Infection, Thyroid Disease, and Autoimmunity*

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I. Introduction

TRADITIONALLY, it was assumed that infectious agents induced disease by causing direct tissue damage (for example via secretion of exotoxins and endotoxins). However, we now know only too well that infectious agents play a role in the induction of noninfectious consequences, including malignancies (for example EBV and Burkitt's lymphoma, HTLV-1, and adult T cell leukemia), acquired immunodeficiency syndrome (human immunodeficiency virus), peptic ulcer (Helicobacter pylori), and autoimmune diseases. Infectious agents have been implicated in the pathogenesis of a variety of autoimmune diatheses, namely, rheumatic fever, Reiter's syndrome, systemic lupus erythematosus (SLE), myasthenia gravis, insulin-dependent diabetes mellitus, Sjogren's syndrome, and the autoimmune thyroid diseases. This review examines the pertinent data relating to the possible role of infecting organisms in the development of autoimmune thyroid diseases (AITD), with an emphasis on human disease, focusing on the mechanisms by which infection could trigger Graves' disease and other thyroiditides. It must be remembered, however, that the role of infection in precipitating human AITD remains purely hypothetical and that precipitating factors may not be either infectious or external.

II. Infections of the Thyroid Associated with Autoimmune Phenomena

A. Human subacute thyroiditis

First defined by De Quervain (1), subacute thyroiditis is a self-limited inflammatory disorder of the thyroid gland. The disease is most prevalent in females in a ratio of 3-6:1. The illness is usually characterized by a sudden onset of neck pain and tenderness, fever, malaise, and variable changes in thyroid function tests, and usually lasts several weeks to several months (2). However, painless subacute thyroiditis occurs regularly and recurrent subacute thyroiditis has been reported (3).

1. Viral infection and the etiology of subacute thyroiditis. Older literature first suggested a viral etiology for human subacute thyroiditis. Clinically the disease has several characteristics typical of viral infections including a typical viral prodrome with myalgias, malaise and fatigue, absence of leukocytosis, and usually a self-limited course. Additionally, clusters of the disease have been reported during outbreaks of viral infection (4). A higher prevalence of subacute thyroiditis has also been reported during the summer, coinciding with the seasonal distribution of the enteroviruses (5), and in Holland an epidemic of subacute thyroiditis affecting 23 individuals has been described (6). Eylan and colleagues (7) reported 11 patients with subacute thyroiditis diagnosed during a mumps epidemic. These 11 patients were found to have circulating anti-mumps antibodies without clinical evidence of mumps. In two patients the mumps virus was cultured from thyroid tissue obtained at biopsy. Others have also reported an association between mumps virus and subacute thyroiditis (8-10). Different viruses reportedly associated with subacute...
thyroiditis include measles virus, influenza virus, adenovirus, Epstein-Barr virus, and Coxsackie virus (11). In an extensive study reported by Volpe et al. (12), 32 of 71 patients with subacute thyroiditis, who had no evidence of specific viral disease, demonstrated at least 4-fold increases in viral antibodies during their thyroid illness. These viral antibodies included those to Coxsackie, adenovirus, influenza, and mumps virus. Coxsackie viral antibodies were most commonly found, and the changes in their titers most closely approximated the course of the disease. Additionally, Stancak and co-workers (13) reported that in five of 28 patients with subacute thyroiditis tested, a cytopathic virus of pathogenic importance was isolated. This virus was subsequently identified as a human foamy retrovirus (14). Hence, it appears that the thyroid responds with the picture of thyroiditis after invasion by a variety of different viruses and that no single agent is likely to be causal in the syndrome of subacute thyroiditis.

2. Autoimmune phenomena in subacute thyroiditis. Several autoimmune phenomena have been reported in subacute thyroiditis (Table 1). Thyroid autoantibodies [antithyroglobulin (anti-Tg) and antithyroid peroxidase antibodies (anti-TPO)] have been found in 42%-64% of patients with subacute thyroiditis (12, 15). In most of these patients the antibody titer gradually decreased and remained low or disappeared as the disease faded. TSH receptor (TSHR) antibodies have also been reported in patients with subacute thyroiditis (16-19) although changes in antibody titer did not correlate with disease activity (19). There are also reports of the sequential occurrence of Graves' disease and subacute thyroiditis (20-22), and rare cases of Hashimoto's thyroiditis have been reported after classical subacute thyroiditis. Recently, autoantibodies to several novel, and uncharacterized, thyroid antigenic determinants were found in eight of nine patients with subacute thyroiditis tested (23). Moreover, these autoantibodies persisted and their level did not decrease over a period of 39 months after onset of subacute thyroiditis. Such antibodies likely arise secondary to the damage caused by viral infection of the thyroid gland since they are typically polyclonal in nature (23).

Evidence that T cell-mediated immunity against thyroid antigens may play a role in the pathogenesis of subacute thyroiditis has also been reported. During the initial phase of the disease the gland is infiltrated by T cells, and sensitization of T cells against thyroid antigens has been shown in such patients (24-26). However, this sensitization was transitory and, therefore, likely represented a secondary immune response to the inflammatory release of antigen induced by the viral infection of the gland (2).

In summary, several reports have suggested that an autoimmune response develops in subacute thyroiditis. However, from the limited data available it appears that these autoimmune phenomena represent a nonspecific, and transient, response to the inflammatory release of thyroid antigens rather than a specific autoimmune disease triggered by the viral infection. Only in rare cases does autoimmune thyroid disease develop after typical subacute thyroiditis, and most patients with subacute thyroiditis recover completely with no autoimmune sequelae. Hence, individuals must possess additional susceptibility in order to acquire chronic autoimmune thyroid disease. Alternatively, patients susceptible to AITD would not develop classical subacute thyroiditis in response to a hypothetical virus but develop what we recognize as AITD.

