Receptors, Antibodies, and Disease

Melvin Blecher

Abnormal antibody production is now recognized as the basis of specific endocrine and neurological diseases and their complications. Among the autoimmune diseases, the best understood from a mechanistic point of view are myasthenia gravis, Graves' disease, several variants of insulin resistance, and a variant of bronchial asthma. In each of these human disorders, the clinical symptoms can be traced to the actions of autoantibodies produced by a deranged immune system. The autoantibodies produced in these diseases are functionally heterogeneous. They may produce the clinical symptoms of hormone or neurotransmitter insufficiency either by blocking the binding of these agents to target cell surface receptors or by accelerating the internalization and degradation of these receptors. In other cases, the autoantibodies may produce the clinical signs of hormone excess by mimicking the actions of the hormone, in an uncontrolled fashion. In some cases, functionally different types of autoantibodies will appear in the same patient at different stages of the disease. For all of these autoantibodies, of whatever function, assays for their presence in serum are available, in forms suitable for clinical chemists, as well as for researchers; these will be described in this review. In addition to the known anti-receptor autoimmune diseases, there are a large number of other autoimmune diseases for which there is fragmentary evidence that their clinical symptoms have an anti-receptor autoantibody etiology. Several examples of this group will be discussed, and assays suitable for establishing the presence of anti-receptor antibodies in the sera of such patients will be provided. The disorders to be considered are: type I diabetes mellitus, chronic atrophic gastritis, autoimmune Addison's disease, autoimmune hypoparathyroidism, type II pseudohypoparathyroidism, resistant ovary syndrome, connective tissue diseases, and the HLA-B8/DR3 antigen haplotype as a potential marker for autoimmune diseases of the anti-receptor type.


Introduction

Disturbances in immune function have been implicated in the etiologies of many endocrine diseases (1–6). Defects of circulating T lymphocytes, suppressor T cells, macrophages, and immune cell–cell interactions have all been described (7, 8), and although there is fragmentary evidence for viral-based etiologies (9–11), perhaps the best studied are the relationships of abnormal antibody production to specific endocrine and neurological diseases and their complications.

Various antibodies interact with the endocrine system at various steps in the system (Figure 1). For example, antibodies can be directed at the hormone itself, as occurs in insulin-taking diabetics who develop anti-insulin antibodies, resulting in insulin "allergy" or, less-commonly, insulin resistance (12). Alternatively, autoantibodies may be directed at specific endogenous tissues, as evidenced in chronic lymphocytic thyroiditis (Hashimoto's thyroiditis, refs. 4, 13), idiopathic adrenal insufficiency (autoimmune Addison's disease, ref. 14), autoimmune hypothyroidism (15, 16), insulin-dependent (Type I) diabetes mellitus (5, 17, 18), and autoimmune gonadal insufficiency (6, 14). In addition, autoantibodies may be directed specifically or generally at non-endocrine tissues: the gastrointestinal tract (chronic atrophic gastritis, and pernicious anemia (19–21)); the connective tissues (sarcoidosis, unixin resistance (22–24), progressive systemic sclerosis (25–27), systemic lupus erythematosus (28, 29), rheumatoid arthritis (27, 30), dermatomyositis (31, 32)); the liver [autoimmune active hepatitis (33–35)], and platelets [autoimmune thrombocytopenia (36)].

Within the past 12 years a new, exciting, and potentially extensive class of antibody-mediated diseases has been established—one in which a break in immune tolerance to cell surface-receptor sites results in the production of autoantibodies against these (glyco)proteins.

Within the framework of intercellular communications, cell surface-receptors perform two kinds of functions: (a) recognition, in which the receptor specifically binds the chemical transmitter, be it hormone, neurotransmitter, circulating immune complexes, growth factors, viruses, or toxins, and (b) transduction, in which news of the ligand-
receptor interaction is translated by the cell into some biochemical action.

Normally, receptors are perceived as "self" by the immune system, and antibodies to one's own receptors are not produced. When the body perceives receptors as "foreign," an autoimmune reaction can occur, producing antibodies (mainly IgG, but others are also known) to the now-antigenic receptors. Subsequent interaction of autoantibodies with antigenic receptors may produce the clinical manifestations of disease, the anti-receptor autoimmune diseases. Several different kinds of autoimmune reactions involving receptors have been described, and these are related to the two general functions of receptors described above. Those involving humoral mechanisms include: (a) blockade of the receptor's recognition site so that the natural ligand binds either at all or with diminished avidity; (b) interaction of the antibody with the active site of the receptor in imitation of the natural ligand; (c) accelerated internalization and degradation of the receptor, leading to fewer functional receptors; (d) damage of the receptor by the antibody, acting alone or in concert with complement or cytotoxic cells or both; and (e) alteration by the antibody of the function of Fc receptors on cells of the mononuclear phagocyte system (formerly called the reticuloendothelial system) so that circulating immune complexes cannot be destroyed.

In this review, I will consider the best-known anti-receptor autoimmune diseases: myasthenia gravis, in which the acetylcholine receptor at the neuromuscular function is the antigen; Graves' and Hashimoto's diseases, in which antibodies are produced against thyrotropin (TSH) receptors, thyroglobulin, and thyroid endoplastic reticulum; insulin resistance, in which insulin receptors are the antigen; and a variant of bronchial asthma in which antibodies are produced against the β2-adrenergic receptor of lung membranes. I will also consider several other human autoimmune diseases that have the potential for being anti-receptor diseases. For all examples, the assays used in detecting and quantifying the circulating antibodies will be emphasized.

Myasthenia Gravis

Myasthenia gravis (MG) is an autoimmune disorder characterized by weakness in, and easily fatigued, voluntary muscles. The abnormality is located in the neuromuscular junction. It is brought about by the autoimmune production of antibodies (mainly IgG) directed against the acetylcholine (ACh) receptor at the junction.

Basic Features

Motor neurons usually synapse with skeletal muscle fibers at just one point in the middle of the fiber (Figure 2). ACh, the neurotransmitter, is synthesized in the terminals of motor neurons and is packaged into storage vesicles. Arrival of an electrical signal at the nerve terminal releases 150 to 200 quanta of ACh, which diffuses across the synaptic cleft and then binds to specific glycoprotein receptors on the junctional folds. This interaction creates ion channels within the four subunits of the receptor, permitting a flow of Na+ and K+ through the postsynaptic membranes (37). The resulting depolarization triggers an impulse ("action potential"), which is propagated along the muscle fiber membranes, initiating muscle contraction. Neurotransmission ends when ACh is removed from its receptors—in part by diffusion out of the junction and in part by enzymatic degradation by acetylcholinesterase (EC 3.1.1.7).

Transmission in Myasthenia Gravis

In patients with MG, resting and stimulated potentials are both low, despite adequate supplies of ACh to the motor end plates (38). Pioneering studies by Drachman and colleagues at Johns Hopkins (39), Appel and coworkers at Baylor (40), and by Engel and colleagues at the Mayo Clinic (41) accounted for the defect in MG patients.

By use of 125I-labeled α-bungarotoxin, a snake venom toxin that binds with great affinity to ACh receptors, it was shown that even though the ACh receptors in MG muscle are functionally normal, only 10% to 30% of the normal number is present. Thus the ACh released by a presynaptic depolarization would not result in a sufficient number of ACh-receptor interactions to achieve threshold depolarization of the postsynaptic membrane. The question is: What is responsible for this decreased number of ACh receptors?

Immunopathogenesis

Early evidence for an immunological basis for MG included:

(a) the frequency of thymic involvement (5–15% of the patients also have thymoma, 70–80% thymic hyperplasia) (42);
(b) the beneficial effects of thymectomy, and of immunosuppressant drugs and plasmapheresis (43);
(c) altered T-lymphocyte and B-lymphocyte function;
(d) development (44) of an animal model of MG by immunizing rabbits against purified ACh receptors from electric eels; and
(e) production of clinical myasthenia and reduced numbers of ACh receptors in mice by injection of human myasthenic serum (45); this same transfer phenomenon is seen in about 20% of neonates born to mothers with MG (46).

Subsequently, antibodies (chiefly IgG) directed against the patient's own ACh receptors were directly demonstrated in the blood of myasthenics. Such antibodies are detectable in about 90% of all MG patients, and in 100% of those with thymoma and thymic hyperplasia (Table 1, refs. 38, 47).
Mechanism of Action of Antibodies

Anti-ACh receptor antibodies interact with sites on the receptor distinct from those to which ACh binds (Figure 2)—and do so without competitively or allosterically inhibiting the binding of the neurotransmitter (37). The antibodies produced in various patients are heterogeneous in the sense that they are directed to different immunodeterminant regions on the receptor glycoprotein. These immunogenic determinants have been mapped by several groups (37, 48, 49) by using a library of monoclonal antibodies to ACh receptors. Although major and minor determinants have been identified, these workers could not detect, among antibodies from various patients, any especially pathogenic species of antibody that preferentially interacted with the major immunogenic determinants. Thus, no correlation has yet been established between the severity of the disease and the pattern of antibody specificity.

All species of MG antibodies tested appear to have the same mechanism of action, although there undoubtedly is quantitative heterogeneity among different species of MG antibodies. The reduction of ACh receptor number in myasthenic muscle is brought about by a combination of antigenic modulation (50–52) and antibody-targeted, complement-induced focal lysis of the post synaptic membrane (53, 54). Antigenic modulation is achieved by the following sequence of events: cross-linking of ACh receptors by the (Fab)2 fragment of myasthenic IgG; rapid lateral movement of the complex to form clusters (similar to "capping" in lymphocytes) in clathrin-coated depressions in post synaptic plasma membranes; internalization of the receptor–IgG complex; and reutilization of a fraction of the receptor mass, and lysosomal degradation of the remainder. Presumably, internalization of ACh receptors is a normal process, and myasthenic IgG merely accelerates it.

When antibody-induced receptor degradation greatly exceeds the capacity of muscle cells to regenerate new ACh receptor molecules, there is a net reduction of receptors in MG muscle. And when both antigenic modulation and focal lysis permanently compromise the ability of muscle cells to make new receptors, this could explain why some patients with long-standing disease fail to improve, even when antibodies are removed by plasmapheresis.

Assays for MG Antibodies and Clinical Heterogeneity

Until recently there was little statistical support for a correlation between the concentration of anti-receptor antibodies in MG sera and the degree of clinical severity when groups of patients were analyzed (55, 56). This has been considered the fault of the assays used in the past, because for individual patients such a correlation is much stronger. For example, a mildly-affected patient who shows weakness only in extracocular muscles will have a lower antibody titer than will the patient with generalized muscle weakness. Further, longitudinal studies in individual patients yield a strong long-term correlation during and after plasmapheresis and after immunosuppressive therapy (57).

As the lack of correlation reported by many clinicians may be more apparent than real, and may be merely an artifact of the usual type of antibody assay, it might be valuable to consider the nature of the assays currently used.

