitre first led to the hypothesis that some forms of thyroid hyperplasia might be due to growth-promoting antibodies (Doniach 1976). The actual occurrence of growth-stimulating immunoglobulins (TGI) was first established by Drexhage et al. (1980) using a sensitive cytochemical bioassay (Drexhage et al. 1983). These autoantibodies are now known to be a cause of goitre formation in Graves' disease, in two thirds of sporadic non-toxic nodular goitres (Van der Gaag et al.

1985b, Smyth et al., in press), and in a proportion of Hashimoto goitres (Drexhage et al. 1980). The original method used for the detection of TGI is still very labor-intensive and is not suitable for extensive population studies. Advances in this direction have been made by measuring ^{3}H-thymidine incorporation into reconstituted rat thyroid follicles incubated with suitable patient's sera, but at the expense of a much lower sensitivity of detection (Chiovato et al. 1983). The right balance between 'ease' in performing the assay and 'sensitivity' should be achieved by measuring TGI on the FRTL-5 cells (an immortalized rat thyroid cell line): this assay is based on a rise of the mitotic index from 0 to 10% when these cells are incubated with the patient's autoantibodies (Ealey et al. 1985).

Stimulating antibodies are also entering into the field of gastric autoimmunity. In experiments performed in rats, Dobi & Lenkey (1982) initially showed that immunoglobulins from patients with hyper-secretory duodenal ulcer stimulated gastric secretion in the animal stomach and the size and the number of gastric parietal cells were increased. Furthermore, in some families in which duodenal ulcers occurred in association with hyper-gastrinemia and elevated levels of pepsinogen I, hypertrophy of gastric parietal cells was documented, and other members of these families had thyrotoxicosis and/or atrophic gastritis (Taylor et al. 1981). This raised the possibility that, as in thyroid autoimmunity, both destructive and stimulating antibodies might also occur in gastric autoimmunity. This prompted Franca de Lazzari to examine whether a subgroup of patients with duodenal ulcer do indeed possess stimulating autoantibodies. This was investigated by determining the ability of patients' immunoglobulins to stimulate cyclic-AMP production by parietal cell-enriched suspensions prepared from the stomachs of young male guinea pigs (De Lazzari et al., submitted). Thirteen out of 30 patients had immunoglobulins with stimulatory effects in this assay, which suggested that they could act either on histamine (H_2)-receptors on gastric parietal cells or on chief cells to stimulate pepsinogen production. In either case, their role in maintaining and perpetuating the gastric secretion would be of pathogenic importance. However, more than half of our cases with stimulating antibodies did not respond to anti-H_2-R drugs. This might indicate *in vivo* occupancy of the target receptor by antibody and suggests the potential prognostic value of the test in predicting the responsiveness to specific treatment in these patients.

A careful analysis of the characteristics of the patients studied indicated that stimulatory gastric autoimmunity is not directly analogous to autoimmune thyroid diseases. In thyroid autoimmunity, there is a remarkable overlap between destructive and stimulatory features and females are mainly affected. In pernicious anemia the sex ratio is more equal, but in the case of duodenal ulcer there is a total reversal of the ratio, especially in hyper-secretors, with men being most frequently affected. Moreover, immunofluorescent autoantibody tests in our recent report, and in previous population studies, suggest that destructive and stimulatory autoantibodies against the stomach are not correlated: for example,

duodenal ulcer patients show a low prevalence of parietal cell antibodies, even when compared with healthy controls. Gastric autoantibodies with growth stimulatory effects are presently under investigation. Table II summarizes the known receptor-stimulating antibodies in human disease, including the latest addition concerning adrenal Cushing's syndrome (Teding van Berkhout et al. 1986).

'BLOCKING' ANTIBODIES: A LENGTHENING LIST

Non-stimulatory blocking antibodies can bind to the same receptors as the stimulating antibodies discussed above and hence cause clinical symptoms. Myasthenia Gravis is still the prototype disease involving receptor-blocking autoantibodies in which an extensive loss of acetylcholine receptors occurs (reviewed by Compston & Vincent 1985, Dawkins & Garlepp 1985). Similarly, TSH-receptor-associated 'blocking' antibodies may stop the pathway of thyroid hormone synthesis, or the growth of thyroid cells. The latter thyroid growth blockers are found in primary myxedema at all ages, while in atrophic thyroiditis autoantibodies impair thyroid hormone synthesis (reviewed by Konishi et al. 1984) or prevent re-growth of thyroid follicles despite increased pituitary output of TSH (Drexhage et al. 1981). Both types of blocking antibodies are also involved in neonatal hypothyroidism, causing different clinical symptoms. Blockers of function transmitted through the placenta cause temporary hypothyroidism (Matsuura et al. 1980), but the growth blockers interfere with normal development of the thyroid in the fetus and are responsible for almost half the cases of athyreotic cretinism (Van der Gaag et al. 1985a).