B. Congenital rubella infection and AITD

Congenital rubella infection has been associated with an increased frequency of endocrine gland failure including diabetes mellitus (27, 28), GH deficiency (29), Addison's disease (30), and thyroid dysfunction (31). It has been reported that the incidence of diabetes mellitus and impaired glucose tolerance may be as high as 20% within the second decade of life in patients with congenital rubella (27). Although only occasional cases of thyroid disorder after congenital rubella infection have been reported, the spectrum of thyroid dysfunctions reported is surprisingly broad. Ziring and co-workers (30) have reported a case of congenital rubella and hypothyroidism secondary to Hashimoto's thyroiditis in which immunofluorescent studies of thyroid tissue demonstrated staining for rubella virus antigen, and three additional cases of congenital rubella and acquired hypothyroidism secondary to Hashimoto's thyroiditis have since been reported (32-34). Other thyroid disorders that have been reported to be associated with congenital rubella include thyrotoxicosis (35-37) and idiopathic hypothyroidism (37-39).

The mechanisms responsible for thyroid dysfunction induced by prenatal rubella infection are poorly understood. Destruction of thyroid cells by persistent rubella virus infection, precipitation of an autoimmune reaction, or both have been proposed (31). We evaluated the prevalence of thyroid autoantibodies in 241 children with congenital rubella syndrome. Anti-TPO or anti-Tg antibodies were found in 34% of the patients (40). Similarly, Clarke et al. (41) have found thyroid autoantibodies in 23.3% of adolescents with congenital rubella and in only 12% of the controls. Additionally, T cell abnormalities have been reported in patients with congenital rubella syndrome (37).

Since most reports have shown no evidence of active rubella infection at the time of thyroid dysfunction in these patients, it is likely that an ongoing autoimmune reaction has ensued, which may explain delayed thyroid dysfunction occurring many years after congenital rubella infection has subsided. Furthermore, the diversity of thyroid disorders

TABLE 1. Evidence for autoimmunity after subacute thyroiditis

| 1. Clinical evidence |
| Graves' disease developed after subacute thyroiditis (20-22) |
| Hashimoto's disease developed after subacute thyroiditis (11) |
| 2. Serological evidence |
| Appearance of anti-Tg antibodies (in 42% of the sera tested) (12) |
| Appearance of anti-TPO antibodies (in 42% of the sera tested) (12) |
| Appearance of TSHR antibodies (16-19) |
| Production of autoantibodies to novel thyroid antigens (23) |
| 3. Cellular autoactivity |
| Sensitization of T cells against thyroid antigens (24-26) |
linked to congenital rubella parallels that associated with adult AITDs. However, it is not known whether the thyroid autoimmunity induced by congenital rubella is initiated by infection of the affected target cells or is secondary to a more generalized infection of the immune system.

C. Animal models of infectious thyroiditis

Mice. Further evidence for viral involvement in the etiology of the AITDs comes from animal models. Klavinskis et al. (42) have demonstrated that lymphocytic choriomeningitis virus can persist in the thyroid gland of mice neonatally infected with the virus. Furthermore, the virus that was shown to persist mainly in the thyroid epithelial cells in which thyroglobulin is synthesized induced a reduction in the level of thyroglobulin messenger RNA and circulating thyroid hormones, but there was no thyroid cell destruction. In contrast, mice infected with reovirus type 1 have been shown to develop a thyroiditis characterized by focal destruction of acinar tissue, infiltration of the thyroid by inflammatory cells, and production of autoantibodies directed against thyroglobulin and thyroid microsomes (43). By the use of recombinants between reovirus type 1 and type 3 (the latter does not induce thyroiditis in mice) Onodera and Awaya (44) were able to identify the segment of the reovirus type 1 genome responsible for the induction of autoantibodies to thyroglobulin. This segment, termed S1, encodes a polypeptide that binds to surface receptors and determines the tissue tropism of the virus (44).

Chickens. The obese strain (OS) of chickens develop a hereditary spontaneous autoimmune thyroiditis characterized by obesity and hyperlipidemia and histologically by lymphocytic infiltration of the thyroid gland (45). Recently, a new endogenous retrovirus (ev 22) was found to be expressed in OS chickens but not in healthy normal inbred strains (46). Breeding experiments have now shown that ev 22 is inherited autosomally in a dominant manner (46). However it is unclear whether ev 22 is directly involved in the pathogenesis of spontaneous autoimmune thyroiditis in OS chickens or is a genetic marker of the disease as, for example, the murine minor lymphocyte stimulating-related (Mls) retroviruses may be (47).

A thyroiditis similar to that which develops spontaneously in OS chickens has also been induced in normal chickens by retroviral infection. Carter and Smith (48) have demonstrated that infection of 10-day chicken embryos with avian leukemia virus (ALV) resulted in hypothyroidism within 3 weeks of hatching. The hypothyroidism was manifested by marked stunting, hyperlipidemia, and low levels of T3 and T4. Histological examination of the thyroids from infected chickens showed an extensive lymphocytic infiltration.