The antibody is usually measured by an immunoprecipitation technique (58–60). ACh receptor, obtained from amputated human calf muscle or from denervated rat muscle, is solubilized by non-ionic detergent and allowed to react with 125I-labeled α-bungarotoxin; the toxin binds to the ACh receptor at a site distinct from that at which ACh interacts (Figure 3). The complex is incubated with the patient’s sera, and the 125I-labeled α-bungarotoxin–ACh receptor–antibody complex is then precipitated by either excess of a second antibody such as goat anti-human IgG (58) or by protein A (59). The radioactivity counted in the washed pellet provides a measure of the amount of anti-ACh receptor antibody in the original serum specimen; titers are expressed in moles of toxin binding sites precipitated. A control for the assay is provided by initial blockade of toxin binding sites (e.g., by unlabeled toxin in excess, or by benzoquinonium chloride or d-tubocurarine) before the labeled toxin is added (60).

Although methodological details, and thus the values obtained for ACh receptor binding by normal sera, vary among laboratories, generally sera of non-MG persons bind no more than 30 pmol of 125I-labeled α-bungarotoxin–ACh receptor per liter (61). False positives are rarely encountered. When positive results are defined as titers > 30 pmol/ L of serum, more than 90% of MG patients show positive results.

In about 30% of MG sera, antibodies can be shown to bind to the regions of ACh receptor that bind α-bungarotoxin (61). In the assay described, this site is inaccessible to antibodies because it is already occupied by the toxin. However, these antibodies can be detected reliably by a modified assay in which the patient’s serum is added to ACh receptor before labeled toxin is added (61). In less than 1% of MG patients, these are the only anti-ACh receptor antibodies demonstrable in serum.

Of the 9% of patients who have clinical signs of MG but no demonstrable anti-receptor antibodies in their sera, the disease in most is of recent onset or is mild, often confined to the extra-ocular muscles. However, in the absence of anti-receptor antibodies in the serum, or in biopsied motor endplates (62), it cannot be stated with certainty that a patient with a clinical defect in neuromuscular transmission has the autoimmune form of MG.

Drachman et al. (63) developed a combination assay for MG antibodies that they claim correlates highly with the clinical severity of the disease. This assay combines the traditional immunoprecipitation method described above and an assessment of the abilities of immunoglobulins to accelerate the degradation of ACh receptors in a skeletal muscle tissue-culture system. Data from the two assays are combined into an "index of immunoglobulin activities," which correlated with the clinical status of the patient in
98% of the MG patients they tested.

**Autoimmune Thyroid Disorders**

Graves' and Hashimoto's diseases are thyroid disorders of autoimmune etiology (5, 7, 8, 64, 65). Sera of most patients with hyperthyroid Graves' disease contain autoantibodies directed against TSH receptors of thyroid cell plasma membranes, while sera of most patients with hypothyroid Hashimoto's thyroiditis contain autoantibodies against thyroglobulin (Tg)—the thyroid colloid protein that is the precursor of the two thyroid hormones, thyroxin (T4) and triiodothyronine (T3)—and against endoplasmic reticulum membranes of the thyroid cell, commonly called "microsomes" (M).

In Graves' disease the autoantibodies that are detected in from 6% to 95% of patients have been given a myriad of names that reflect both the assays used to detect them and the idiosyncrasies of the investigators. From the approximately 30 names and abbreviations of Graves'-disease autoantibodies that appear in the literature, four suffice to classify the unique antibody activities:

(a) long-acting thyroid stimulator (LATS), the first of the Graves' immunoglobulins to be discovered (66, 67), but which appears in only a minority of Graves' patients when unconcentrated sera are used for assays;

(b) thyroid-stimulating antibodies (TSAb), a generally accepted (68) name applied to a property of most Graves' autoantibodies, that of stimulating the metabolism of human thyroid tissue in vitro in a manner similar to that of TSH;

(c) long-acting thyroid stimulator protector (LATS-P), a subclass of Graves' autoantibodies that prevents binding and neutralization of LATS by human thyroid tissue (64, 69); and

(d) thyrotropin-binding inhibitor (TBI), which describes the ability of Graves' autoantibodies to compete with TSH for binding to sites on thyroid cell surfaces, but which says nothing about whether the blocking simply of TSH binding is accompanied by TSAb-like activity or whether blocking leads to hypothyroidism (70).

Autoantibodies to thyroglobulin and microsomes are not limited to Hashimoto's disease. A high titer of anti-thyroglobulin autoantibodies is found in about 55% of Hashimoto-disease patients and in about 25% of Graves'-disease patients. The complement-fixing antibodies to microsomes are found in about 95% of Hashimoto-disease patients, in 80% of Graves'-disease patients, and in 90% of patients with idiopathic (autoimmune?) myxedema (71–73). Autoantibodies to thyroglobulin are found less frequently and with variable titers in patients with thyroid cancer; in other autoimmune disorders such as systemic lupus erythematosus, rheumatoid arthritis, autoimmune Addison's disease, autoimmune chronic active hepatitis, insulin-dependent diabetes mellitus, and autoimmune Type A chronic atrophic gastritis; and in 10% to 20% of normal individuals, especially elderly women. As the thyroglobulin and microsomal autoantibodies reflect thyroid cell destruction, the most common endocrinopathy in disorders in which such antibodies dominate, e.g., in chronic Hashimoto's thyroiditis, consists of variable degrees of loss of thyroid reserve and primary hypothyroidism. Much less common is the syndrome of "Hashitoxicosis," in which the pathology resembles Hashimoto's thyroiditis, but the clinical picture is one of hyperthyroidism. It is possible that, in this uncommon syndrome, TSAb is being produced concurrently with the anti-Tg and anti-M antibodies, and is acting on unaffected thyroid cells.

**Clinical Features**

**Graves' disease.** This is a common, gene-linked disorder of unknown etiology. It occurs in about 0.4% of all women. In keeping with autoimmune diseases generally, it is more common, by four- to fivefold, in women than men. There is definite familial aggregation in this disorder, and a high incidence of concordance in identical twins. About 80% of Graves'-disease patients in the United States have the HLA-DR3 and HLA-B8 histocompatibility haplotype (Table 1).

Clinically, Graves' disease presents with a characteristic triad of manifestations: hyperthyroidism with diffuse toxic goiter, infiltrative ophthalmopathy, and infiltrative dermopathy. These three manifestations rarely appear together, and each runs its separate course. These manifestations may appear in other autoimmune thyroid disorders; occasional patients with Hashimoto's thyroiditis or with primary idiopathic myxedema will present with typical Graves' ophthalmopathy, and such patients will have anti-TSH receptor antibodies of the strictly blocking variety, i.e., TBI.

Although the diffuse toxic goiter of Graves' disease is a major cause of hyperthyroidism, one should be aware that this complex of clinical symptoms can arise in a variety of circumstances other than Graves' disease, such as in a functional thyroid tumor. Thus, tests designed to assess the integrity of the homeostatic control of thyroid function—i.e., thyroid suppression tests and tests for the response of the

<table>
<thead>
<tr>
<th>Table 1. General Features of Anti-Receptor Autoimmune Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antigen</strong></td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td><strong>Antibody</strong></td>
</tr>
<tr>
<td><strong>Prevalence of autoantibodies</strong></td>
</tr>
<tr>
<td><strong>Clinical effect</strong></td>
</tr>
<tr>
<td><strong>Immunopathogenesis</strong></td>
</tr>
<tr>
<td><strong>Polyautoimmune associations</strong></td>
</tr>
<tr>
<td><strong>Major HLA associations</strong></td>
</tr>
<tr>
<td><strong>Sex predominant</strong></td>
</tr>
</tbody>
</table>
pituitary to thyroliberin (TSH releasing hormone)—are of little use in the differential diagnosis of Graves’ disease. Abnormal results in such tests are common to all varieties of hyperthyroidism. However, except in cases of autonomous functioning foci, such as in thyroid tumors, it is only in Graves’ disease that homeostatic disruption exists in the absence of thyrotoxicosis, i.e., TBI.

Hashimoto’s thyroiditis. Hashimoto’s disease, or autoimmune thyroiditis, is a common disease. Its prevalence (currently 1% and 2%) appears to be increasing, and it increases with age. The disease is sex-linked (four times more common in women than men) and race-oriented (four times more common in whites than blacks) and, although there may be familial aggregations of the disease, this tendency is not as marked as with Graves’ disease.

In the thyroid gland of the patient with Hashimoto’s thyroiditis there are variable degrees of depletion of colloid from the follicles, infiltration with lymphocytes, germinal-center inflammation, and fibrosis. Goiter and disturbances of thyroid function dominate the clinical picture. Many such patients are euthyroid, but variable degrees of loss of thyroid reserve (i.e., hypothyroidism) are common. Indeed, infiltration by lymphocytes, destruction of thyroid function, high titers of anti-thyroid autoantibodies, and increase in TSH in serum are directly correlative to each other in patients and in animal models of Hashimoto’s thyroiditis.

Basic Features and Immunopathogenesis

For a better understanding of the clinical consequences of the actions of Graves’ immunoglobulins, the significance of the presence of Hashimoto’s anti-thyroid antibodies, and the basis of assay for anti-TSH receptor antibodies, it might be helpful to briefly review thyroid biochemistry.

TSH is the major regulator of the anatomical and functional state of the thyroid. Removal of TSH stimulation by, for example, hypophysectomy, is followed by decreased synthesis and secretion of thyroid hormones and hypovascularity and atrophy of the gland. The reverse effects are produced by stimulatory concentrations of TSH, or of factors acting like TSH (Figure 4).

Secretion of TSH by the adenohypophysis is stimulated by thyroliberin, a tripeptide produced in the hypothalamus and reaching the adenohypophysis via the blood vessels of the hypothalamic–pituitary portal system. Thyroid hormones (chiefly T₃) inhibit the TSH secretory mechanism by a direct action on the adenohypophysis (Figure 4); it is still only conjecture that T₂ and T₄ also regulate the hypothalamic production of thyroliberin. In any case, T₃ and T₄ homeostatic feedback controls TSH production, the threshold for this feedback effect apparently being set by thyroliberin.

As a result of the interaction of TSH, a 27 500-dalton glycoprotein, with a specific glycoprotein receptor (Mₛ = 280 000) located in the plasma membrane of thyroid cells, adenylate cyclase (EC 4.6.1.1) is activated. Cyclic AMP is thereby produced, and a cell machinery is thereby set into motion that increases the transport of iodide into the cell, incorporates it into thyroxin-like structures within the thyroglobulin protein, stores thyroglobulin within colloid droplets, produces T₄ and T₃ by proteolysis of thyroglobulin, and releases these hormones to the general circulation.

Clinically, this system is self-limiting. Production of T₃ and T₄ in excess of the body’s requirements feeds back to decrease the production of TSH by the adenohypophysia; conversely, underproduction of thyroid hormone signals increased production of TSH.

If, as the result of some pathological situation, the body produces a new factor that behaves like TSH and continues to produce it regardless of the amount of T₃ and T₄ being secreted by the thyroid gland, the thyroid hormone-producing thyroid cells will hypertrophy and the patient will be profoundly hyperthyroid. Furthermore, results for the thyroid suppression tests and the usually used stimulation with thyroliberin will be abnormal. In the former the serum TSH will be vanishingly low in the face of increased iodide uptake and thyroid hormone production. In the latter the response to thyroliberin will be subnormal or absent because of the profound feedback inhibition on the pituitary by T₃ and T₄. This is the situation in the most common variant of Graves’ disease, and the factors responsible are autoantibodies to TSH receptor.