Anti-insulin receptor antibodies were first observed in patients with extreme insulin resistence and associated acanthosis nigricans by their ability to inhibit binding of ^{125}I-insulin to peripheral blood mononuclear cells (reviewed by Kahn et al. 1982). More recently, antibodies to insulin receptors were described in a group of untreated, newly diagnosed diabetic patients using a rat adipocyte binding assay (Maron et al. 1983). The latter findings are still awaiting confirmation. However, comparison of the data relating to these two conditions indi-

TABLE II
Known receptor-stimulating antibodies in human diseases

Disease	Antigen	Effect	Reference
Graves' thyrotoxicosis	TSH-R	T_3-T_4	Adams (1958)
Graves'/Non-Toxic Goitre	?	Growth	Drexhage et al. (1980)
Adrenal Cushing's disease	ACTH-R ?	Steroids Growth	Teding Van Berkhout et al. (1986)
Duodenal Ulcer	H_2-R	Acid	De Lazzari et al. (submitted)

cates that, although they apparently involve similar antibodies directed against the same receptor, these antibodies produce different clinical effects and do not have the same immunological characteristics. In contrast to the insulin-resistant diabetic syndrome, in newly-diagnosed juvenile diabetic patients resistance to the injected hormone is uncommon and whereas the receptor antibodies were IgG in the former condition they were found to be predominantly IgM in the latter. Interestingly, both types of antibodies had a stimulating effect in the different assays used when this was assessed in the early phase of their incubation *in vitro*. This stimulation may cause subsequent down-regulation of insulin-receptor expression, and lead to the insulin resistance in the severe clinical syndrome.

As in destructive thyroiditis, other atrophic organ-specific autoimmune disorders seem to involve receptor blocking antibodies. This is emphasized by the demonstration of gastrin-receptor blockers in fundal gastritis (Loveridge et al. 1980) and the latest development in this area is the identification of immunoglobulins with similar properties in Addison's disease (Wulffraat et al. 1986). Table III summarizes these and other less frequent cases of blocking antibodies against hormone receptors in relation to disease.

ANTI-HORMONE ANTIBODIES: WHAT IS THEIR SIGNIFICANCE?

It is well-established that autoimmune responses can often occur against large peptides: hormone precursors, such as Tg, and intrinsic factor (IF) are perhaps the best example. Antibodies to IF have an important influence on the outcome of fundal gastritis when they are secreted into the gastric juice and precipitate the onset of pernicious anemia by neutralizing the residual traces of IF made by the atrophic mucosa. However, these antibodies have no effect on the function of viable parietal cells and do not affect acid secretion (Doniach et al. 1981).

TABLE III
Known receptor-blocking antibodies in human diseases

Disease	Antigen	Reference
Myasthenia Gravis	Acetylcholine-R	Lindstrom et al. (1976)
Insulin-Resistant Diabetes with Acanthosis (Type B)	Insulin-R	Flier et al. (1976)
Renal Insufficiency (some cases)	Parathormone-R	Juppner et al. (1978)
Fundal AI Gastritis	Gastrin-R on parietal cells	Loveridge et al. (1980)
Asthma (some cases)	Beta-2-Adrenergic-R	Fraser et al. (1981)
Gonadal Deficiency	Gonadotrophin-R	Escobar et al. (1982)
Atrophic AI Thyroiditis	TSH-R (c-AMP)	Endo et al. (1978)
	?TSH-R (Growth)	Drexhage et al. (1981)
Addison's Disease	ACTH-R	Wulffraat et al. (1986)

Much less frequent is the appearance of anti-T3, T4 and TSH antibodies (reviewed by Schatz & Doniach 1983). These autoantibodies are normally detected by their interference in the measurement of native hormone by radioimmunoassay (RIA) but, rarely, they also give rise to clinical symptoms.

In the context of pancreatic autoimmunity, the spontaneous development of insulin autoantibodies (IAA) is gaining prominence. The full 'insulin autoimmune syndrome' was initially described in Japan (reviewed by Hirata 1983) but has been documented in other countries to a lesser extent (Burden & Rosenthal 1983). The patients originally described in Japan were treated for thyrotoxicosis with methimazole (Okabe et al. 1983). They also had hypoglycemic attacks which spontaneously remitted after withdrawal of the anti-thyroid drug. Recently, insulin autoantibodies have been described in newly diagnosed patients with Type I diabetes (Palmer et al. 1983) and in some polyendocrine non-diabetic cases (Wilkin & Nicholson 1984). Using immunoglobulin class-specific conjugates in an ELISA, Dr. Betty Dean identified similar specificities in susceptible members of our diabetic families (Dean et al. 1986). IgG-IAA were found to be significantly associated with complement-fixing (CF)-ICA (Bottazzo et al. 1980) and 9 out of 12 CF-ICA$^+$ individuals developed acute diabetes during an 8-yr follow-up study (reviewed by Bottazzo et al. 1986). One out of 18 first degree relatives who where CF-ICA-negative but positive for conventional ICA, also possessed IgG-IAA and progressed to overt disease. However, IgM-IAA, when detectable, did not confer an increased risk for Type I diabetes. Our results support recent observations suggesting that IAA measured by RIA are preferentially of the IgG class (Srikanta et al. 1986). However, the incidence of IAA in populations at risk appeared to be lower when measured by RIA than by ELISA. Furthermore, our own results differ from those of other investigators who also used an ELISA (Wilkin et al. 1985) in that they did not find an association of IAA with ICA. This latter discrepancy may be explained by the separate analysis of IgG-IAA and IgM–IAA in our study. Recently we have observed elevated levels of IgM-IAA in a high proportion of children with serological evidence of recent viral infections (Bodansky et al., in press). The significance of these findings is now under investigation but they may reflect a cross-reactivity between insulin and an environmental antigen leading to the production of IAA. These new data are of great theoretical interest in relation to pancreatic autoimmunity since it is obvious that we are not dissecting all the possible immunological components whose interplay masks the basic destructive process against beta cells.