Rats. Experimental autoimmune thyroiditis can be induced in the FVG/c strain of rats by thymectomy and irradiation (49). Studies on the influence of the gastrointestinal flora on this experimental model have revealed that rats maintained under specific pathogen-free conditions were significantly less susceptible to induction of autoimmune thyroiditis (50). Moreover, oral administration of antibiotics followed by administration of intestinal contents from conventionally reared rats significantly enhanced their susceptibility to the disease. These studies indicate that the composition of the gut flora influences the susceptibility of rats to the induction of autoimmune thyroiditis. However, it is not clear at present whether the disease is triggered by an infecting organism in the gut flora or by an unknown toxic agent or immune stimulant. Recently, Ebner et al. (51) have successfully induced lymphocytic thyroiditis in rats by immunizing them with Yersinia enterocolitica-purified outer membrane protein but further controls are required to analyze the specificity of such data. In summary, the data strongly suggest that infectious agents can induce thyroid dysfunction in susceptible experimental animals supporting the earlier discussion of such a possibility in humans.

III. Autoimmune Thyroid Diseases Associated with Infectious Etiology

A. Graves' disease and infection

Graves' disease is an autoimmune disease characterized by hyperthyroidism and diffuse goiter with or without the associated ophthalmopathy and dermopathy (52). Graves' disease is an organ-specific autoimmune disease caused by the production of TSH receptor autoantibodies (TSHR-Ab). These autoantibodies stimulate the TSHR to increase iodide uptake and cAMP production, inducing production and secretion of excess thyroid hormones (53).

1. Infectious agents in the pathogenesis of Graves' disease (Table 2). We have been unable to find evidence for linkage of AITD to the HLA gene region using serological HLA typing (54) and HLA-DQ restriction fragment length polymorphisms (55). Since there is a well confirmed population association with HLA-DR3 (56), also seen in our own family studies, but an absence of linkage, the familial clustering of the disease may just as well be associated with an environmental risk, such as an infectious agent, or with another unknown susceptibility gene. Recently, several studies have suggested that infectious agents may be involved in the mechanisms triggering the breakdown of tolerance for the TSHR in Graves' disease.

Table 2. Evidence for involvement of infection in the pathogenesis of Graves' disease

<table>
<thead>
<tr>
<th>Evidence for infection</th>
<th>Reference</th>
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<tbody>
<tr>
<td>1. Epidemiological evidence</td>
<td>Clustering of Graves' disease (58, 64)</td>
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<tr>
<td></td>
<td>Seasonality in the development of Graves' disease (57)</td>
</tr>
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<td></td>
<td>Lack of linkage despite association to HLA DR 3 (54-56)</td>
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<tr>
<td></td>
<td>Increased prevalence of nonsecretors (58-61)</td>
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<tr>
<td>2. Serological evidence</td>
<td>Serological evidence for a recent infection in Graves' patients (62)</td>
</tr>
<tr>
<td></td>
<td>Antibodies to Influenza B virus (63)</td>
</tr>
<tr>
<td></td>
<td>Antibodies to Yersinia (in up to 72% of the patients) (71, 73)</td>
</tr>
<tr>
<td></td>
<td>Presence of serum retroviral factors (83)</td>
</tr>
<tr>
<td>3. Evidence at the molecular level</td>
<td>Presence of retroviral sequences in Graves' thyroid tissue (80)</td>
</tr>
<tr>
<td></td>
<td>66% homology between HIV-I nef protein and human TSHR (85, 86)</td>
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</table>
disease. Cox et al. (57) found seasonal trends in the diagnosis of Graves' disease but their data failed to reach statistical significance. In another study, Phillips et al. (58) found that the incidence of thyrotoxic patients who had TSHR and anti-TPO varied markedly between towns in England, but this study remains to be confirmed. Moreover, a significantly increased prevalence of nonsecretors (individuals with inability to secrete the water-soluble glycoprotein form of the ABO blood group antigens into saliva) was reported in patients with Graves' disease (59–61). Since nonsecretors are known to have increased susceptibility to infection (60), these findings lend further support to the notion that an infective agent may play a part in the pathogenesis of Graves' disease. Indeed, Valtonen and co-workers (62) found serological evidence for a recent bacterial or viral infection in 36% of newly diagnosed Graves' patients and in only 10% of controls. An increased frequency of antibodies to the influenza B virus has also been found in patients with thyrotoxicosis (63). To date, only one report of "clustering" of Graves' disease patients exists (64).

Recently a TSH-binding protein was reported to exist in a number of gram-positive and gram-negative bacteria suggesting the presence of TSHR antigen (65). Moreover, five of 12 Graves' immunoglobulin G (IgG) preparations displaced radiolabeled bovine TSH from the bacterial binding proteins (65). These findings imply that anti-TSHR antibodies could be produced by cross reaction between bacterial proteins and the TSHR.

2. Yersinia infection and Graves' disease. Primarily known to cause outbreaks of food poisoning, Yersinia enterocolitica has also been implicated in the pathogenesis of Reiter's syndrome and is associated with various autoimmune phenomena (66). Studies on patients with Y. enterocolitica infections have demonstrated that the sera of these patients contained autoantibodies to thyroid epithelium (67, 68) although these may be of the "natural" autoantibody variety (69, 70). Conversely, a large proportion of patients with Graves' disease and autoimmune thyroiditis were reported to have antibodies to Yersinia (71), and many different Yersinia antigens cross-react with thyroid antigens (72) although with uncertain affinity. Wenzel et al. (73) found antibodies to plasmid encoded release proteins of Y. enterocolitica in 72% of Graves' disease patients, in 81% of patients with recurrent disease, but also in an unmatched 35% of controls. Such data need to be confirmed. While Weiss et al. (74) demonstrated a saturable binding site for TSH on Y. enterocolitica, and the binding of radiolabeled TSH to Yersinia has been shown to be inhibited by Graves' immunoglobulins (75), none of these observations have shown that infection with Yersinia leads directly or indirectly to the development ofAITD. However, it is possible that the hypothesized cross-reaction between Yersinia antibodies and thyroid antigens is secondary to a recurrent epitope or a reflection of a related but unknown infection (76). Recently a 200 base pair fragment of Yersinia complementary DNA was successfully amplified using TSHR oligonucleotide primers and a radiolabeled TSHR probe gave several discrete bands of hybridization with digested Y. enterocolitica UNA but under low stringency conditions (77).