On the other hand, if the factors being produced merely block the binding of TSH to its thyroid receptors, without mimicking its action, then hypothyroidism will result. This is the situation often seen in the ophthalmic variant of Graves’ disease, and the factors are anti-TSH receptor autoantibodies of the blocking variety (TBI).

In contrast to the situations in Graves’ disease(s), in which one can ascribe specific biochemical activities to the various immunoglobulins produced, the situation in Hashimoto’s thyroiditis is less well defined. Here, the autoantibodies produced by invading B-lymphocytes are primarily directed against cell structures (i.e., thyroglobulin and the endoplasmic reticulum) other than the plasma membrane TSH-receptor, although the latter type of autoantibody also appears in some Hashimoto’s disease patients (see above). The etiology of anti-thyroglobulin and antimicrosomal and autoantibodies is still not clear, but thyroid tissue destruction and hypothyroidism are hallmarks of the classical chronic Hashimoto patient, and it seems reasonable to suggest that these autoantibodies are simply end products of thyroid cell destruction by a deranged immune system.

We know very little about the mechanism of action of anti-TSH receptor antibodies. We know that the Graves’ TSAb-types of immunoglobulins are IgG (subclasses 1, 2, 3, and 4).

Ed. note added in proof: The author of this review has asked that we inform readers that he disagrees with editorial changes bringing the nomenclature of the peptide hormones (e.g., thyrotropin, thyroliberin) into harmony with recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature published in 1973 in J Biol Chem 258, 3215–3216 and Eur J Biochem 55, 485–486. He believes that these recommendations have not been accepted by endocrinologists and biochemists and that their implementation is therefore inappropriate.

Fig. 4. Relationships between TSH and the thyroid gland normally (left) and in Graves’ disease (right).

A negative influence of thyroid hormones on the hypothalamic production of thyroliberin (thyrotropin-releasing hormone, T-RH) is still conjectural; ①, stimulating effect; ②, inhibitory effect; cAMP, adenosine 3’5’-cyclic monophosphate. For other abbrevs., see footnote 1
Thyroid Cyclic 1142 McKenzie
Monoclonal explanations preparations example, human activities presentations ant-TSH presentations interfere in influence present ing, membranes has results in wide mechanisms used as its hormone and as the parent molecule.

Assays for Thyroid Autoantibodies

Anti-TSH receptor antibodies. The wide variety of assays used (Table 2) and the many different apppellations given to autoantibodies to thyroid membrane (64, 73) attest to the many uncertainties in this field. Progress in elucidating the mechanism(s) of action of these autoantibodies has been delayed by these uncertainties. Lack of correlation between results by different methods for identical clinical samples has been the rule rather than the exception. In some cases the reasons for this are defined, in others not. For example, in some systems factors other than antibodies to thyroid membranes can influence the binding of 125I-labeled TSH to thyroid membrane receptors. In particular, IgG from normal subjects—and even thyroglobulin—can influence TSH binding, and high concentrations of thyroglobulin are often present in the sera of Graves’ patients. Do these factors influence all receptor binding assays, and do they also interfere with bioassays based upon the TSH-mimetic actions of Graves’ autoantibodies? These questions are under investigation.

There is also the question of cross reactivity of human anti-TSH autoantibodies with thyroid membrane preparations from other species. This question is an important one because of the obvious desirability of being able to use non-human tissue for assays, but it appears to be more of a problem in bioassays than in receptor binding assays. For example, there appears to be no species specificity in TBI activity as measured with porcine, ovine, or human TSH receptors (74), but specificity may become evident when human TSAb activities are assessed in thyroid membrane preparations from different species (75, 76). Whatever the explanation for the discrepancies—heterogeneity of auto-antibodies or of binding sites—they have made much of the literature on Graves’ disease difficult to follow.

Assays for thyroid receptor autoantibodies can be classified as bioassays or binding assays (Table 2). As the names suggest, bioassays, whether in vivo or in vitro, have as their endpoint the quantification of some biochemical product of TSH action—for example, radiiodine as T4 released from the mouse thyroid, or adenylate cyclase activation and cyclic AMP accumulation (77). Such assays are based upon the TSH-mimetic action of the most common of the Graves’ autoantibodies. On the other hand, the binding assays are based upon competition between 125I-labeled TSH and the patient’s immunoglobulins for binding to thyroid preparations. The precise nature of the binding sites has been controversial. In addition to the plasma membrane glycoprotein receptor, a candidate receptor consists of thyroid cell membrane gangliosides (78), although this has been disputed (79). Of only academic interest is the controversy over whether mere binding to membrane sites, with no metabolic changes, represents a “function” of a hormone or an autoantibody.

Assays based upon estimation of the concentration of cyclic AMP in cultured thyroid cells have much to recommend them. They are probably the most precise and possibly the most sensitive of the bioassays. Use of intact cells and measuring a biochemical consequence of cell–immunoglobulin interaction make such assays more “physiological.” The recent availability (80) of a continuously-cultured cloned line of rat thyroid cells (FRTL-5) that reportedly respond to over 90% of hyperthyroid Graves’ IgG preparations exhibiting stimulatory activity and the successful use (81) of frozen primary culture of human thyroid cells make it quite feasible to use this technique routinely to assay for TSAb.

Recently described competitive-binding assays for receptor, in which highly purified particulate and detergent-solubilized human thyroid membranes are used, are also more precise and less affected by normal IgG than were earlier membrane assays (82–88).

Enzyme-linked immunosorbent assays and other solid-state assays for Graves’ immunoglobulins are feasible and under active development in several laboratories. One is

<table>
<thead>
<tr>
<th>Method</th>
<th>Recommended term for antibody</th>
<th>Parameter measured</th>
<th>Thyroid tissue source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioassays</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McKenzie</td>
<td>LATS</td>
<td>Iodine uptake</td>
<td>Mouse</td>
<td>(76, 77)</td>
</tr>
<tr>
<td>Cyclic AMP</td>
<td>TSAb</td>
<td>Increase in cAMP in cultured FRTL-5 cells</td>
<td>Rat</td>
<td>(78)</td>
</tr>
<tr>
<td>Cyclic AMP</td>
<td>TSAb</td>
<td>Increase in cAMP in primary thyroid cell cultures</td>
<td>Human</td>
<td>(79)</td>
</tr>
<tr>
<td>Cyclic AMP</td>
<td>TSAb</td>
<td>Adenylate cyclase activation in plasma membranes</td>
<td>Human</td>
<td>(77, 80)</td>
</tr>
<tr>
<td>Thyroid cell growth</td>
<td>None</td>
<td>Stimulation of growth of cultured FRTL-5 thyroid cells</td>
<td>Rat</td>
<td>(90)</td>
</tr>
<tr>
<td>Binding assays</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LATS-P</td>
<td>LATS-P</td>
<td>Prevention of binding of LATS by thyroid tissue</td>
<td>Human</td>
<td>(81, 82)</td>
</tr>
<tr>
<td>Radioreceptor assay</td>
<td>TBI</td>
<td>Inhibition of binding of 125I-TSH to particulate or solubilized plasma membranes</td>
<td>Human</td>
<td>(83–86)</td>
</tr>
<tr>
<td>ELISA</td>
<td>None</td>
<td>Binding of patient’s IgG to ganglioside-coated plates, then binding of second antibody linked to chromogen or 125I</td>
<td>Human</td>
<td>(None)</td>
</tr>
</tbody>
</table>

Monoclonal assays

Blocking (TBI) and stimulating (TSAb) monoclonal antibodies to the human TSH receptor, produced by hetero hybridomas (mouse myeloma cells × active Graves’ peripheral lymphocytes), are currently being used to develop new assays.

*See footnote 1 for spelled-out abbreviations.

1142 CLINICAL CHEMISTRY, Vol. 30, No. 7, 1984
based upon the competition between 125I-labeled TSH and blocking autoantibodies for binding to microtiter-plate wells coated with poly-L-lysine and thyroid membranes. Another is based upon the theory that among the heterogeneous array of immunoglobulins produced in Graves patients are some that bind only to the TSH glycoprotein receptor and others that bind to gangliosides in the thyroid plasma membrane (89). Microtiter plates coated with glycoprotein receptor or ganglioside will, according to this hypothesis, bind either TSAb or TBI. The amount of immunoglobulin bound can be quantified by the addition of anti-human IgG antibody, linked either to 125I or to a reporter enzyme (90).

It has been recently suggested (91) that goiter may be linked to the presence of growth-stimulating antibodies that act through the thyrotropin receptor but use mechanisms other than stimulation of thyroidal adenylate cyclase. This suggestion has given new use to a general assay for such antibodies that involves the stimulation of the growth of FRTL-5 cultured rat thyroid cells, as measured by the uptake of labeled thymidine (92). This growth assay reportedly compares well with human thyroid cell systems in detecting cyclic AMP-stimulating antibodies in (about 90% of patients with active Graves' disease), but it is said to possess the major advantage (over human cell systems) of measuring growth-stimulating factors, including autoantibodies.

Assays for anti-thyroglobulin and anti-microsomal auto-antibodies. The technique most widely used for detecting anti-thyroglobulin (anti-Tg) antibody in the sera of patients with thyroid autoimmune disorders is passive hemagglutination involving tanned erythrocytes coated with Tg (93). Although this is a sensitive and reliable procedure for routine clinical practice, it has its limitations: it allows only a semi-quantitative determination of anti-Tg antibody; its sensitivity may be inadequate for special investigation purposes such as in vitro studies of antibody production by Hashimoto's peripheral lymphocytes; its sensitivity may be inadequate to screen sera for Tg antibodies prior to assay for Tg itself; and in some patients it gives negative values where Tg antibody measured by RIA gives positive values (reviewed in 94).

Most RIA methods are based on the co-precipitation of 125I-labeled Tg with antibody to Tg by specific anti-human immunoglobulin antisera (95) or on the competitive inhibition of binding of radiolabeled anti-Tg antibody to plastic wells coated with Tg (96). The latter is a sensitive and quantitative assay, but it suffers from interference by circulating Tg, which produces falsely positive results (94). To avoid this interference, a "sandwich" RIA is available (97), in which anti-Tg antibody bound to Tg-coated plastic wells is quantified by binding of 125I-labeled Tg. A similar solid-phase enzyme-linked immunosorbent assay technique in which Tg is coupled to β-d-galactosidase has also been described (98). More recently, use of anti-human immunoglobulin antibody conjugated to alkaline phosphatase has been proposed for detecting anti-Tg antibodies bound to a solid phase (99).

A new solid-phase immunoradiometric assay for autoantibody to thyroglobulin has been developed recently by Pinchera and colleagues at Pisa. In this sensitive and quantitative method (94), anti-Tg antibody in serum binds to plastic wells coated with human Tg, and the amount of the patient's bound anti-Tg antibody is then quantified by adding purified 125I-labeled antibody to human IgG. With use of only 1.0 μL of serum, anti-Tg was detected in over 81% of patients with Hashimoto's thyroiditis or idiopathic myxedema, in 46% of patients with Graves' disease, and in only 4% of normal controls. For 1.0-μL samples there was no difference between the passive hemagglutination test and immunoradiometric procedure in the number of positives; but where patients were passive-hemagglutination negative, even with 100 μL of serum, the majority were positive by the solid-state assay. Another advantage of the method is that it precludes false-positive results in the presence of increased circulating Tg.