The contribution, if any, of anti-hormone antibodies to the clinical picture of autoimmunity is not known. However, a destructive role for anti-insulin antibodies, for example, is certainly feasible given the observation that cultured animal (Kaplan et al. 1983) and human (Pujol-Borrell et al. 1986b) beta cells express insulin on their plasma membrane. Another possible, but not mutually exclusive role for anti-hormone antibodies is suggested by their theoretical anti-

idiotypic relationship to anti-hormone receptor antibodies (reviewed by Roitt 1984) which were discussed in the previous two sections. Thus, the idiotype of an anti-hormone antibody could be the substrate for the generation of 'internal image' anti-idiotypic antibodies whose binding sites would resemble the epitopes of the hormone itself. Some of the latter antibodies should therefore bind to the hormone receptor. For example, anti-idiotypic antibodies to anti-TSH might bind to the TSH receptor. This could explain the generation of some forms of thyroid stimulating/blocking antibodies, and possibly account for certain cases of intermittent or recurrent hyperthyroidism (Raines et al. 1985). The feasibility of this scheme centred around TSH has been experimentally demonstrated in both rabbits (Beall et al. 1986) and mice (Gafni et al. 1986). A similar murine model relating to anti-insulin/insulin-receptor antibodies has also been extensively investigated (Schechter et al.1984).

   Little direct evidence has so far emerged for the clinical relevance of these schemes, apart from isolated cases, including a Type I diabetic whose serum contained antibodies to both insulin and the insulin receptor (Shoelson et al. 1986). Testing predisposed first degree relatives of patients for anti-hormone and anti-hormone receptor antibodies may lead to further evidence. It may also be informative to carefully analyze circulating immune complexes which occur in patients at the acute onset of organ-specific autoimmune disorders (although only at low concentrations) including Graves' disease (De Bruin et al. 1984) and Type I diabetes (reviewed by Pozzilli & Di Mario 1984). Finally, it should be borne in mind that, due to the mutuality of idiotypic interactions, the converse situation to that described above is perfectly feasible in which anti-hormone receptor antibodies precede, and give rise to, anti-hormone antibodies.

EPITHELIAL EXPRESSION OF MHC CLASS II MOLECULES: AN IMPORTANT PATHOGENIC FACTOR IN ORGAN-SPECIFIC AUTOIMMUNITY

Although much remains to be learned about effector mechanisms in organ-specific autoimmunity, still less is known about the precise etiology of autoimmune diseases. It is apparent, however, that the causes are complex, with contributions from both genetic and environmental factors. This is mirrored in the variety of animal models of autoimmune diseases which have been developed: at the one extreme are inbred strains in which the majority of individuals develop particular diseases at a similar age, regardless of their environment and at the other extreme are models in which disease is induced by deliberate immunization of animals which would otherwise remain healthy. It is thus clear that no single animal model can fully mimic the corresponding human diseases. This emphasizes the importance of investigating all aspects of human autoimmunity in order to determine those features which are central to the pathogenesis.

Epithelial expression of Class II in autoimmunity

An interesting property of thyroid epithelial cells (thyrocytes) is their ability to express major histocompatibility complex (MHC) Class II molecules when preparations of human thyroid cells are cultured with phytohemagglutinin or other plant lectins (Pujol-Borrell et al. 1983). The relevance of this finding to autoimmunity is suggested by the fact that, although thyrocytes are normally HLA Class II-negative, they do express these molecules in patients with Graves' thyrotoxicosis and Hashimoto's thyroiditis as well as having enhanced expression of HLA Class I products (Hanafusa et al. 1983).

The importance of this inappropriate HLA Class II expression is indicated by the well-known involvement of these molecules in the presentation of antigens leading to T cells stimulation. These considerations led to the hypothesis that the inappropriate expression of MHC Class II molecules by epithelial cells might enable these cells to present their own surface molecules to autoreactive T cells, by-passing a requirement for "conventional" antigen-presenting cells like macrophages and dendritic cells (Bottazzo et al. 1983). Such a process could make an important contribution to the potentiation, and also possibly the intiation of the autoimmune process.