Further evidence supporting the role of Yersinia infection in thyroid autoimmunity comes from the study of rats which demonstrated induction of lymphocytic thyroiditis after immunization with the Yersinia enterocolitica purified outer membrane protein (51). In addition T cell-mediated immunity toward Yersinia enterocolitica has been reported in patients with Graves' disease (78). However, similar data have been demonstrated in rheumatoid arthritis (79). The possibility of Yersinia-specific immune responses being merely reflective of the "natural" immune response deserves serious consideration in view of the low affinity interactions and lack of predictability in the published studies. Moreover, most patients with Yersinia infection (including those who produce anti-TSHR antibodies) do not develop Graves' disease. Hence, it is possible that, while Yersinia antigens that are homologous to the TSHR can induce production of anti-TSHR antibodies, this immune response may be transitory except in susceptible individuals.

3. Endogenous and exogenous retroviruses and Graves' disease. Retroviruses have been implicated in the induction of Graves' disease but data supporting this view remain unsubstantiated. Recently, Bottazzo and colleagues (80) reported the existence of retroviral sequences in the thyroid and peripheral blood mononuclear cells of Graves' patients. DNA extracted from thyroid glands of five Graves' disease patients was hybridized with a probe containing the gag region of HIV-I. The results showed positive bands in each patient's thyroid DNA which apparently were absent in control samples. However, these results were not confirmed by Burman and colleagues (81) or by Tominaga et al. (82). An uncharacterized retroviral-like factor (p15E) has also been detected in the serum of Graves' patients; the absence of p15E in controls suggested involvement of endogenous retroviruses in the pathogenesis of the disease (83). Tas et al. (84) have suggested that the origin of the p15E-related factors may be an exogenous infection with an as yet unknown retrovirus possessing determinants with structural homology with the p15E, or an endogenous retrovirus. Supporting these findings are the studies in OS chickens discussed above which also demonstrated expression of a new retrovirus (ev 22) in susceptible chickens' thyroid tissue (46).

In order to help explain a possible relationship between retroviruses and thyroid autoimmunity, a homolog has been suggested between the HIV-I Nef protein and the human TSHR (85). There was 66% homology demonstrated within a 166 bp region encoding a unique portion of the human TSHR with a segment in which seven of 10 consecutive amino acids were identical. However, when sera from 10 patients with Graves' disease and 10 controls were tested for reactivity against an 18 amino acid peptide containing this segment of homology, there was no significant difference in the degree of interaction (86). Nevertheless, this does not exclude a conformational B cell epitope or the presence of a linear T cell epitope.

D. Human autoimmune thyroiditis and infection

Human autoimmune thyroiditis is characterized by infiltration of the thyroid by lymphocytes, gradual destruction of
the gland associated with cytotoxic T cells, and production of various secondary polyclonal thyroid autoantibodies, notably anti-TPO and anti-Tg (87). The etiology of Hashimoto’s disease is still unknown; however, both genetic and environmental factors have been implicated in the pathogenesis of the disease (88). Serological evidence for a recent bacterial or viral infection has been demonstrated in patients with Hashimoto’s thyroiditis (62), and lymphoid thyroiditis has been described after immunization with group A streptococcal vaccine (89). Recently, it has been reported that autoimmune thyroiditis may be associated with significantly increased frequency of antibodies that react to HIV-1 Western blot proteins in patterns not diagnostic for HIV infection (90). These data suggested that autoimmune thyroiditis patients were infected with non-HIV retrovirus producing antibodies to a viral protein cross-reactive with HIV proteins (91). Such a retroviral infection may be the triggering factor leading to the development of thyroid autoimmunity (see below). Viral-like particles have been detected in thyroids of humans with AITDs; however, since these virus-like particles were also demonstrated in normal thyroids and in other tissues, their significance is unclear at present (92).

_Yersinia enterocolitica_ infection has also been implicated in the pathogenesis of Hashimoto’s thyroiditis as well as Graves’ disease. Wenzel et al. reported that antibodies against _Yersinia_ release proteins (RP) raised in rabbits showed specific bands on Western blots with thyroid epithelial cell homogenates, and one band could be blocked by purified TPO (93). Further evidence suggesting that infection is involved in the etiology of Hashimoto’s thyroiditis comes from studies of T cell function in the disease. It has been recently reported that the lymphocytes in Hashimoto’s thyroids have restricted antigenic response, suggesting their triggering by a specific antigenic stimulus, perhaps an infection (94). Clearly, isolation of infecting organisms from thyroid tissue is needed to substantiate these indirect data.

IV. Lessons from Infectious Triggers in Autoimmune Disease

The central feature of the immune system is specificity. The specificity of the immune response is maintained by complex mechanisms that include the unique structures of secreted and nonsecreted antigen receptors on lymphocytes and the elaborate interactions that occur between cells involved in the immune response. Together they form an interconnecting regulatory network that controls immune reactions. Infectious agents may induce autoimmunity by influencing any of the stages of the immune response, namely, the encounter with an antigen, the recognition of an antigen as non-self, the activation of the various effector arms of the immune system, and the regulation of the immune response.