Several alternative techniques are available for detecting antibodies to thyroid microsomes (anti-M). The passive hemagglutination method for anti-M antibodies is probably the most convenient procedure for routine clinical use (100). Anti-M antibodies may also be detected by a competitive binding radioassay that has proven to be a very sensitive and useful procedure for clinical investigation (101). In this technique, the patient's autoantibodies to M in serum bind to plastic microtiter plates coated with human thyroid microsomes; the amount of autoantibody bound is quantified by a competitive binding assay, involving 125I-labeled anti-M IgG and unlabeled anti-M IgG. A new solid-phase immunoradiometric assay (102) for anti-M antibody has evolved from the 1978 method (101), also from Pinchera's laboratory. In the current method (102), plastic microtiter plates are coated with human thyroid microsomes that have been solubilized with the surfactant Triton X-100. The anti-M antibody in the equivalent of 1.0 μL of patient's serum binds to the microsomes, and the amount bound is quantified by the subsequent binding of radiolabeled antibody to human IgG. By this technique, these workers could detect anti-M antibodies in all of their patients with Hashimoto's thyroiditis or with idiopathic myxedema, in 86% of Graves' patients, in 11% of patients with other non-autoimmune thyroid disorders, and in 8% of normal controls (102). At this sample volume one can expect good correlations with the passive hemagglutination method, but by using larger volumes of serum (up to 100 μL), the method could be used to detect anti-M antibody in many patients for whom results of the passive hemagglutination test were negative (102). The solid-phase immunoradiometric assay is 15 to 30 times more sensitive than the competitive binding radioassay.

Clinical Applications of Assays

All workers in the field probably agree that measuring antibodies to TSH receptor is important to an understanding of the mechanism of Graves' disease, but considerable controversy surrounds the use of such assays. Some clinicians consider them of little assistance in diagnosis and management of patients, and believe them too time-consuming, technically difficult, and expensive to be useful on a routine basis.

It is argued that Graves' disease can easily be diagnosed by clinical signs and assays for TSH, T3, and T4, without resorting to antibody assays. In addition, opponents cite data that suggest not all Graves' patients present with antibodies against the TSH receptor. Percentages range from a low of 50% to a high of 95%, depending upon the quality of the assay and selection of the patients. Further, in some patients, particularly those with the ophthalmic variant, receptor antibodies inhibit TSH binding but do not activate adenylate cyclase. Indeed, such blocking antibodies probably contribute to the hypothyroidism in those 10% to 15% of Hashimoto's-disease patients in whom anti-TSH receptor antibodies are found.

Further confusion may arise when patients have stimulatory and blocking antibodies simultaneously, and the balance between the two changes either spontaneously or as the result of various treatment regimens. Other variables include the ability of "normal" IgG to block TSH binding to thyroid membranes in some cases; the transient appearance of receptor antibodies in patients with thyroid cancer or
subacute thyroiditis, but without Graves' or Hashimoto's disorders; and the unpredictable clinical course that patients with intermediate antibody concentrations tend to follow after therapy with anti-thyroid drugs is stopped.

Despite these drawbacks, there are compelling arguments in favor of the use of antibody assays in Graves' disease. Such assays can be valuable in confirming that the hyperthyroidism is caused by Graves' disease; in distinguishing the ophthalmic variant of Graves' disease from an orbital tumor; in determining whether the infant of a pregnant woman with Graves' disease is at risk of developing hyperthyroidism in utero or neonatally; and in following the course of therapy with either anti-thyroid drugs or radioactive iodine, or results of partial thyroidectomy.

Admittedly, it is difficult to use antibody assays to predict the course of therapy in patients with low to undetectable concentration of antibody (i.e., either continued remission or relapse after withdrawal of antithyroid drugs). But virtually all patients with intermediate to high concentrations of antibody do relapse under these circumstances, including many who become euthyroid on anti-thyroid drugs. Furthermore, we know that Graves' disease is associated with HLA-DR3 and HLA-B8 in Caucasians, and that patients who are of this haplotype are very likely to relapse (see below). The Hall and Rees-Smith group in Cardiff has found that combining HLA typing with receptor antibody measurement has predicted remission or relapse in 95% of their cases (70).

As to the disadvantages due to the technical difficulty and expense of the antibody assays themselves, this is a criticism based on outdated states of the art. The newer assays (Table 2 above) are well within the technical capabilities of good clinical chemistry laboratories, and the solid-phase type of assays will extend the feasibility to many laboratories.

**Insulin Resistance**

Ininsensitivity to insulin is the hallmark of a class of endocrinopathies characterized by clinical signs of insulin deficiency in the face of normal or supranormal circulating concentrations of the hormone. Clinically, this brings to mind the image of the insulin-treated patient who remains hyperglycemic while receiving large doses of exogenous insulin. Although the patient qualifies as insulin resistant, without more information one knows nothing about the basis of the resistance. Furthermore, insulin has diverse cellular actions that may result from more than one biochemical mediator and that may even involve more than one receptor type. Thus resistance to one action of insulin (e.g., its hypoglycemic effect) need not necessarily be associated with resistance to other important actions such as antilipolysis, amino acid uptake, stimulation of cell growth, and stimulation of glycogen and lipid synthesis. Discordance in the degree of resistance in these various pathways may have great clinical importance.

**Pathophysiology**

The basis of the resistance will largely determine the nature of its treatment. Although there appear to be many clinical states of insulin resistance (Table 3), these can be classified into three general mechanisms: (a) abnormalities of the insulin molecule; (b) circulating antagonists of insulin action; and (c) target-cell defects in the mechanisms of insulin action. Although insulin resistance of the autoimmune variety, which is the subject of this review and which will be considered in detail below, falls within classification b, one should bear in mind that these categories overlap. Consequently, this review will first deal briefly with the other classes of causes of insulin resistance.

**Abnormal insulin.** According to receptor theory, an abnormal insulin molecule that has decreased intrinsic activity as compared with its receptor-binding ability would be expected to produce a state of hormone resistance. Although suspected for years, it was only recently demonstrated for the first time, and as yet in only a single patient, that a structurally abnormal insulin does in fact exist (103). The patient was a Type II diabetic with fasting hyperglycemia and hyperinsulinemia, but with normal sensitivity to exogenous insulin. The patient's insulin had a single amino acid substitution in the active site of the molecule, and this was associated with an 85% reduction in bioactivity in an in vitro assay with adipocytes. This disorder is probably rare.

**Circulating antagonists of insulin action.** Virtually all patients treated with exogenous insulin (bovine, porcine, or even human) develop insulin-binding IgG molecules within several months. These antibodies have not proven to be significant in most patients, although in a small minority (0.1%) antibody titers rise and clinically significant insulin resistance ensues (104). This resistance is usually self-limited, but it may be circumvented by substituting less-immunogenic forms of insulin (insulin produced by recombinant DNA technology may be useful in this connection) or by therapy aimed at the immune response itself (i.e., with prednisone). Anti-insulin antibodies limit the access of insulin to its receptors, but no receptor abnormalities have been described in this setting.

Cortisol, somatotropin, glucagon, and catecholamines are each capable of producing states of insulin resistance (105). These hormones may produce insulin antagonism by a variety of mechanisms, including (a) actions on peripheral tissues to influence the concentrations of important substrates such as fatty acids that may antagonize insulin action; (b) actions to stimulate hepatic enzymes that counter the action of insulin, such as those that mediate gluconeogenesis and glycogenolysis; (c) actions to limit insulin production by the pancreatic B-cell; and (d) actions to impair directly insulin-sensitive processes in target tissues, including effects on the glucose transport system and on the expression of the insulin receptors. It is difficult to assess the role of insulin receptor changes in insulin resistance caused by an excess of these hormones. One example will suffice to make the point. In early studies, in vivo glucocorticoid excess (in concentrations sufficient to produce immune system suppression) was found to diminish receptor affinity for insulin in hepatocytes and adipocytes (106, 107). However, a major role for receptor alterations in the production of

---

**Table 3. Clinical States of Insulin Resistance**

<table>
<thead>
<tr>
<th>Major</th>
<th>Minor (partial list)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus, Type II*</td>
<td>Syndromes of acanthosis nigricans and insulin resistance*</td>
</tr>
<tr>
<td>Obesity*</td>
<td>Ataxia telangiectasia*</td>
</tr>
<tr>
<td>Insulin antagonism*</td>
<td>Leprechaunism*</td>
</tr>
<tr>
<td>Cushing's syndrome*</td>
<td>Cachexia (malignant and anorexia nervosa)*</td>
</tr>
<tr>
<td>Acromegaly*</td>
<td>Liposarcoma diabetes*</td>
</tr>
<tr>
<td>Pregnancy*</td>
<td>Leukemia*</td>
</tr>
</tbody>
</table>

*Receptor assessed.
steroid-induced insulin resistance has been questioned, for several reasons:
(a) When cells are exposed to glucocorticoids in vitro, insulin receptor changes are found in some, but not all, studies (108, 109).
(b) In many in vivo studies of insulin receptors on circulating monocytes or erythrocytes in humans, observations were diverse and conflicting (110, 111).
(c) Unexpected differences among different steroid preparations were also noted.
(d) The glucocorticogenic and hyperglycemic actions of adrenal steroids suffice to account for the insulin resistance, without involving the participation of insulin receptors in the mechanism of resistance.

Although autoantibodies to the insulin receptor are present in the circulation, the insulin resistance that they produce is more logically considered later, together with the target-tissue defects in insulin action.

Target Cell Defects

Obesity. After the radioimmunoassay for insulin was developed, it became evident that nondiabetic obese individuals may have high circulating concentrations of insulin, both in the fasting and the postprandial states (112). The hypothesis that such individuals were resistant to the hypoglycemic action of insulin was confirmed in subsequent studies (113). The clinical significance of insulin resistance in nondiabetic obese individuals has not been defined, but many studies of this phenomenon were motivated by the obesity that characterizes 80% to 90% of adults with Type II diabetes, a disorder also characterized by insulin resistance (see below).

Experimental and clinical studies have established clearly that obesity involves a defect at the insulin receptor level. Less insulin is bound to its target-tissue membrane receptors in this condition, whether the obesity is genetic or acquired (114–116), and this decrease is ascribable to a decreased number of available receptors. The major factor regulating the number of insulin receptors in obesity appears to be the concentration of insulin in the circulation. Amelioration of the hyperinsulinemia through diet or treatment with streptozotocin or dioxane corrects the receptor defect (113)—and this will occur even where the obesity persists, which stresses that obesity per se is not the proximate cause of the observed receptor defect.

Although the insulin receptor deficiency in obesity is indisputable and its cellular mechanism is fairly well understood, the relationship between the receptor deficiency and the target cell resistance to insulin is not straightforward. Part of the uncertainty is due to the existence of "spare receptors" on cell surfaces and the fact that certain of insulin's actions are stimulated maximally when only a small fraction of the total receptor population is occupied with insulin, whereas other insulin effects are stoichiometrically related to the number of receptors occupied. Another part of the uncertainty stems from in vitro observations in genetically obese animals. These demonstrate that the predominant abnormality responsible for the cellular insulin resistance is a post-receptor defect in the intracellular pathway of glucose metabolism (117, 118) and that this defect is causally related to the chronic hyperinsulenic state in animals (119) and man (120).