The applicability of our findings in thyroid to a variety of autoimmune diseases is indicated by the growing list of autoimmune conditions in which abnormal expression of HLA Class II molecules by specific cells of the target organs has been demonstrated (Table IV). This list can be roughly divided into three categories, the first of which includes the autoimmune endocrinopathies involving the thyroid or pancreas. The second category of organ-specific conditions to be included are those involving the gut where the situation is somewhat different

TABLE IV
Aberrant HLA Class II expression in autoimmune diseases

Disease	Cells aberrantly expressing Class II molecules	Reference
Autoimmune thyroid diseases	Thyroid epithelium	Hanafusa et al. (1983). Jansson et al. (1985). Aichinger et al. (1985).
Type I (insulin-dependent) diabetes mellitus	Pancreatic beta cells	Bottazzo et al. (1985). Foulis & Farquharson (1986).
Inflammatory bowel disease	Gut epithelium	Selby et al. (1983)
Autoimmune protracted diarrhea of infancy	Immature jejunal enterocytes	Mirakian et al., unpublished.
Alopecia areata	Hair folllicular cells	Messenger et al. (1984).
Primary biliary cirrhosis	Bile duct epithelium	Ballardini et al. (1984).
Sjörgren's syndrome	Salivary ducts	Lindhal et al. (1985).

because Class II is normally synthesized by mature villous enterocytes of the small intestine (Selby et al. 1981). This physiological expression could play an important role in the handling of environmental antigens from micro-organisms of the gut, and/or the education of gut-associated intra-epithelial lymphocytes. By contrast, abnormal Class II expression by cells of the gut could upset the normal immune regulatory mechanisms in these tissues and play a similar role in autoimmune activation to that postulated in the thyroid. A third category includes primary biliary cirrhosis (PBC) and Sjögren's syndrome. These conditions are not fully organ-specific in the sense that, although they affect particular tissues (i.e. the bile ducts and exocrine glands, respectively), they are characterized by organ non-specific autoantibodies: mitochondrial antibodies in PBC and antibodies to the ribonucleoprotein La/SS-B in Sjögren's syndrome. In these diseases, Class II expression by epithelia of the affected tissues could contribute to the tissue localization of the attack and also, possibly, to the autoantibodies produced if, for example, bile duct epithelial cells can express mitochondrial antigens on their surface.

Put into a wider context, the model we have proposed for the role of inappropriate epithelial Class II expression in autoimmunity accords with the suggestion of a more general relationship between aberrant or excessive MHC Class II expression and pathogenesis (Unanue et al. 1984). For example, in rheumatoid arthritis the large number of Class II+ macrophages/dendritic cells in affected joints may stimulate excessive T cells activation (Janossy et al. 1981, Klareskog et al. 1982). Similarly, classical antigen-presenting cells may play a critical role in autoimmune chronic active hepatitis (CAH), since, in contrast to the bile duct epithelium in PBC discussed above, the target hepatocytes in CAH show very little Class II expression, but Kupffer cells are increased in numbers and Class II positivity in the affected tissue (Ballardini et al., submitted).

Evidence of a role for Class II in autoimmune pathogenesis

In order to determine whether HLA Class II+ thyrocytes can really function as antigen-presenting cells, their ability to stimulate a cloned human T cell line was investigated in collaboration with Drs. M. Londei and M. Feldmann. The line employed, HA1.7, is specific for a defined peptide fragment (p20) of the influenza A hemagglutinin molecule, which it recognizes in association with DQw1 (Lamb & Feldmann 1983). Class II+ thyrocytes from a Graves' disease patient of the appropriate HLA type were indeed found to stimulate proliferation of HA1.7 in the presence of p20, but not an irrelevant peptide of hemagglutinin, and this presentation was blocked by monoclonal anti-Class II antibodies (Londei et al. 1984). However, unlike autologous monocytes, the thyrocytes were unable to present the whole virus (fixed or live), suggesting that they cannot process

complex antigens for presentation at the cell surface. This did not necessarily mean that thyroid cells would be unable to present their own surface molecules as autoantigens since, by their very nature, these are inserted in the plasma membrane where they could be recognised by T cells together with Class II molecules. Indeed, other experiments showed that autoreactive cloned T cell lines, derived from the activated lymphocytes infiltrating Graves' disease thyroids, proliferated upon exposure to autologous thyrocytes, but were not stimulated by either autologous peripheral blood mononuclear cells or allogeneic thyrocytes (Londei et al. 1985). Furthermore, the interaction with autologous thyrocytes could be blocked with monoclonal anti-Class II antibodies. This experiment thus demonstrated directly that thyrocytes are capable of presenting directly their own autoantigens in an MHC Class II-restricted, tissue-specific fashion to autoreactive T cells infiltrating the diseased thyroid.

Consistent with the above experiments are findings in a murine system, in which the I-A restricted primary sensitization of murine lymphocytes by syngeneic thyrocytes generates activated T cells capable of inducing thyroiditis (Charreire & Michel-Bechet 1982) and also thyroid-specific cytotoxic T cells (Salamero & Charreire 1985).

The enhanced expression of HLA Class I molecules by thyrocytes in ATD should also not be overlooked, since this could facilitate killing by Class I-restricted CD8$^+$ autoreactive T cells. Indeed, CD8$^+$ T cells with natural killer activity are the principal clones derived from Hashimoto's disease thyroid glands (Del Prete et al. 1986, Londei et al. 1986).

If HLA Class II expression by thyrocytes really does play an important role in the pathogenesis, then as well as permitting demonstrations of direct interactions between thyrocytes and T cells, the occurrence of this aberrant expression would be expected to correlate with other features of the autoimmune pathology. In this regard, we have analyzed a large series of patients and found a significant relationship between HLA Class II expression by thyrocytes and the occurrence of circulating autoantibodies to thyroglobulin and thyroid microsomal antigen (Todd et al. 1986b, Lucas Martin et al., submitted). This relationship was not restricted to cases of overt thyroid autoimmune disease, thus suggesting a role for thyrocyte Class II expression in autoimmune pathogenesis at the subclinical as well as clinical levels.