A. Viral-induced changes in self-antigen expression

Viral infection, theoretically, may 1) alter self, 2) lead to the virus becoming a persistent endogenous antigen, or 3) cause the revelation of previously nonexposed, or rarely exposed, antigens.

Alterations in normal body components can develop during infections as a result of the release of bacterial or viral products, the expression of viral antigens on host cells, or the tissue injury caused by the accompanying inflammatory reaction (95). For example, it has been shown that Coxsackie virus B4 infection produces diabetes in mice by inducing increased expression of a 64,000-M islet autoantigen in infected mice (96). The 64,000-M antigen is believed to be the primary target protein in the anti-β-cell autoimmune reaction which characterizes the insulitis of insulin-dependent diabetes mellitus (97). Further evidence that viral-induced modification of self-constituents may cause autoimmune disease comes from studies of Whittingham et al. (98) who reported a patient who developed Sjogren’s syndrome after protracted infectious mononucleosis. The authors were able to show that the autoimmune reaction developed as a consequence of the association of viral RNA with the La nucleoprotein resulting in a break in immunological tolerance and induction of anti-SSB antibodies leading to autoimmune sialoadenitis. The mechanism of altered self has not been reported to operate in thyroid autoimmunity. However, one observation of interest is the finding of Graves’ disease and Graves’ ophthalmopathy in patients who have received external radiation over the thyroid region. The radiation may have altered thyroid antigens or their expression, thereby inducing an autoimmune reaction (99). However, it cannot be ruled out that the development of Graves’ disease in these patients was a consequence of immune dysregulation induced by irradiation of the thymic region.

Another mechanism by which an infecting organism, mainly viruses, may alter self constituents and induce autoimmunity is via persistent expression of viral antigens on host cells. This phenomenon has been shown to occur in infections with endogenous retroviruses. Adams et al. (100) have reported that if endogenous retroviral expression does not develop neonatally and is delayed until adulthood, then an autoimmune reaction ensues. As discussed earlier, endogenous retroviral protein expression has been reported in autoimmune thyroid disease (80, 83, 90) and in Sjogren’s syndrome (101–103), and it has been shown that when transgenic mice expressing LCMV antigens in pancreatic β-cells are challenged with LCMV they produce lymphocytic infiltrates in their β-cells and develop signs of insulin-dependent diabetes mellitus (104, 105).

Additionally, antigens not normally exposed to the immune circuit may become significant antigens for the first time. This may be important in viral-induced murine encephalitis and cardiac myositis, and in the formation of sperm antibodies after vasectomy (106).

B. Molecular mimicry

Molecular mimicry is defined as structural similarity between antigens coded by different genes. Molecular mimicry has long been implicated as a mechanism by which microbes can induce autoimmunity (107). The best known example of molecular mimicry and autoimmunity is rheumatic fever, in
which antigenic cross-reactivity between cardiac tissue and streptococcal polysaccharides is believed to induce an autoimmune reaction targeted at the heart valves (108). Antigenic similarity between infectious agents and host cell proteins is common, and in one analysis of 600 monoclonal antibodies raised against a large variety of viruses it was found that 4% of the monoclonal antibodies cross-reacted with host determinants expressed in uninfected tissues (109). The clinical importance of molecular mimicry between mycobacteria and self-antigen was highlighted by the observation that patients treated with Bacillus Calmette-Guérin immunotherapy developed arthritis (110). Furthermore, anti-DNA antibodies were shown to have amino acid sequence homology with anti-Klebsiella pneumoniae Waldenström monoclonal antibody (111), and when normal peripheral blood mononuclear cells were stimulated with Klebsiella antigens they secreted a common anti-DNA idiotype (the 16/6 Id) (112). The 16/6 Id has also been found in the serum of patients with the parasitic infections filariasis and schistosomiasis (113).

Mice infected with reovirus type 1 developed an autoimmune polyendocrinopathy and generated a panel of autoantibodies directed against normal pancreas, pituitary, and gastric mucosa, suggesting an antigenic similarity between a reoviral antigen and an endocrine tissue antigen (114, 115). Serreze and colleagues (116) report that antibodies directed against the p73 antigen, an endogenous retroviral gene product, are cross-reactive with anti-insulin antibodies. The authors suggested that anti-p73 autoantibodies are involved in inducing \( \beta \)-cell destruction in NOD mice. Likewise, Talai et al. (117) have demonstrated that 22 of 61 SLE patients produced antibodies to the p24 gag protein of HIV-1. Moreover, Sm (a ribonucleoprotein involved in the generation of mRNA) was shown to partially inhibit the antibody binding of p24 gag, suggesting immunological cross-reactivity between the retroviral antigen p24 gag and the autoantigen Sm (117).

Homology between microbial and host tissue antigens does not necessarily mean that an autoimmune response will emerge upon infection with that microbe. In order to prove that a sequence homology leads to autoimmunity it is necessary to show that challenging the host with the microbial antigen leads to an autoimmune response unrelated to direct infection of the target tissues. An example of such an experiment was provided by Oldstone and co-workers. They have used myelin basic protein, which has been shown to have significant homology with several viral proteins including hepatitis B virus polymerase (118). When the authors injected a hepatitis B virus polymerase-derived peptide into rabbits, the animals developed lesions in the central nervous system similar to the autoimmune disease induced by injection of myelin basic protein (119).