Type II diabetes. As in obesity, the mechanism responsible for insulin resistance in Type II diabetes was probed by studying insulin receptors and at the same time assessing the shape of the in vivo dose–response curve for insulin-mediated glucose disposal. Circulating monocytes and erythrocytes, which are not important target tissues of insulin action, and freshly isolated subcutaneous adipocytes and cultured skin fibroblasts, which are major target organs of insulin with respect to glucose homeostasis, have been the tissues primarily used for studies of insulin receptors in these patients. In general, patients with impaired glucose tolerance and overt diabetes have fewer insulin receptors per cell than do controls (121–122). Insulin resistance is more severe in patients with overt fasting hyperglycemia than in those with impaired glucose tolerance, but the severity of the binding defect is similar in the two groups (121). This was the first hint that defects apart from receptor binding might be important components of the insulin resistance of Type II diabetes. Thus, in patients with impaired glucose tolerance and mild insulin resistance, the diminished insulin sensitivity could be attributed to a decreased number of cellular receptors (123). But in patients with Type II diabetes and more severe insulin resistance there is both a receptor defect and a post-receptor defect, and the latter is probably the dominant abnormality (123).

Neither the biochemical nature of the post-receptor defect nor the cause of the abnormality are known. Although most Type II diabetics are obese, the fact that obese and non-obese Type II diabetics have similar degrees of insulin resistance suggests that, whatever the additive effect of obesity, insulin resistance is a function of the diabetes itself.

Extreme insulin resistance. In contrast to obesity and diabetes, in which insulin resistance is typically modest, there are syndromes in which resistance is extreme. Representative of this category are patients with both acanthosis nigricans and severe insulin resistance (124). These are uncommon disorders, but these syndromes have led to important insights regarding the mechanism of action of insulin.

Although clinically diverse in their manifestations, patients with these symptoms nearly all manifest the skin lesion, acanthosis nigricans, which appears to be a cutaneous manifestation of severe target-cell insulin resistance, regardless of specific etiology. It is not known whether acanthosis nigricans occurring with malignancy has the same basis. However, the presence of acanthosis nigricans should raise suspicion of insulin resistance even in nondiabetic patients, as compensatory hyperinsulinemia might prevent the development of overt signs of diabetes.

Type B Syndrome of Insulin Resistance with Insulin Receptor Autoantibodies

Autoantibodies to insulin receptor were first discovered in 1975 by Flier and colleagues at NIH (125) during the evaluation of several patients with extreme insulin resistance. Since then, fewer than 75 cases have been discovered throughout the world, an incidence that probably more reflects a lack of diagnosis than a lack of cases. Clinically, there is a female preponderance, and most of the patients have been black, although several cases are Caucasians or Japanese. The mean age of onset is 43, with a range of 12 to 78 years of age (Table 1).

Immunopathogenesis. The clinical syndrome is characterized by three primary features.

The most striking is marked insulin resistance accompanied by such signs of uncontrolled diabetes as polyuria, polydipsia, polyphagia, and weight loss—and by hyperinsulinemia. Plasma insulin concentrations are 10- to 100-fold normal in both the basal and the stimulated state. In some patients, this endogenous hyperinsulinemia is sufficient to overcome the insulin resistance, and glucose tolerance is normal or only slightly impaired. In others, the resistance to insulin is so severe that they fail to respond to as much as 100 000 units of insulin per day (126). In still other patients
there is, surprisingly, a frank hypoglycemia associated with the insulin resistance (126). This heterogeneity in the clinical picture will be discussed below in connection with evidence for heterogeneity of the antibodies to insulin receptor that are produced in this syndrome.

The second clinical feature of patients in this group is the presence of a generalized immunological disease. That is to say, most of these patients have symptoms or laboratory-test results suggestive of autoimmune disease, including alopecia, vitiligo, arthralgias and arthritis, splanchnogaly, Raynoud's phenomenon, enlarged salivary glands, increased erythrocyte sedimentation rate, leukopenia, increased γ-globulins, and anti-nuclear and anti-DNA antibodies (126). One third of the cases could be classified as having a specific autoimmune syndrome such as Sjögren's syndrome or systemic lupus erythematosus. However, evidence for organ-specific autoimmunity, such as the presence of anti-thyroid autoantibodies, is rare.

The third feature of the disease is the presence of acanthosis nigricans in most, but not all, patients. Although the pathophysiological relationship of this skin sign to the other features is uncertain, its clinical course seems closely tied to the insulin resistance.

Mechanism of action of insulin receptor autoantibodies. The basis of the insulin resistance in this syndrome was first elucidated by Flier and colleagues (125) when they measured insulin binding to circulating monocytes from affected patients. They noted a profound reduction of binding affinity of normally reactive tissues—a decrease resulting from an increase in the spontaneous rate of dissociation of the hormone from cells. Subcutaneous adipocytes (in which insulin affects metabolism) and circulating erythrocytes (in which insulin probably does not have important effects upon metabolism) showed a similar defect.

Evidence that the syndrome was due to autoantibodies to insulin receptor first came with the discovery that such patients' sera blocked the binding of insulin to a wide variety of insulin target cells, thus mimicking in vitro the defect observed in vivo (127). Subsequently, the blocking antibodies were identified as IgG (126). (Although IgM antibodies to the insulin receptor are known, these have been found only in ataxia telangectasia; see below.) The following observations provide evidence that the autoantibodies in this syndrome bind to the insulin receptor:

(a) The antibodies inhibit insulin binding in vitro to a wide variety of tissues from different species.

(b) The antibodies immunoprecipitate detergent-solubilized insulin receptor quantitatively.

(c) They mimic most of the metabolic effects of insulin in vitro—although insulin-like activity is transient in vitro (see below).

(d) 125I-labeled patients' IgG binds to insulin target cells in proportion to the number of insulin receptors, and this binding is inhibited by insulin analogs in order of their affinity to the receptor.

(e) Target cells bind protein A only when the cells are first treated with patients' sera, or with IgG fractions isolated from them.

The assays used in such studies are shown in Figure 5. When tested in vitro, anti-insulin receptor autoantibodies mimic most if not all of insulin's metabolic actions in vitro. However, these paradoxical insulin-like activities are only transient. When cells are exposed to the autoantibodies for prolonged periods, the insulin-like effects are lost, the cells become insulin resistant, and the in vitro effects of the autoantibodies then resemble those observed in vivo (129).

There are exceptions to the general rule that the acute insulin-mimicking effects of autoantibodies give way under chronic conditions to an insulin-resistant state. Within two months, one famous NIH patient (B-2) who for three years had been extremely insulin resistant became insulin-sensitive and hypoglycemic (130). Her hypoglycemia recurred after insulin was discontinued, and the patient required intravenous infusions to maintain blood glucose. Eventually, she died of intractable hypoglycemia. Another patient (B-12) presented with fasting hypoglycemia as the sole manifestation of autoantibodies to the insulin receptor (130). Therapy with prednisone restored the serum glucose, measured during fasting, to normal values within 48 h, with no detectable change in titer of autoantibodies; after 10 weeks of this therapy, the titer of anti-insulin receptor antibodies declined by 100-fold and the prednisone could be discontinued without recurrence of hypoglycemia. These two cases demonstrate that anti-receptor antibodies must be considered in the differential diagnosis of hypoglycemia, especially in patients with other manifestations of autoimmunity.

In no case of hypoglycemia accompanying autoantibodies to insulin receptor has the cause of the hypoglycemia been found. Without immunosuppressive therapy, the autoantibody titers remained unchanged, as was their insulin-like activity in short-term bioassays. During the hypoglycemic phase of patient B-2's disease, however, there was a marked increase in insulin binding due to a proliferation of low-affinity binding sites. This proliferation of receptors may have resulted in an amplification of the effects of endogenous insulin, but it is more likely that the nature of the autoantibodies themselves changed to become permanently insulin-like, much as autoantibodies to TSH receptor in Graves' disease may be TSH-mimetic throughout the course of the disease.

The biological activity of receptor autoantibodies, unlike their ability to block insulin binding, depends upon their bivalency (132). Purified patient IgG and Fab(1') fragments derived therefrom are fully active on a molar basis compared to insulin, whereas monovalent Fab fragments can
inhibit insulin binding to receptors but have little or no bioactivity. This has led to the suggestion that cross-linking of insulin receptors or receptor aggregation is important in both antibody and insulin action (Figure 5).

The fate of cell-bound insulin has been determined by both biochemical and morphological means. Briefly, it appears that initially insulin is bound diffusely over the cell surface. After a short period, during which it is presumed that cross-linking of receptors occurs, insulin–receptor complexes diffuse laterally and congregate in discrete areas of the cell surface, over the well-known clathrin-coated pits. Under physiological conditions, aggregation is followed by internalization, and the intracellular receptor and insulin are degraded in lysosomes. A portion of the internalized receptors can be reinserted into the plasma membrane for continued use. Cell-bound antibodies to the insulin receptor appear to follow the same course. Indeed, on lymphocytes the anti-insulin receptor autoantibodies cap, and actually co-cap, with insulin, before internalization and degradation in lysosomes (133).

It has not escaped the attention of workers in this field that the mechanism for the internalization of the insulin receptor is similar to the mechanisms in antibody-mediated acceleration of the internalization and degradation of acetylcholine receptors in myasthenia gravis. This suggests that autoantibody–insulin receptor interaction might lead to the same result. However, as noted above, the number of insulin receptors is not significantly reduced in insulin resistance with acanthosis nigricans Type B. It is more likely that the antibodies in the typical patient simply block the binding, and therefore the action, of insulin.

This all suggests that in this disorder a heterogeneous array of autoantibodies to the insulin receptor is produced, and that different molecular species of antibodies act differently. One species simply blocks insulin binding, and leads to insulin resistance and diabetes. Another species is insulinomimetic in vivo and brings about fasting hypoglycemia. A third species may, after internalization into target cells, induce a massive proliferation of insulin receptors, thereby accentuating insulin's actions—at least those involving glucose disposal.

**Ataxia Telangiectasia**

This complex, multisystem disease is characterized by progressive ataxia, telangiectasias, diverse abnormalities of the immune system, susceptibility to neoplasia, and insulin-resistant diabetes (134). The disease is transmitted as an autosomal recessive trait. The estimated prevalence of ataxia telangiectasia (A-T) is one in 40,000. Over 1% of the U.S. population are heterozygotes or carriers of the A-T trait. Because of its multifaceted clinical symptomatologies, A-T is an important and intriguing syndrome for most medical subspecialties; in addition, this disease provides a unique opportunity to probe the interrelationships between the immune system and specific malignancies.

**Clinical and Immunological Features**

The initial presentation and subsequent clinical course are similar in most A-T patients. Cerebellar ataxia is the first symptom in the young child. The ataxia slowly progresses, resulting in confinement to a wheelchair by age 10 to 14 years. At three to eight years of age most patients display an increased incidence of sinopulmonary infections, and these become more persistent and severe with time. Death typically occurs between ages 10 to 30 years, due to bronchopulmonary infection or lymphoreticular malignancy, or both. Other clinical features are summarized in reference 133.

Abnormalities of both humoral and cellular immunity are hallmarks of A-T. Most patients have no detectable or decreased concentrations of IgA and IgE, normal concentrations of IgG, and a low-molecular-mass (8s) form of IgM. Disturbances of cellular immune function are evidenced by poor responses to common skin antigens, delayed skin-graft rejection, mild lymphocytopenia, lymphocyte depletion in lymph nodes, and absent or hypoplastic thymic tissue. Although patients with the lowest incidence of infections tend to have the mildest immunological defects, no single parameter of immunological function is predictive of susceptibility to infection.