A more detailed analysis of a similar type was performed in Graves' disease patients in whom we examined expression of the HLA-D subregions DR, DQ and DP. The incidence and intensity of Class II subregion expression by thyrocytes was found to vary between patients, with DR being most expressed, followed by DP, and DQ least expressed. In this analysis, the most significant relationships were observed between high serum titres of thyroglobulin autoantibodies and thyrocyte expression of HLA-DQ, and between autoantibodies to microsomal antigen and HLA-DR. These findings are consistent with different HLA-D

subregion products expressed by thyrocytes being dominant in stimulating responses to different thyroid autoantigens (Todd et al. 1986b).

Regulation of epithelial Class II expression

In view of its potential role in autoimmune pathogenesis, it was important to investigate the regulation of HLA Class II expression in the epithelial cells expressing these molecules in autoimmune diseases. What has clearly emerged from our collaborative studies with Dr. M. Feldmann is that such regulation is complex, with a variety of factors modulating Class II expression, and differences in susceptibility depending on the cell type. These principles are best exemplified by our studies on thyrocytes in relation to thyroid autoimmunity, and on pancreatic islet cells in relation to Type I diabetes mellitus. Interferon (IFN)-gamma has been found to induce or enhance Class II expression by a variety of cell types and, indeed, we found that recombinant human IFN-gamma induced strong surface and cytoplasmic expression of Class II molecules in cultured normal human thyrocytes (Todd et al. 1985b). The surface expression was detectable within 24 h and as little as 1 U/ml was effective, thus being within the physiological range. By contrast, IFN-alpha and IFN-beta did not induce Class II expression although, like IFN-gamma, they did enhance HLA Class I expression by the thyrocytes. IL-2 had no effect on thyrocyte expression of either Class I or Class II.

These findings suggest that IFN-gamma may well be an inducer of the Class II expressed by thyrocytes in ATD: the production of this lymphokine by activated autoreactive T cells infiltrating the gland could enable the spread of Class II expression by the epithelial cells, and hence the propagation of the autoimmune process. IFN-gamma can also determine the quality of Class II subregion expression by thyrocytes. Thus, low doses of IFN-gamma (5–10 U/ml) induce expression of DR, but very little DP or DQ, whereas DR, DP and DQ are all induced in a high proportion of thyrocytes cultured with 500 U IFN-gamma/ml (Todd et al. 1986a and in preparation). This may help to explain the heterogeneity of thyrocyte HLA-D subregion expression by Graves' disease patients, noted above.

All cells are responsive to a variety of regulatory stimuli, and it would be naive to assume that IFN-gamma is the only stimulus relevant to epithelial Class II expression: indeed, investigations in Type I diabetes highlight the involvement of other factors. In this disease, the insulin-producing beta cells, which are the target of the pathogenic process, aberrantly express Class II molecules, although the other islet endocrine cells and the exocrine cells of the pancreas remain Class II⁻ (Bottazzo et al. 1985, Foulis & Farquharson, in press). By contrast, only a small proportion of cultured human beta cells are induced to express Class II by rIFN-gamma, although the exocrine and ductal cells in these cultures become strongly Class II⁺ (Pujol-Borrell et al. 1986c). However, induction of Class II in cultured

islet cells can be achieved with a combination of IFN-gamma and tumor necrosis factor (TNF) or lymphotoxin (LT), although TNF or LT alone have no effect (Pujol-Borrell et al. 1986a, and submitted for publication). Unlike the *in vivo* pathological situation, Class II was induced in the glucagon cells and exocrine/ ductal cells, as well as in the beta cells of the pancreatic cultures. This raises the possibility that synergism between IFN-gamma and TNF or LT is not the mechanism initiating beta cells Class II expression in diabetes, although it could have a potentiating effect. Alternatively, effective beta cell specificity could be explained by localized release of these mediators *in vivo* with their short half-life limiting their sphere of action, possibly together with yet another signal acting specifically on beta cells. In any case, it is clear that our *in vitro* systems do not fully reproduce the *in vivo* situation as yet.

   A requirement for combinations of factors to optimally induce epithelial Class II expression should limit the circumstances in which such activation occurs, and hence the opportunities for autoimmune stimulation. This is clearly applicable to the pancreatic islet, where either signal alone is ineffective, but could also apply to the thyroid, particularly in circumstances where the exposure to IFN-gamma is limited. As in the islets, TNF synergizes with IFN-gamma to induce Class II expression by thyrocytes (Todd et al., in preparation). However, a more potent enhancer of thyrocyte Class II expression *in vitro* is thyroid stimulating hormone (TSH). This is most effective in combination with a suboptimal dose of IFN-gamma, and although TSH added alone to thyroid cultures has some effect, this is probably secondary to activation of Class II genes by some other means (Todd et al. 1986c and in preparation). The enhancing effect of TSH is apparent on the expression of all three HLA-D subregions, and is mimicked by dibutyryl-cyclic AMP, suggesting that cyclic AMP is the second messenger for this activity, as it is for many effects of TSH on thyrocytes. The optimal *in vitro* concentration of TSH for Class II stimulation is 0.1 mU/ml, which is within the range of serum concentrations in hypothyroid patients with Hashimoto's disease. The raised TSH levels in these patients might therefore exacerbate the pathogenic process by enhancing the aberrant Class II expression within the thyroid. Thyroid stimulating antibodies, which mimic the actions of TSH, could have similar effects in Graves' disease.