As discussed earlier, molecular mimicry has been reported between Yersinia enterocolitica and the TSHR based on the observed cross-reaction between sera from Yersinia and Graves patients (68, 71). Moreover, a saturable binding site for TSH was demonstrated on Y. enterocolitica (74). Recently, Wolf and co-workers (120) have demonstrated that IgG of individuals convalescing from Yersinia infections produced concentration-dependent inhibition of TSH binding to thyroid membranes and stimulation of adenylate cyclase activity. This study demonstrates that IgG from patients with Yersinia infections can react directly with the TSHR, perhaps as a consequence of cross-reactivity between antigenic determinants on Y. enterocolitica and the TSHR. However, since none of these patients demonstrated thyroid dysfunction, a definite association between Y. enterocolitica infections and increased incidence of Graves’ disease has not been demonstrated. The pathogenetic importance of Yersinia infection in the development of Graves’ disease, therefore, remains to be unravelled.

Molecular mimicry has been suggested between retroviral sequences and the TSHR (86); however, these findings remain to be confirmed. Another finding suggesting a possible role for molecular mimicry inAITD was that 42% of sera from lepromatous leprosy patients contained anti-Tg antibodies compared to 3% in the controls (121).

C. The superantigens

Superantigens are powerful T cell stimulatory molecules that bind to major histocompatibility complex (MHC) class II determinants. The complex of MHC proteins and superantigen thus formed is recognized by particular T cells which then become activated. The individual superantigens activate a restricted proportion of T cells which is decided by the T cell receptor (TCR) V gene used (47). The recognition of antigens bound to MHC proteins involves all the variable components of the TCR (i.e. the V, D, and J \( \alpha \) and \( \beta \)-molecules); however, superantigens stimulate T cells exclusively via the V \( \beta \) chain of the TCR (122). Recent data have suggested that while normal antigens bind to the MHC class II molecules within a well-defined cleft, thus creating a complex that is recognized by the variable regions of the TCR, superantigens bind to the MHC molecule at the external surface of their \( \beta \)-pleated sheets (Fig. 1). In this way a superantigen can bring the surfaces of the TCR bearing the specific V \( \beta \) antigen it recognizes, and the antigen presenting cell expressing MHC class II molecule, into close contact. This association activates T cell clones expressing only that specific V \( \beta \) chain (123). Superantigenic stimulation of a V \( \beta \) specific T cell clone can lead to stimulation, anergy, or deletion of that particular clone, depending on the developmental state of the T cell (47).

Superantigens may be extrinsic or intrinsic (Table 3). Extrinsic superantigens constitute a group of bacterial toxins (e.g. staphylococcal, streptococcal, and mycoplasmal toxins) that cause various syndromes, notably toxic shock syndrome. These bacterial toxins were found to induce disease by stimulating T cells via the V \( \beta \) chain of the TCR (123). Intrinsic superantigens have only been reported in the mouse and comprise the minor lymphocyte stimulating (Mls) antigens. Mls are antigens that induce a mixed lymphocyte reaction between MHC identical lymphocytes. Recently, it was discovered that Mls antigens stimulate T cells bearing particular TCR V \( \beta \) chains, in a similar manner to the extrinsic superantigens and were encoded by several murine endogenous
MIS and/or enterotoxins

FIG. 1. Superantigenic stimulation of T cells is achieved by binding to the TCR and the MHC class II molecule on the antigen presenting cell (APC) outside the antigen binding sites. [Reproduced with permission from H. Acha-Orbea and E. Palmer: Immunol Today 12:356-361, 1991 (47).]

TABLE 3. Exogenous and endogenous superantigens and their target Vβ specific T cells

<table>
<thead>
<tr>
<th>Exogenous</th>
<th>Endogenous</th>
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<tr>
<td>Superantigen</td>
<td>Human V-β specificity</td>
</tr>
<tr>
<td>SEA</td>
<td>?</td>
</tr>
<tr>
<td>SEE</td>
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SEA-SEE, Staphylococcus aureus enterotoxins A-E; TSST1, toxic shock syndrome toxin 1; Mls, minor lymphocyte stimulating antigens. [Derived from data in Refs. 47 and 123.]

As first envisioned by Jerne (126), auto-anti-idiotypic antibodies and T-suppressor cells (127) are generated in the course of the normal immune response to a foreign invading pathogen, and serve to regulate the normal immune response. Anti-idiotypic antibodies produced during the primary immune response to an exogenous pathogen carry the internal image of the epitopes on the pathogen which bind to its receptor in the host. Consequently, the development of receptor binding anti-idiotypic types can be harmful to the host and initiate autoimmunity (128). For example, the antibodies to reovirus type 3HA (HA3) have been studied extensively (129). Williams and co-workers have been able to produce in BALB/c mice a monoclonal antibody (called 9BG5) which could strongly bind to reovirus type 3 and neutralize its infectivity. Moreover, an anti-idiotypic monoclonal antibody was produced which was found to bind to 9BG5 and to inhibit its interaction with HA3. The workers then demonstrated that the anti-idiotypic bore the internal image of the receptor binding epitope on reovirus type 3; this anti-idiotypic could induce changes in cells upon binding to their HA3 receptors similar to those induced by the virus itself (129).