**Diabetes mellitus**. Studies by Bar and colleagues at the Diabetes Branch at the NIH have shown that the insulin resistance in A-T, as in patients with Type B acanthosis nigricans, is caused by circulating anti-insulin receptor antibodies (134). However, in contrast to the Type B patients, the autoantibodies in A-T are a low-molecular-mass IgM, which may be monoclonal (Table 1).

Studies of the two index cases by the Diabetes Branch at NIH revealed a pattern of symptoms of diabetes similar to those exhibited by the Type B acanthosis nigricans patients. The index cases were young adult siblings who were first evaluated because of fasting hyperglycemia and glycosuria. At that time, both patients also had symptoms typical of A-T and, in addition, had acanthosis nigricans. Both patients had marked endogenous hyperinsulinemia during oral glucose tolerance testing, and were also resistant to exogenous insulin.

When circulating monocytes and cultured skin fibroblasts were examined for insulin receptor status, an assay of 125I-labeled insulin binding demonstrated an 80% to 85% decrease in receptor affinity in monocytes (but not in cultured fibroblasts), and an increased concentration of low-affinity receptors at the expense of a 60% reduction in high-affinity insulin receptors.

Whole plasma or immunoglobulin-enriched plasma from these patients inhibited the binding of 125I-labeled insulin to receptors on human placental plasma membranes and IM-9 lymphoblastoid cells (134). Harrison et al. (135) subsequently identified the inhibitory factor in A-T plasma as an IgM, specifically belonging to the low-molecular-mass (8s) IgM class. Immune depletion of plasma by anti-human IgM or by antisera to λ-type light chains removed the binding inhibition activity; this suggests a monoclonal antibody. In addition, Harrison et al. (136) also applied a new immunoprecipitation assay for antireceptor antibodies which improves the sensitivity and retains the high specificity. In this assay, the insulin receptors in Triton X-100-solubilized placental membranes are pre-labeled with 125I-labeled insulin, incubated with serum or antibody fractions, and finally precipitated by a second antibody (Figure 5). Because several insulin-receptor autoantibodies of Type B and A-T patients bind to receptor determinants outside of the insulin-binding domains, the immunoprecipitation method has the capacity for measuring those autoantibodies that would not be detected in the 125I-labeled insulin binding inhibition assays. The rationale is similar to that for MG assays (Figure 3).

On applying this new assay, it was found that after exposure to A-T serum, the labeled insulin receptor was precipitated only by antisera directed against IgM or λ chains, but not against κ chains, heavy chains of IgG, or IgA. Further, the serum of the two patients' mother, an obligate A-T carrier, was positive for the IgM autoantibody, although she was symptom free. If other carriers of A-T are found to have such anti-receptor antibodies, it would suggest that they may be markers of the carrier state and could
provide a practical and valuable basis for a screening test for A-T heterozygosity. Subsequent examination of sera of nine other A-T patients from several families revealed the presence of the same anti-insulin receptor 7s IgM autoantibodies.

Finally, it should be noted that several other autoantibodies have been reported in A-T, including those directed against brain tissue, smooth and striated muscle, bile canaliculi, IgA, mitochondria, and basement membrane (134). The anti-insulin receptor autoantibodies may therefore represent one of several abnormal autoantibodies, suggesting a more generalized defect in antibody production or antigen processing in this syndrome.

**Bronchial Asthma**

A topy is a state characterized by a constellation of signs that include familial predisposition to asthma, allergic rhinitis (hay fever), eczema or urticaria (skin wheals), an increased concentration of IgE in serum, and abnormal autonomic reactivity. The last classically encompasses decreased sensitivity to β-adrenergic agonists and increased sensitivity to α-adrenergic and cholinergic agonists (137).

It has been suspected for at least 60 years that the above-mentioned conditions share a common immunopathological basis. Consistent with this notion is the even older observation that the immediate hypersensitivity of atopic individuals to allergies is passively transferred by blood or serum. However, the effector role of the immune system in atopy is not well defined beyond the demonstration that IgG provoked by allergies triggers release of smooth-muscle contractile (such as histamine) from mast cells. As will be detailed below, there is now evidence from two laboratories for a direct connection between allergy and immunity, namely, the presence in the serum of some asthmatics of autoantibodies to lung β2-adrenergic receptors and an association of such antibodies with autonomic dysfunction (138-141).

**Atopy and Autonomic Function**

Asthmatic patients have an impaired response to β-adrenergic agonists. The reduced effect in asthmatics of isoproterenol or other β-agonists on glycogenolysis, lipolysis, and cyclic AMP production, or on pulse pressure has been correlated with enhanced effects of acetylcholine and histamine on bronchial constriction (reviewed in 142, 143).

In the late 1960s, Szentivanyi (144) proposed the β-adrenergic theory of asthma: decreased β-adrenergic sensitivity of bronchial smooth muscle, mucus glands, and mucosal vessels causes an imbalance in autonomic control, leading to bronchospasm. This theory was supported by many studies showing impaired cyclic AMP response to β-agonists in circulating leukocytes from asthmatics (reviewed in 143). Although leukocytes are not bronchial smooth muscle cells, it is known from studies of other endocrinopathies such as insulin-resistant diabetes mellitus that receptor anomalies on circulating leukocytes can fairly represent the pathological situation in true target cells (see above).

Such studies raised the question of whether the β-receptors of target tissues from asthmatic patients had an intrinsic defect. Subsequent direct binding studies with use of β-adrenergic ligands of high specific activity showed clearly that cells from untreated and symptomatic asthmatics indeed exhibit defects in β-receptor binding, reflected both in reductions in numbers of receptors and in lowering of binding affinities (143). In subsequent studies, the decrease in β-receptor binding in atopic individuals was linked to an increase in α-adrenergic receptor binding (143). As atopic individuals are frequently hypersensitive to α-adrenergic agonists, Szentivanyi has expanded his theory to a "dual receptor imbalance" theory (143), according to which the bronchospasm in asthma is due to concurrently decreased β-adrenergic sensitivity and increased (or unchecked) α-adrenergic sensitivity.

**Atopy, autonomic function, and autoimmunity.** In a collaborative effort between Harrison and Kaliner at the NIH and Venet and Frassetto at State University of New York at Buffalo, sera of atopic patients from the Clinical Center at NIH were tested for the presence of autoantibodies to lung membrane β-adrenergic receptors. The hypothesis to be tested was that β-blocking autoantibodies could be responsible for the decreased β-adrenergic sensitivity in such patients. In two small studies (138, 139), autoantibodies were detected in sera of three of 19 normal subjects, four of 17 asthma patients, one of nine "allergic" patients, one of 17 patients with cystic fibrosis, and in none of eight patients suffering from allergic rhinitis. Two primary assays for the autoantibodies were used. In one, sera were assayed for immunoglobulins inhibiting the specific binding of [125I]-labeled hydroxybenzylpindolol (a high-affinity β-adrenergic antagonist) to β-receptors in isolated mammalian lung membranes. In the other, autoantibodies of the IgG class in these sera were demonstrated by indirect precipitation of detergent-solubilized lung β-receptors (cf. Figure 5 for a similar assay). Most importantly, these investigators showed that the presence of these autoantibodies was associated with autonomic abnormalities; such patients required more than the usual doses of infused isoproterenol (a powerful β-agonist) to raise their pulse pressure or plasma cyclic AMP concentration, and less than the usual doses of phenylephrine (an α-agonist) to dilate their pupils. They were also abnormally sensitive to the effects of the cholinergic agent, carbachol, on pupillary contraction. A role for autoantibodies as β-receptor antagonists was further supported by a showing that human lung cells (VA-13), cultured in the presence of globulins from antibody-positive subjects, had a markedly impaired cyclic AMP response to isoprenaline (140). More recently, we confirmed, in a pediatric bronchial asthma population, the essential point of the studies of Venter and colleagues; furthermore, the number of patients tested was sufficiently large to eliminate the statistical difficulties in the previous studies (138, 139). Blecher et al. (141) have used this hydroxybenzylpindolol binding assay with plasma membrane β-adrenergic receptors from canine lung to test for β-blocking autoantibodies in the sera of 376 asthmatic patients and 58 non-asthmatic patient controls. This binding inhibition assay, coupled with a variant test in which the binding assay was performed on selected sera before and after immunodepletion (removal of IgG and IgA), permitted the conclusion that about 5% of the total asthmatic population studied produced β-blocking autoantibodies. Although in the studies of Fraser et al. (139) it was not possible to distinguish clinically those asthmatic patients who had the autoantibody from those who did not, our studies (141) suggest that autoantibodies are more likely to be produced in the more severely ill asthmatic child; that is, 8.8% of a high-risk group of asthmatic patients produced large amounts of the β-blocking autoantibody, whereas only 3.4% of the general asthma population did so (Table 1).

A unifying hypothesis. The findings from both groups of investigators are consistent with the notion that β-adrenergic receptor autoantibodies play a primary role in mediating autonomic dysfunction, at least in a subgroup of the juvenile, and possibly adult, population of bronchial asthmatics. The most simple mechanism is impairment by autoantibod-
ies of the β-receptor-mediated relaxation of smooth muscle in the airways, and the concomitant unmasking of the opposing influence of other mediators such as α-receptor agonists and acetylcholine. It will be important, however, to delineate the relationship of β-adrenergic function to other features of atopy, for instance the triggering of histamine release from mast cells by IgE. In addition, it will also be important to determine whether autoantibodies to other receptors are involved. For example, autoantibodies against airways α-adrenergic receptors, if produced, could be mimetic of norepinephrine's α-agonist activities, thereby exacerbating bronchospasm.

Atopy must now be placed within the spectrum of autoimmune disorders. This concept is supported by the demonstration of defective suppressor T lymphocytes in atopic subjects (145). Clearly, "forbidden clones" that recognize self-antigens may be more pervasive than was hitherto suspected. On the other hand, if autoimmunity is a naturally regulated state (146), perhaps it should be viewed as a continuum, as suggested by Harrison et al. (140), from a normal state of regulated autoimmunity, through the emergence of non-pathogenic autoantibodies with advancing age or after exposure to certain drugs, to the emergence of pathogenic autoantibodies in classic autoimmune states. Although the latter autoantibodies usually mediate tissue destruction in conjunction with mononuclear cells or complement, there may be less-extreme situations, exemplified by atopy, where the manifestations are due solely to the specific effects of autoantibodies.

The Foreseeable Future

In 1960, Simpson (147) established the basis for research into receptors, antibodies, and disease when he postulated that antibodies to the ACh receptor at the neuromuscular junction could account for the clinical symptoms of MG. Eleven years later, Lennon and Carnegie (148) developed, on the basis of data derived from studies of experimental autoimmune encephalomyelitis, a hypothesis of "immunopharmacologic block." According to this hypothesis, in certain organ-specific diseases "immunocytes," antibodies produced against cell surface receptors for peptide hormones or neurotransmitters, would complex with such receptors, thereby blocking the normal function of those—relat-ed—cells. Although Lennon and Carnegie applied their hypothesis specifically to multiple sclerosis, they suggested that it could apply equally well to MG and a wide variety of autoimmune diseases of secretory organs.