   Since IFN-gamma is a lymphocyte product, induction of epithelial Class II by IFN-gamma alone, or in combination with synergizing factors, must involve immune mechanisms. These could be related or unrelated to the autoimmune activation: in the latter case involving a response to a local viral infection, for example. However, the possibility that other mechanisms could also stimulate epithelial class II expression should be considered, particularly since, as already noted in relation to diabetes, Class II induced *in vitro* by mechanisms involving IFN-gamma can be found in a wider range of cell types than it is in the diseased tissues. One possibility is that certain viruses might directly induce Class II. This

is supported by recent experiments in our laboratory in which epithelial cell lines were derived from thyroid monolayers by transfection with a plasmid containing the early region of SV-40 viral DNA: a proportion of the cells in these lines showed constitutive Class II expression (Belfiore et al. 1986 and in preparation). A different example is provided by the finding that rat astrocytes express Class II following non-infective interaction with a murine neurotropic coronavirus (Massa et al. 1986).

Another possibility is that factors other than IFN-gamma, derived from non-lymphocytic cells (e.g. macrophages or endothelial cells), might activate Class II genes. Precedents for this include the production of a novel form of interferon by macrophages infected with lentiviruses (Kennedy et al. 1985) and the production of an Ia-inducing factor by a murine macrophage tumor cell line treated with IFN-gamma (Walker et al. 1984).

Turning to the other side of the coin, we have also investigated mechanisms whereby epithelial Class II expression could be down-regulated, since these could be just as important as stimulating mechanisms in determining the level of Class II expression and the course of autoimmunity. Epidermal growth factor (EGF) has been reported both to stimulate thyroid growth in culture and suppress TSH-stimulated processes, such as efflux and organification of iodide (Westermark et al. 1983). In the present context, we found that EGF suppressed, by at least 50%, Class II expression by cultured thyrocytes induced by IFN-gamma alone, or IFN-gamma + TSH (Todd et al. 1986c and in preparation).

The implications of these various investigations are summarized in Fig. 1. First of all, it appears likely that a variety of courses may lead to epithelial Class II expression. This may depend not only on those substances which directly activate Class II genes, but also on synergizing co-factors. The importance of the latter's contribution will be determined by the nature and amount of the primary inducer (e.g. IFN-gamma), and the particular cell type involved. Furthermore, the nature and levels of the various stimulators will influence not only the quantity of Class II expressed, but also its quality in terms of HLA-D subregion expression. Secondly, the extent and duration of Class II expression will be affected by the balance between Class II enhancing and suppressive influences, which may thereby contribute to the severity and duration of autoimmune attack. The autoimmune processes will themselves contribute to the position of this balance: some of these effects will be direct, e.g. by autoreactive lymphocytes infiltrating the diseased tissue producing lymphokines, including IFN-gamma and lymphotoxin; others will be indirect, e.g. changes in hormone production by the thyroid as a result of autoimmune processes will effect the levels of TSH and EGF (for further details, see Todd et al. 1986c). Finally, the effect of a particular factor may vary with the cell type: for example, whereas EGF suppresses Class II expression by thyrocytes (Todd et al. 1986c, and in preparation), it has been found to enhance Class II expression by human monocytes (Acres et al. 1985).
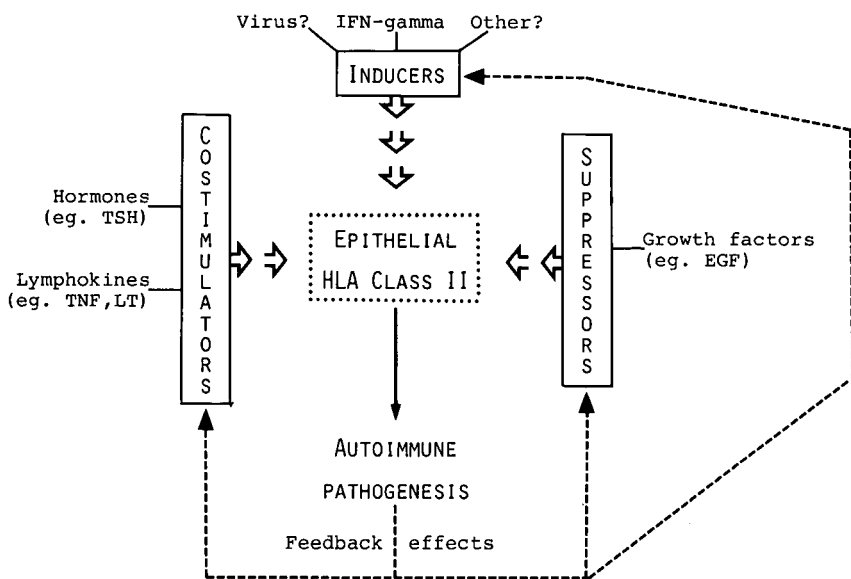
Figure 1. Modulation of epithelial HLA Class II expression in relation to autoimmunity.