D. Alterations in the idiotypic network

As first envisioned by Jerne (126), auto-anti-idiotypic antibodies and T-suppressor cells (127) are generated in the course of the normal immune response to a foreign invading pathogen, and serve to regulate the normal immune response. Anti-idiotypic antibodies produced during the primary immune response to an exogenous pathogen carry the internal image of the epitopes on the pathogen which bind to its receptor in the host. Consequently, the development of receptor binding anti-idiotypic types can be harmful to the host and initiate autoimmunity (128). For example, the antibodies to reovirus type 3HA (HA3) have been studied extensively (129). Williams and co-workers have been able to produce in BALB/c mice a monoclonal antibody (called 9BG5) which could strongly bind to reovirus type 3 and neutralize its infectivity. Moreover, an anti-idiotypic monoclonal antibody was produced which was found to bind to 9BG5 and to inhibit its interaction with HA3. The workers then demonstrated that the anti-idiotypic bore the internal image of the receptor binding epitope on reovirus type 3; this anti-idiotypic could induce changes in cells upon binding to their HA3 receptors similar to those induced by the virus itself (129).
Therefore, the damage caused by certain viruses need not involve their direct effect on target cells but rather an immunological attack by anti-idiotypic antibodies on viral receptors. There are now reports supporting the notion that anti-idiotypic antibodies may also act as the consequence of chronic immune activity.

E. Immune complex formation

Antigens produced by an infectious organism can form immune complexes with antibodies generated against them, thus leading to the development of antibody complexes that recognize and were able to activate the TSHR (131). Furthermore, monoclonal antibodies to the TSHR have been claimed to be produced after TSH immunization (132).

Immune complex formation has also been implicated in the pathogenesis of Graves' disease based on the findings that immunization of animals with TSH has led to the development of anti-idiotypic antibodies that recognized and were able to activate the TSHR (131). Furthermore, monoclonal antibodies to the TSHR have been claimed to be produced after TSH immunization (132).

F. Heat shock proteins and thyroid autoimmunity

Cells in different organisms respond to elevated temperatures by synthesizing new proteins termed heat shock proteins (hsp). Hsp synthesis can be induced not only by heat shock but also by other stressful stimuli, including exposure to oxidative radicals, alcohol or heavy metals, anoxia, and infection (139). Since hsp 60 is highly conserved among different species and are widespread in bacterial cells, it is believed that they carry vital cellular functions during stress and in resting conditions (reviewed in Ref. 140). Therefore, hsp 60 is produced in small quantities under normal conditions. By interacting with other intracellular proteins, altering their folding and unfolding, hsp serve four vital cellular functions: 1) hsp assist in the assembly of polypeptides into their final tertiary structures (141); 2) hsp assist in intracellular transport of other proteins by maintaining them in a conformation suitable for transport into cellular organelles (e.g. mitochondria) (142); 3) hsp can bind and temporarily inactivate other proteins such as the steroid receptor (143); and 4) hsp participate in protein degradation (140).

Hsp have been shown to be strongly immunogenic and, in view of their high degree of conservation between different species and their presence in many infectious agents, they may be involved in the induction of autoimmune diseases (Fig. 3). During the course of a viral infection the antibody and T cell response to the microbe’s hsp is induced. These antibodies and T cells may then cross-react with self-hsps containing conserved epitopes. Moreover, the stress of the infection itself and the inflammation accompanying it may generate increased synthesis of self-hsps, thereby enhancing the autoimmune response (144). Self-hsp production can also be induced by a concomitant viral infection. Increased levels of anti-hsp antibodies have been reported in SLE (145), and in rheumatoid arthritis (146). However, it is likely that self-hsp production is not the primary event triggering autoimmunity, but a secondary response to the tissue damage induced by the autoimmune process itself.

Recent studies have suggested a role for hsp in the pathogenesis of insulin-dependent diabetes mellitus. Cohen and co-workers have demonstrated that a pancreatic β-cell target antigen in NOD mice is a molecule cross-reactive with hsp 65 of Mycobacterium tuberculosis. The authors have shown that the onset of β-cell destruction in the mice was associated with spontaneous development of anti-hsp 65 T lymphocytes and antibodies (147). Only a few studies have examined the possible association of hsp 70 with AITD although this is an active area of investigation. Ratanachaiva-vong et al. (148) reported an association between Graves' disease and a specific restriction fragment length polymorphism (RFLP) of the hsp 70 gene (148). Additionally, hsp 72 was recently demonstrated in thyroid specimens from

<p>| Table 4. Some heat shock protein families and their functions |
|-------------|--------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Family</th>
<th>Major cellular functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsp 90</td>
<td>Maintenance of proteins (e.g. steroid receptor)</td>
</tr>
<tr>
<td></td>
<td>in inactive state</td>
</tr>
<tr>
<td>hsp 70</td>
<td>Assist in protein assembly of multimeric complexes</td>
</tr>
<tr>
<td>hsp 60</td>
<td>Assist in protein transport into organelles</td>
</tr>
<tr>
<td>hsp 27</td>
<td>Unclear</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>Protein degradation</td>
</tr>
</tbody>
</table>

[Reproduced with permission from D. S. Latchman: J R Cell Physic. 25:295–299, 1991 (140).]
be induced to express MHC class II antigens by recombinant (for a review see Ref.154). Rat and human thyroid cells can unusual MHC class II molecule expression in thyroid cells have, therefore, investigated the mechanisms leading to this absent in normal tissues (153). We and many other workers demonstrated in thyroids from patients with AITD which was DR antigens by thyroid epithelial cells has been demon-

...on therapeutic intervention. Indeed, expression of HLA-

...in thyroids from patients with AITD which was DR antigens by thyroid epithelial cells has been demon-

...for the first time, in nonimmune cells (e.g. epithelial cells), and this can lead to presentation of autoantigens and activation of autoreactive T cells. Indeed, expression of HLA-DR antigens by thyroid epithelial cells has been demonstrated in thyroids from patients with AITD which was absent in normal tissues (153). We and many other workers have, therefore, investigated the mechanisms leading to this unusual MHC class II molecule expression in thyroid cells (for a review see Ref.154). Rat and human thyroid cells can be induced to express MHC class II antigens by recombinant γ-interferon, tumor necrosis factor-α, and TSH itself (155). The known accumulation of T cells in the thyroid tissue of patients with AITD may have initially been induced by an infection, and such T cells may subsequently act as a source of cytokines which induce MHC class II antigen expression by the thyroid epithelial cells. Moreover, Kawakami et al. (156) have recently shown that in vivo induction of MHC class II molecules on thyrocytes by interferon-γ can induce autoimmune thyroiditis in susceptible mice.