The Lennon–Carnegie hypothesis has been amply validated. One need only look for this purpose to the wide-ranging discussions at a 1962 Ciba Foundation Symposium on receptors, antibodies, and disease (149), to the fact that 25% of the text of a recent book on receptors and human disease is devoted to anti-receptor autoimmune diseases (150) to a book devoted entirely to antibodies and receptors (151), and, of course, to the present "state-of-the-art" review.

The purpose of this section is to develop the hypothesis that the anti-receptor autoimmune diseases thus far described are merely, as the expression goes, "the tip of the iceberg," and that a tendency to produce antibodies against "self" receptors may be the cause of yet other diseases for which the etiologies currently are unknown. This hypothesis, for which the supporting evidence has been reviewed in detail recently by myself (27, 150) and others (151, 152), was born of the awareness that there is a tendency for autoimmune diseases to cluster in a single patient or in a single type of patient. Thus, in those patients in whom anti-receptor antibodies are known to be, or suspected of being, responsible for one member of the constellation of symp-toms, it is logical to proceed investigatively on the presumption that other members of the constellation may have a similar etiology.

Type I Diabetes Mellitus

A clinical association between Type I diabetes mellitus (formerly known as insulin-dependent or juvenile diabetes mellitus) and autoimmune disorders has been repeatedly documented. The disease cluster may involve the adrenal cortex, neuromuscular junctions, parathyroid, liver, and gastrointestinal tissues (Table 4). In addition, Type I diabetes mellitus is frequently associated with autoantibodies to pancreatic islet (17, 160–162) and insulinoma (163) cells. In addition, examination of sera from Type I diabetics with polyendocrine disorders by an immunofluorescence technique has revealed organ-specific autoantibodies against pancreatic islet, adrenal cortex, thyroid, gastric parietal cells, gastric intrinsic factor, smooth muscle, mitochondria, and nuclei (160).

Because the pancreatic autoantibodies detected in the studies of Bottazzo et al. (160) were directed against both α and β islet cells, one could contemplate aberrant production of glucagon or insulin, or both, in such autoimmune disorders as the result of (a) interaction of autoantibodies with plasma membrane receptors for regulatory concentrations of glucose or (b) interaction of autoantibodies with the plasma membrane domains responsible for the Ca2+-mediated exocytosis of glucagon and (or) insulin molecules. This hypothesis receives support from several recent reports:

(a) Antibodies directed against the pancreatic islet cell plasma membrane have been detected in sera of Type I diabetes (164).

(b) Autoantibodies in newly-diagnosed diabetic children immunoprecipitate specific human pancreatic islet cell proteins of Mr = 64 000 and = 38 000 (16).

(c) Immunoglobulin fractions isolated from the sera of six newly-diagnosed Type I diabetic children all profoundly inhibited the ability of d-glucose to induce insulin release from perfused rat islet cells (165).

(d) When the immunoglobulin fraction of plasma from five children positive for islet-cell surface antibody, newly diagnosed as being insulin-dependent diabetics, was injected into immunosuppressed BALB/C mice, pancreata were produced in which insulin production was diminished; the same immunoglobulin fractions induced complement-dependent cytotoxicity in a rat islet-tumor-cell line (166).

In all autoimmune disease clusters having diabetes mellitus as a component, the presence of anti-insulin receptor antibodies directed against insulin's target tissues is a

| Table 4. Autoimmune Disorders Associated with Type I Diabetes Mellitus |
|-----------------------------|----------------|
| Disorder                   | Reference no. |
| Idiopathic hypoparathyroidism | 153            |
| Idiopathic Addison's disease | 154            |
| Hashimoto's thyroiditis     | 155            |
| Myasthenia gravis           | 156            |
| Pernicious anemia, with antibodies to intrinsic factor and parietal cells | 157 |
| Schmidt's syndrome (primarily idiopathic Addison's disease with chronic lymphocytic thyroiditis) | 158 |
| Polycystic disease (rheumatoid arthritis, chronic ulcerative colitis, idiopathic thombo- cytopenia purpura, bronchial asthma, thyrotoxicosis, acute hepatitis) | 159 |
distinct possibility. Indeed, quite recently Maron et al. (167), in a study of 22 patients with Type I diabetes mellitus, found in the sera of 10 of these patients anti-insulin receptor antibodies of the IgM class as detected by inhibition assays of 125I-labeled insulin binding and lipogenesis inhibition assays, both with use of rat epididymal adipocytes. The clinical consequences and theoretical implication of these recently discovered autoantibodies may be important, although their role, alone or together with viral infection and islet cell antibodies, in the pathogenesis of Type I diabetes mellitus is not currently understood.

The presence of organ-specific antibodies against thyroid, adrenal cortical, and gastric parietal tissues in patients with polyautoimmune diabetes mellitus could be tested by assays predicated on the presumption that the antibodies produced will include those directed against cell surface receptors for trophic hormones in those tissues. Thus, the methods described above to assay for Graves' disease immunoglobulins could be adapted for anti-thyroid autoantibodies in polyautoimmune diseases. Methods to assay for anti-adrenal cortex antibodies might be based on the presumption that subclasses of these immunoglobulins are directed against the corticotropin receptor. Blockade of the binding of radiolabeled corticotropin to isolated adrenal cortical membranes (168) or impairment of the ability of corticotropin to activate adenylate cyclase or to stimulate steroidogenesis in suspensions of adrenal cortical cells (169) would be suitable bases for assays for such autoantibodies. These assays will be considered below in connection with a discussion of autoimmune Addison's disease.

Diabetic patients with parietal cell autoantibodies tend to present with diffuse Type A fundal gastritis (170, 171). When chronic, this syndrome includes, among other characteristics (Table 5), hypergastrinemia and autoantibodies to a glycoprotein intrinsic factor, to a parietal cell lipoprotein, and to thyroid epithelial cells (172). The course of hypergastrinemia can readily be followed by RIA (173). As for the autoantibodies against intrinsic factor, two types have been identified (167). One is a blocking type that combines with the intrinsic factor–vitamin B12 binding site and prevents union of the vitamin with intrinsic factor. This autoantibody could be assayed by its ability to impede the binding of labeled cobalamin to intrinsic factor. For assay purposes, use could be made of the observation that polyethylene glycol precipitates the intrinsic factor–vitamin B12 complex in the presence of serum from patients with pernicious anemia who have autoantibodies against intrinsic factor (174). The other type of autoantibody is a binding type that combines with intrinsic factor at a site presumably remote from that to which vitamin B12 binds and therefore does not inhibit vitamin B12–intrinsic factor complex formation. As the ternary complex (autoantibody–intrinsic factor–vitamin B12) cannot bind to tissues, an assay based upon an inhibition of the binding of the intrinsic factor–labeled-cobalamin complex to isolated ileal plasma membranes would appear to be suitable.

Addison's Disease

Organ-specific autoantibodies have been demonstrated repeatedly in idiopathic autoimmune Addison's disease (175). The pattern here is one of reduced production of corticosteroids in response to corticotropin despite above-normal concentrations in plasma. This pattern is similar to that observed in tubercular Addison's disease or congenital adrenal hyperplasia, but the basis for the autoimmune version undoubtedly is different.

A clue to this difference may be found in those patients with idiopathic Addison's disease who also present with a constellation of autoimmune endocrine disorders (Table 6). Neufeld et al. (6) have described two patterns. Type I is the autoimmune polyglandular syndrome that may result from a thymic disfunction and that is characterized by adrenal insufficiency, hypoparathyroidism, and chronic mucocutaneous candidiasis, frequently associated with chronic active hepatitis, early-onset pernicious anemia, alopecia, and primary hypogonadism. Type II is the version in which only Type I diabetes mellitus and thyroid autoimmune disease accompany the adrenal insufficiency. The Type II syndrome, for which the range of age of onset is broad and which shows a marked increase in incidence of the HLA-B8 antigen (Type I exhibits no consistent HLA association), is essentially Schmidt's syndrome expanded to include diabetes.

The nature of the antigens producing the autoantibodies, although unknown at present, should not be difficult to determine. The lack of tissue or gender specificity for steroid cell autoantibodies found in serum in the Type I syndrome (175) could be due to antibody heterogeneity. Indeed, tissue adsorption studies showed that there were two distinct populations of adrenal-cortical autoantibodies in some patients. One type is bound only to adrenal cortical membranes, the other type is cross-reactive with all steroid-producing tissues (175). Thus it might be possible to fractionate serum immunoglobulins to autoimmune Addison's syndrome by specific tissue adsorption techniques or by isoelectric focusing. Of peripheral interest is the possibility that the cross reactivity among the various steroid-producing cells observed could be due to a common antigenic structure or receptor protein shared by all such cells.

Hypoparathyroidism

Many atrophic diseases (idiopathic Addison's disease, Hashimoto's disease, pernicious anemia, alopecia, chronic mucocutaneous candidiasis, moniliasis) are associated with idiopathic hypoparathyroidism. Lymphocytic infiltration of the parathyroid gland occurs when animals are injected with isologous parathyroid tissue. Immunofluorescence demonstrates autoantibodies specific for parathyroid tissue in a significant number of patients with idiopathic hypo-

| Table 5. Features of Type A Chronic Atrophic Gastritis |
|-----------------|---------------------|
| **General:**     | Hypergastrinemia, pernicious anemia with impaired B12 absorption, Hashimoto-type thyroid lesions |
| **Autoimmune:** | Antibodies to parietal cells (incidence 90%), intrinsic factor (75%), thyroid epithelial cells (66%), nucleic (26%), adrenal cortical and parathyroid tissues (minor incidence) |
| **Associations:**| Major: Hashimoto's thyroiditis, Type I diabetes mellitus, vitiligo Minor: Idiopathic Addison's disease and hypoparathyroidism |

<table>
<thead>
<tr>
<th>Table 6. Autoimmune Addison's Disease and Associated Autoimmune Disorders*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal cortical antibodies (incidence 73%)</td>
</tr>
<tr>
<td>Gonadal steroid cell antibodies (Type I, 80%; Type II, 13%)</td>
</tr>
<tr>
<td>Thyroid tissue antibodies (hypothyroidism)</td>
</tr>
<tr>
<td>Parathyroid tissue antibodies (hypoparathyroidism)</td>
</tr>
<tr>
<td>Schmidt's syndrome (primarily, autoimmune Addison's disease</td>
</tr>
<tr>
<td>coupled with chronic lymphocytic thyroiditis; secondarily, also</td>
</tr>
<tr>
<td>with Type I diabetes mellitus (7%), ovarian failure, pernicious</td>
</tr>
<tr>
<td>anemia)</td>
</tr>
</tbody>
</table>

* Data taken from ref. 172.
parathyroidism. These facts constitute incriminating evidence that this disease entity is attributable to, or associated with, an autoimmune process (153).

The mechanism and induction of this disease remains unknown. It is not known if the diminished production of parathyrin (parathyroid hormone) in this disorder is caused by antibodies generated against the β-adrenergic receptor or by the membrane calcium-binding protein (both of which are linked to the production of cyclic AMP), or against intracellular components of the biosynthetic system for the polypeptide hormone, parathyrin. One could conceive of an assay for the former type of antibody based upon its ability to impede the activation of adenylate cyclase in parathyroid cells or plasma membranes by parathyrin secretogogues such as isoproterenol.