SPECULATIONS AND PROSPECTS FOR THE FUTURE

In recent years, the unexpected results of novel investigations in organ-specific autoimmunity have required us to modify our conception of the mechanisms involved. For example, the target cells no longer appear to be 'passive', as previously thought, but their role is now seen to be much more prominent with the demonstration that they can express HLA Class II molecules. The current debate is thus focused on whether the process should be visualized as 'homicidal', i.e. attack by common environmental factors and autoreactive immunocytes, either separately or in combination, on 'unsuspecting' target cells, or as 'suicidal', with the target making itself vulnerable by expression of Class II and enhanced expression of Class I molecules (Bottazzo 1986). The latter possibility certainly has a conceptual advantage in that both the afferent and efferent limbs of the autoimmune response then take place within the same location, i.e. at the surface of the target cells themselves. This may be contrasted with the conventional, but more complex models which require release of surface autoantigens from damaged target cells, their presentation by classical antigen-presenting cells in distant specialized lymphoid organs, and the subsequent re-circulation of activated autoreactive lymphocytes to the target tissues. These steps clearly pose a number of logistical problems.

With regard to the forces promoting infiltration of the target tissues, an

important part could be played by capilliary endothelial cells, which physiolog-ically constitute a discrete and selective barrier between the blood and the tissues. In organs affected by autoimmunity, these structures are hypertrophic and strong-ly Class II-positive, as indicated by the observations in diabetic pancreases where this phenomenon occurs selectively around islets which are otherwise normal (Bottazzo et al. 1985, Foulis & Farquharson, in press). This suggests an important role for the capilliaries in facilitating the 'homing' of potentially autoreactive lymphocytes (Gallatin et al. 1983, Naparstek et al. 1984). The enhanced Class II expression by endothelial cells could be important in this process, and might possibly enable these cells to present antigens which are cross-reactive with those expressed by the target endocrine cells. We have already mentioned the possibility that the endothelium might also secrete factors affecting MHC expression by the adjacent epithelial cells.

An intriguing question is what might be the role of epithelial Class II expression in non-autoimmune situations? It is apparent that such expression can contribute to autoimmune pathology, but the fact that these cells have the capacity to express Class II at all and that, in thyrocytes for example, Class II is relatively easily induced, suggests that this may be advantageous to the organism in certain circumstances. Indeed, some epithelia, particularly where exposed to external environments, are normally Class II$^+$: we have already mentioned possible roles for such physiological Class II expression by epithelium of the gut. Pathological situations in which an immune response to certain cells would be desirable is when these cells are virally infected or malignantly transformed. Expression of Class II by such cells would facilitate an immune response to the viral or tumor antigens, and the subsequent destruction of the same cells would abrogate the spread of infection or growth of the tumor. The possibility that certain viruses might directly induce Class II expression has been discussed, and Class II ex-pression by thyroid papillary carcinomas has been noted (Lloyd et al. 1985, Lucas-Martin et al., submitted). Only when the balance of mechanisms regulating Class II expression and/or immune activation is upset would the phenomenon progress to overt autoimmunity.

Other important factors in autoimmune pathogenesis could include, firstly, suppressor T cells, which may normally tip the balance in favor of self non-responsiveness. The possible existence of organ-specific suppressor T cells (Topliss et al. 1983, Vento et al. 1985) remains attractive, although it is presently difficult to devise strategies for their isolation and unambiguous characterization. Turning to the possible involvement of anti-idiotypic responses, it is hard to envisage these making a major contribution to destructive autoimmunity, as has been suggested (Plotz 1983), particularly since the lack of MHC restriction of such responses is difficult to reconcile with the observations of aberrant MHC ex-pression by the target cells (reviewed by Bottazzo et al. 1984). On the other hand, we have discussed how the idiotype theory could explain the generation of

antibodies to hormone receptors and it is possible that growth stimulating anti-
bodies of this type could account for the regeneration of target tissues which is
postulated to occur during the long latency period preceding the onset of clinical
symptoms in many autoimmune diseases (Bottazzo 1984).

Advances in immunological techiques are facilitating a more detailed under-
standing of human organ-specific autoimmunity. For example, the recent appli-
cation of T cell cloning (Hohlfeld et al. 1984, Londei et al. 1985) has greatly
facilitated dissection of the processes involved in the autoimmune attack (re-
viewed by Feldmann et al. 1985). With regard to Graves' disease, intrathyroidal
lymphocytes from autoimmune glands proved to be the best source of starting
material for the establishment of thyroid-specific, autoreactive cloned T cell lines.
(This followed several unsuccessful attempts over the years using peripheral blood
lymphocytes from patients affected by the same disorders). Once again, epithelial
HLA Class II expression proved to be a key factor, since autologous Class II$^+$
thyrocytes were successfully employed to stimulate expansion of the autoreactive
T cells (Londei et al. 1985). On the other hand, major problems still exist in
applying T cell cloning technology to diabetes. Lack of sufficient numbers of
insulin cells for *in vitro* studies is one of the main limitations, but most important
is the fact that the pancreas cannot be biopsied, so that the T cells most relevant
to the diabetic process (i.e. those involved in the insulitis process) are not avail-
able. The T cell clones which would most probably be derived from the diabetic
pancreas are cytotoxic ones, since these appear to be the cells which dominate
the infiltrate and finally destroy the beta cells. With regard to thyroid autoimmu-
nity, only CD4$^+$ (?helper) T cell clones have so far been derived from the infiltrate
of Graves' disease thyroids, whereas CD8$^+$ clones with cytotoxic activity similar
to natural killer cells are more easily raised from Hashimoto's glands (Londei et
al. 1986, Del Prete et al. 1986). By analogy, the latter situation should also apply
in 'destructive pancreatic insulitis'.