However, we have also demonstrated that cultured rat thyroid cells (derived from a rat thyroid cell line) infected with reovirus types 1 and 3 were induced to express class II MHC antigens in a dose-dependent manner in the absence of T cells (157). Furthermore, a viral thyroiditis, caused by infecting either the thyroid or the immune cells, has been demonstrated in an avian model as discussed earlier (48). Thyroid follicular epithelial cells bearing MHC class II determinants have been shown to be able to present preprocessed viral peptide antigens to cloned human T cells (158) but, unfortunately, the thyroid cells utilized contained other potential antigen presenting cells. Further evidence came from studies in which thyroid-reactive T cell clones were specifically reactive to cloned autologous thyroid cells in the total absence of antigen presenting cells (159).

Viruses are known to be able to induce class II MHC molecule expression independent of cytokine secretion. Massa et al. (160) have shown that a neurotropic murine hepatitis virus can induce expression of Ia antigen on astrocytes in tissue culture directly and not through release of cytokines. Retroviruses have also been shown to enhance class I MHC expression (161). There are also reports showing reduced expression of MHC molecules by cells infected in vitro with some viruses (162–164). Recently, it has been reported that cytomegalovirus (CMV) infection of primary cultures of thyroid cells resulted in induction of HLA-DR expression on thyroid follicular cells (165) and, as mentioned, we have shown induction of MHC class II antigens by reovirus infection of rat thyroid cells (156). These findings support the view that infection may induce MHC class II molecule expression on thyroid cells for the first time and that these cells may act as antigen presenting cells and may be involved in the induction of AITD. T cell accumulation and cytokine secretion would then perpetuate the disease process.

**Table 5.** Possible mechanisms of induction of AITD by an infecting organism

| 1. Molecular mimicry between the TSH receptor and: Yersinia enterocolitica (88, 71, 74, 120) |
| 2. Superantigenic stimulation of autoreactive lymphocytes to thyroid antigens (125) |
| 3. Production of anti-idiotypic antibodies reactive with the TSHR (130–131) |
| 4. Formation of immune complexes (135–138) |
| 5. Induction of immune response to thyroid hsp (148–151) |
| 6. Induction of class II MHC antigens on thyrocytes (154) |
V. Conclusions

The literature examined in this review points to the possible involvement of infectious agents in the pathogenesis of AITD. Thyroid diseases thought to be of infectious etiology (e.g. subacute thyroiditis, congenital rubella syndrome) have been shown to be associated with thyroid autoimmune phenomena. Vice versa, classical AITD have sometimes been shown to be associated with infectious agents. Various mechanisms have been proposed to explain induction of autoimmunity by infection, but it seems that three possibilities may be important in individuals susceptible to developing AITD (see Table 5 and Fig. 4). Namely, molecular mimicry (perhaps to retroviruses), polyclonal T cell activation (by an endogenous superantigen or on an infecting organism), and MHC class II antigen induction. It seems reasonable that all three mechanisms operate together or separately in different individuals. However, it should be remembered that the association between AITD and infections may also be merely coincidental although the animal models strongly suggest otherwise. It should also be remembered that patients who develop AITD after infection may not present with the same clinical disease as those who fail to develop AITD. Perhaps subacute thyroiditis is the clinical response of a patient not destined to develop chronic thyroid disease. To address this question, and more, studies utilizing direct approaches (e.g. isolation of the infecting organisms from thyroids of patients with AITD and induction of AITD in experimental animals by viruses) are badly needed.

VI. Summary

The etiology of the AITDs remains unclear but it is now generally believed that both genetic and environmental factors contribute to their development. Some recent findings have begun to directly and indirectly implicate the possibility of infectious agents in the pathogenesis of AITD, and these data serve as the basis for this review. Classical AITD (i.e. Graves' disease and Hashimoto's thyroiditis) has been shown to be associated with a variety of infectious agents (e.g. Yersinia enterocolitica, retroviruses) while infections of the thyroid gland (e.g. subacute thyroiditis, congenital rubella) have been shown to be associated with thyroid autoimmune phenomena. However, the causative role of infectious agents in AITD has not been definitively demonstrated in humans although AITD can be induced in experimental animals by certain viral infections. Infectious agents may induce thyroid autoimmunity by a variety of diverse mechanisms, such as inducing modifications of self-antigens, mimicking self-molecules, inducing polyclonal T cell activation (for example by superantigens), altering the idiotypic network, forming immune complexes, and inducing expression of MHC molecules on thyroid epithelial cells. While indirect data suggesting involvement of the infecting organisms in the pathogenesis of human AITD is abundant, only a limited number of studies have employed direct approaches. Such a direct approach would involve isolation or molecular identification of the potentially infecting organisms from the thyroid gland and the subsequent induction of AITD in an experimental model.

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