Pseudohypoparathyroidism

Fuller Albright coined the term "pseudohypoparathyroidism" (PHP) to describe hypoparathyroidism caused by target-organ resistance to the action of parathyrin as contrasted to a simple deficiency of the hormone (176). The resistance to parathyrin in PHP Type I is thought to be due to a defect in the hormone receptor–adenylate cyclase complex, as such patients do not have the brisk rise in urinary cyclic AMP excretion that normally follows administration of this hormone (177). In PHP Type II, a much rarer disorder, the urinary cyclic AMP response is normal, but the phosphaturic response is defective (178).

Two laboratories appear to have uncovered the defect in PHP Type I. Bourne and colleagues at the University of California–San Francisco (179–181) and the Spiegel–Aurbach group at the NIH (182, 183) have found that the guanine nucleotide regulatory protein that couples the parathyrin receptor to the activation of adenylate cyclase is generally deficient in the tissues of those PHP Type I patients who also have the somatic features of Albright’s hereditary osteodystrophy. Not only does this deficiency in renal tubular membranes explain the blunted response to parathyrin observed in such patients, but the generalized deficiency of this non-tissue-specific (184) guanine nucleotide regulatory protein helps to explain the resistance to several other hormones—e.g., TSH, glucagon, and gonadotropins (184, 185)—in these patients, and perhaps also the deficient prolactin response to parathyrin observed in hypocalcemic and normocalcemic PHP (186).

PHP Type II not only is less common than PHP Type I, but also there is fragmentary evidence that Type II has a different basis, an autoimmune etiology. Autoantibodies that block the binding of parathyrin to membrane (human cultured lymphocytes) receptors for the hormone were detected in sera (especially in the IgG fraction) in 49 of 50 uremic patients with secondary hyperparathyroidism (patients with elevated C-regional parathyrin) (15). The production of cyclic AMP by parathyrin stimulation of adenylate cyclase in bovine renal tubular membranes also was reduced by the blocking antibodies.

More recently, evidence of anti-renal tubular membrane autoantibodies (IgG), was reported in a patient with PHP Type II and Sjögren’s syndrome. The autoantibodies were detected by their ability to block parathyrin-induced phosphaturia when infused into rats (187), and they had no effect on parathyrin-induced increases in urinary cyclic AMP. If these reports are confirmed, we may be able to add PHP Type II to the list of anti-receptor autoimmune diseases.

Resistant Ovary Syndrome

The “resistant ovary syndrome” is characterized by (a) primary or secondary amenorrhea before age 30; (b) chromosomal complement 46XX and normally-developed secondary sexual characteristics; (c) numerous ovarian follicles of normal morphology; (d) increased endogenous production of the gonadotropins follicitropin (follicle-stimulating hormone) and lutropin (luteinizing hormone); and (e) resistance of ovarian follicles to even excessive stimulation with exogenous human follicitropin plus lutropin (188–191).

Possible explanations for the resistance include (a) the generation of antibodies against gonadotropins; (b) increased production of prolactin, which seems to exert an anti-gonadotrophic action on ovarian follicles; and (c) the generation of blocking autoantibodies against the ovarian gonadotropin receptors.

Explanations a and b appears not to be viable because, in three separate studies, no antibodies against follicitropin or lutropin were found (188–190), and values for prolactin in serum were normal (188, 189). However, in two patients with hypergonadotrophic amenorrhea and myasthenia gravis, a serum immunoglobulin with properties of an IgG was found that inhibited the binding of radiolabeled follicitropin to its receptors in rat testicular plasma membranes (190). This observation suggests that circulating anti-follitropin receptor autoantibodies may be responsible for the observed clinical follitropin resistance in the gonadotropin-resistant ovary syndrome.

It is also of interest that, in keeping with the “clustering” hypothesis described at the beginning of this section, the patients producing the anti-follitropin receptor antibodies had a second autoimmune disorder—myasthenia gravis (190). Although not examined in these studies, one would anticipate that autoantibodies to acetylcholine receptor would also be found in sera from these patients.

Likewise, in a case reported several years ago of a woman with spontaneous premature menopause due to autoimmune ovarian failure, who presented with increased lutropin concentrations in serum, but normal concentrations of follitropin and concurrent idiopathic Addison’s disease (191), one would predict that the patient’s serum would contain autoantibodies to both corticotropin receptor and lutropin receptor.

HLA-B8/DR3 Haplotype

The association between certain HLA antigen products of human immune response genes and an increased risk for specific diseases has been known for several years (192, 193). One such association is that of the HLA-B8 and HLA-DR3 antigens with diseases involving immunological dysfunction (193, 194). For example, chronic active hepatitis, celiac disease, myasthenia gravis, Graves’ disease, sicca syndrome, systemic lupus erythematosus, diabetes mellitus, idiopathic Addison’s disease, dermatitis herpetiformis (associated with a defect in the Fc receptor in cells of the reticuloendothelial system), and Type II autoimmune polyclanular syndrome are all associated with the HLA-B8/DR3 haplotype (192, 194–197).

The presence of the HLA-B8/DR3 antigens in patients with immune diseases may provide important diagnostic and prognostic advantages to the clinician. Several examples will make this clear.

The presence of HLA-B8/DR3 was reported to be very useful in predicting relapse in hyperthyroid Graves’ disease patients after cessation of drug therapy (197). Others have not always found this correlation to be strong, but a recent prospective study by Allannic et al. (198) suggests that the strength of the HLA–relapse correlation may be functions of the type and duration of therapy, as well as of the follow-up interval.

Lawley et al. (196) have evidence that HLA-B8/DR3
antigens may be markers for a possible defect in the expression of Fc receptors on a number of circulating and fixed macrophages—receptors that are responsible for the clearance of circulating immune complexes. Not only was the HLA-B8/DR3 haplotype present in 90% of patients with dermatitis herpetiformis (an autoimmune disease characterized by pruritic papulovesicular skin lesions, cutaneous deposits of IgA, and circulating immune complexes), but of these, about 50% had defective Fc receptor function. Further, examination of a population of "normal" subjects, unique only in that they were Rh-positive and possessed the HLA-B8/DR3 antigens, revealed that about half of this group had defective reticuloendothelial system Fc receptors, similar to that seen in dermatitis herpetiformis patients (196). Further, all patients and controls with the marker haplotype had decreased percentages and total numbers of T cells bearing Fc receptors for IgG. Although the basis for the Fc receptor function defect is still unknown, the presence of the defect renders it likely that circulating immune complexes will not be cleared and destroyed normally but will remain in the circulation and be deposited in tissues, producing tissue damage. At the same time, an Fc receptor abnormality on immunoregulatory cells may greatly increase the likelihood of an aberrant immunological response to some antigen, leading to the development of an autoimmune disease.

Connective Tissue Diseases

Insulin resistance can be associated with a variety of connective tissue diseases, including acanthosis nigricans, diffuse scleroderma, Sjögren's syndrome, congenital lipodystrophy, rheumatoid arthritis, and systemic lupus erythematosus. We considered earlier the anti-insulin receptor antibody basis for the insulin resistance in acanthosis nigricans Type B, Sjögren's syndrome, and diffuse scleroderma. A seemingly anomalous situation exists in patients with lipoatrophic diabetes plus acanthosis nigricans in whom anti-insulin receptor antibodies were apparently absent (189).

Systemic lupus erythematosus is a product of an aberrantly regulated immune response. A suppression of cell-mediated immunity is accompanied by enhanced humoral immunity. These abnormalities may account for the multitude of antibodies to nuclei (90% incidence) and other cellular components found in these patients (200) and the deposition of these immune complexes in their renal glomerular and vascular basement membranes. The polyclonal antibody characteristics of this complex disease make it a prime candidate for investigations into the presence of anti-receptor antibodies. Indeed, Pedersen et al. (201) recently reported on a young female patient with this disease accompanied by acanthosis nigricans and massive insulin resistance. Present in her serum was a factor that inhibited the binding and functioning of insulin in isolated normal human subcutaneous adipocytes. Immunofluorescence studies suggested that the factor might be IgG. Undoubtedly, future investigations will establish whether the presence of systemic lupus erythematosus is a "marker" for anti-receptor autoimmune diseases.

A variety of autoimmune signs are associated with Sjögren's syndrome. Such patients usually present with hypergammaglobulinemia (elevated IgM); antibodies to nuclei, thyroglobulin, and salivary ducts; leukopenia; and accelerated erythrocyte sedimentation rates. Often present are rheumatoid arthritis, thrombocytopenic purpura, and chronic active hepatitis. Rarely seen in Sjögren's syndrome are systemic lupus erythematosus, diffuse scleroderma, polymyositis, and periartitis nodosa. To date only one patient with a concurrent anti-receptor autoimmune disease has been reported, a case of extreme insulin resistance due to anti-insulin receptor autoantibodies. However, if Sjögren's syndrome is indeed an important member of the anti-receptor antibody cluster phenotype, further studies with additional patients may uncover other such antibodies.

Rheumatoid arthritides frequently produce "rheumatoid factors," which are immunoglobulins (IgG, IgM, and IgA) produced against the patient's own, probably altered, IgG. "Rheumatoid factors" may also be found in patients with Type I diabetes mellitus, progressive systemic sclerosis, systemic lupus erythematosus, chronic active hepatitis, as well as in a recently discovered autoimmune complex, namely, rheumatic disease (systemic lupus erythematosus, rheumatoid arthritis, chronic inflammatory diseases) with anti-lipomodulin antibodies (203). Although anti-receptor antibodies have not yet been reported in rheumatoid arthritis, the polyautoimmune nature of this complex disorder suggests that such a phenotype is very likely.

There are important ramifications to the concurrent presence of anti-lipomodulin autoantibodies with some rheumatic disorders. Lipomodulin is a protein that inhibits phospholipase A2 (EC 3.1.1.4) and whose synthesis is induced by anti-inflammatory steroid hormones. Phospholipase A2 has been recognized as an important enzyme in the release of prostaglandin precursors, especially arachidonic acid. Lipomodulin autoantibodies will therefore increase the formation of arachidonic acid and, consequently, the formation of inflammatory prostaglandins. This concept is supported by the observation that inhibitors of prostaglandin synthesis such as chloroquines and glucocorticoids are effective therapeutically in patients with the above-mentioned rheumatic diseases (205). Rheumatic arthritis and systemic lupus erythematosus are diseases of chronic inflammation. Prostaglandins play an important role in chronic inflammation in these diseases, as well as in altering lymphocyte function. It is, therefore, not surprising that antibodies against lipomodulin were detected in these inflammatory diseases.

References
12. Kahn CR, Rosenthal AS. Immunoresponse to insulin:
37. Lindstrom JM. Structure of the acetylcholine receptor; specificities of antibodies to it in myasthenia gravis. In ref. 24, pp 178–196.
41. Engel AG, Fumagalli G. Mechanisms of acetylcholine receptor loss from the neuromuscular junction. In ref. 24, pp 197–224.
55. Roses AD, Olanow CW, McAdams MA, Lane RM. No direct correlation between serum anti-ACh receptor antibody levels and the clinical state of individual patients with MG. Neurology 31, 220–224 (1981).
90. Kohn LD, personal communication.