Given the apparent importance of cytotoxic cells in destructive autoimmunity,
therapeutic drugs directed against these cells could obviously be very beneficial.
Autoreactive cytotoxic clones would be a valuable substrate for testing such agents.
Recently developed drugs do not appear to act in this way: thus newly diagnosed
diabetics given cyclosporin A or ciamexone (a new compound used in the attempt
to halt progressive beta cell damage [Bicker et al. 1986]) quickly relapsed after
withdrawal of the treatment (Stiller and Usadel, personal communications) and
cyclosporin A did not prevent an anamnestic anti-islet response in diabetics given
pancreatic transplants from HLA-matched siblings (Sutherland et al. 1986).

A more refined analysis of tissue-derived autoreactive lymphocytes should also
permit investigations into the T cell receptors directed against specific autoanti-
gens. Due to the organ-specific localization of the response, one would expect
the frequency of the relevant T cells in the peripheral blood to be very low. Thus,
T cell clones obtained from the lymphocytes invading the tissue would be the best

material for these studies. One could then accurately characterize the receptors at the genetic level, and also probe whether viral infection and integration might play a role in promoting the expansion of these self-reactive specificites.

Appreciation of the prominent role that the target cells play in autoimmune pathogenesis has highlighted the need for appropriate epithelial cell lines to facilitate further *in vitro* investigation. The usefulness of primary cultures of human epithelial cells is limited by their cellular heterogeneity and short life span. Furthermore, in diabetes research, for example, the lack of sufficient beta cells curtails experimentation. Endocrine cell lines only of animal origin are presently available and therefore the development of human cell lines is a high priority. Physiological mechanisms occasionally differ markedly between rodents and humans but these interspecies variations are most critical when studying immunological phenomena since antigenic differences can interfere with recognition by antibodies, and more especially by T cells. One approach to the development of human epithelial cell lines is exemplified by our experiments in transformation of thyroid cells with portions of the SV-40 genome. This has given insights into possible mechanisms of Class II induction, as discussed in the previous section, but it has also resulted in the establishment of stable cell lines which have been cloned several times. However, these lines no longer display all the features of the original cells. In fact, they have lost TSH receptors, they synthesize thyroglobulin but do not secrete it into the medium and the microsomal/microvillar antigenic system is poorly represented on the cell surface. This implies that future efforts must be concentrated on devising new strategies which will allow cells to grow while maintaining their original features. These include the use of suitable mutant viruses, the implementation of various culture conditions, more effective combinations of nucleic and cytoplasmic oncogene products and the use of the somatic cell fusion technique, so successful in monoclonal antibody technology. Some success with the latter technique in deveoloping thyroid cell lines has been reported by Karsenty et al. (1985).

The feasibility of these and other techniques, together with our present state of knowledge, bode well for exciting advances in the field of organ-specific autoimmunity.

The jigsaw puzzle is taking shape.

SUMMARY

The normally functioning immune system is subject to intricate networks of regulatory mechanisms: it is therefore not surprising to find that autoimmune diseases present a complex pathogenic picture in which the relative contributions of various factors probably determine the precise nature and course of disease.

This is particularly evident in the effector mechanisms of organ-specific autoimmunity which are described in this chapter. These ultimately give rise to the

disease symptoms, and can be directly cytotoxic, or may either stimulate or block functional activity or growth of the target cells. Their various contributions to human diseases are becoming more firmly established, as in Type I diabetes, or are only now being described, as in the case of EC-Ab in protracted diarrhea of infancy and as evidenced by the growing lists of receptor-stimulating or -blocking antibodies. The nature and precise location of relevant autoantigens is also coming under closer scrutiny.

The answers to the question of why these diseases arise in the first place remain more elusive. However, it is again likely that a variety of factors can contribute. The attractive possibility of a role for idiotypic interactions is gaining ground, particularly within the context of antibodies to hormones and their receptors. Another potential mechanism which we believe may be of central importance, particularly in the development of organ-specific destructive autoimmunity, and which we have discussed here in detail, is the aberrant expression of HLA Class II molecules by target cells. Whether this is actually an initiating factor is presently not known, but its potential for promoting pathogenesis both early and late in the process is clear. Furthermore, the complex nature of the regulation of epithelial Class II expression may help to explain the heterogeneity of features and course of disease in different patients with the same underlying pathology. All these advances in our basic understanding of the disease processes should ultimately lead to more effective and specific means of therapeutic intervention.

ACKNOWLEDGMENTS

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