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IMMUNOPATHOLOGY OF THE THYROID GLAND

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There is considerable confusion and disagreement on the criteria required for a disease to be called "autoimmune." Part of this confusion is because of the ambiguity of speech usage. Some persons seek to apply the term "autoimmune" only to those illnesses in which there is reason to believe that immunological mechanisms play a part in the pathogenesis. Others deem it sufficient if an antibody can be demonstrated in the patients' serum or if a substance resembling an antibody is directed toward the ill organ. This antibody formation therefore does not necessarily have anything to do with pathogenesis, which can also represent a secondary phenomenon.

If it is required that immunity mechanisms have pathogenetic significance, it would be logical to use the four criteria established by Witebsky [44]:

1. It must be possible to demonstrate directly in the patient's serum free, circulating antibodies efficaceous at body temperature, or to demonstrate indirectly cell-connected antibodies.

2. It must be possible to demonstrate and preferably to characterize closely the specific antigen against which the antibody is directed.

3. It must be possible to produce antibodies against the same antigen in animal research.

4. Finally, pathological changes must occur in the corresponding organs in similarly actively immunized animals, and these changes should in their nature resemble those seen in the sickness in humans.

Chronic thyroiditis in humans is one of the few illnesses which comes close to meeting these criteria. Many of the problems which are involved in human, autoimmune thyroiditis are best elucidated if knowledge is first obtained of the autoimmune thyroiditis which can be produced in animals.

I. EXPERIMENTAL AUTOIMMUNE THYROIDITIS

The only thyroid antigen which it has been hitherto possible to show as playing any part in experimental autoimmune thyroiditis is thyroglobulin [29]. A series of basic investigations of thyroglobulin's serological properties was made by Hektoen and his coworkers in the 1920s. They found that rabbits immunized with heterological thyroglobulin produced antibodies which were specific for organs but not entirely specific for species. The antibodies almost always gave the strongest reactions with the thyroglobulin used for immunization. This, however, is probably a cross reaction with thyroglobulin from other mammals. These antigens' relationship among thyroglobulin from various mammals caused Hektoen and his coworkers to look on them as antibodies. They found that by immunizing rabbits with thyroglobulin from dogs, bears, or zebras, they obtained antibodies which also reacted with thyroglobulin or thyroid extract from rabbits [13].

The next important advance did not come until 20 years later. With the aid of Freund's immunization technique, Witebsky and Rose succeeded in 1956 in demonstrating that aqueous saline extracts of thyroid from rabbits are an antigen for rabbits [42]. The extracts were very carefully treated to avoid to the greatest possible extent denaturing of the antigens. They demonstrated these antibodies with complementary fixing, precipitation, and hemagglutination techniques. In their first experiment they used thyroid tissue from other rabbits for immunization. It might be thought that antigens would differ in thyroid extracts from different rabbits. To convince themselves that what they actually observed were auto-antibodies and not iso-antibodies, they performed hemithyroidectomies and injected the rabbits with extracts from their own organs. They also produced antibodies which reacted with both autologous and homologous thyroid ex-extract. Corresponding antibodies were also produced if, instead of an unpurified thyroid extract, they used freshly

prepared rabbit thyroglobulin for the immunization.

These actively immunized rabbits not only produced circulating auto-antibodies, however; considerable significant changes also occurred in their own thyroids [30, 44]. The histological changes consisted of infiltration of lymphocytes, plasma cells and eosinophilic leucocytes, and to some extent also destruction and atrophy of thyroid tissue itself, along with fibrosis. The pattern showed a certain similarity to chronic thyroiditis in humans. Serological techniques showed also that quantities of the thyroidally specific antigen were deposited in their organs.

The autoimmune reaction in the animals of the experiment also manifested itself in a third way, by the development of positive cutaneous reactions after intradermal injection of the homologous thyroid extract. This hypersensitivity reaction was of the delayed type. The circulatory anti-bodies first became demonstrable after several weeks.

Experimentally autoimmune thyroiditis was first produced in rabbits, but later it was reported that changes similar in principle had been produced in dogs, guinea pigs, rats, mice, and goats. A prerequisite for success of the experiment is that adjuvants be used in the immunization. In most experiments, Freund's complete adjuvant is used; the incomplete adjuvant is considerably less successful. Other adjuvants have also been tested, e.g., alum. Immunization with alum-precipitated homologous thyroid extract will also produce circulatory auto-antibodies and a delayed hypersensitivity, but it is only when mycobacteria are added to the alum-precipitated antigen that is is possible to produce histological changes [29]. We do not known exactly what these effects of mycobacteria actually are.

Based on the marked difference in occurrence of thyroiditis in the two sexes in humans, studies have been made on whether sex hormones play any part in the development of experimental thyroiditis [20]. The experiments indicate that testosterone can retard the autoimmune response, but the estrogenic hormone had a more variable effect.

Studies have also been made to determine if the experimental radioactively caused thyroiditis is produced by autoimmune phenomena. The treatment of experimental animals with ^{131}I, even in large doses, was not able to produce auto-antibodies [29, 37].

What part do the circulatory antibodies play in the

development of histological changes? In even some of the first experiments, histological changes were occasionally found, but it was not possible to demonstrate thyroid antibodies [30]. Even in the experimental animals in which there were both antibodies and histological changes, there was no significant correlation between the antibody titer and the degree of thyroiditis [29]. Attempts were also made to transfer large amounts of serum or whole blood from actively immunized animals to normal receivers of the same species. Not a single one of the animals receiving a transfusion suffered histological changes in the thyroid [29]. Sclare and Taylor [33] found that the hemagglutinating thyroid antibody in guinea pigs can pass through the placenta and be found in about the same titer in offspring and mother. They found no indications that this mechanism can lead to serious thyroid damage in the offspring, however. Histological investigations also showed changes, namely an increased vacuolation of the colloid.

In summary it may be said that the circulatory antibodies in themselves are hardly responsible for development of the significant changes in the thyroid.

There is strong reason to suspect the immunity mechanisms on which the delayed hypersensitivity reaction is based. In the experimental animals there is a much better correlation between this reaction and the occurrence and degree of histological changes than there is between the occurrence of circulatory antibodies and thyroiditis. The delayed hypersensitivity reaction and thyroiditis occur almost simultaneously [29].

We know that lymphoid cells play a part in the delayed hypersensitivity reaction. It is also known that it is possible to accomplish a passive transmittal of experimental allergic encephalomyelitis with the aid of similar cells. It was thus natural to attempt a passive transmittance of thyroiditis by transferring lymph-node cells from actively immunized animals to normal recipients. This actually succeeded when inbred guinea pigs were used [9]. On the other hand, it was unsuccessful with rats [28] and with inbred rabbits [29]. When animals from strains which are so highly inbred that the animals are practically monozygotic and therefore exhibit complete tissue similarity are used, the transferred lymph-node cells will be able to survive and propagate in the recipients. They may then continue their functions from the donor's organism. In the cited experiment on guinea pigs, however, only thyroiditis and delayed hypersensitivity were found in the receiving animals, but no circulatory

antibodies.

In this regard it may be of interest to mention that lymph-node cells from actively immunized animals in tissue culture continue to produce thyroid antibodies for several weeks [29].

There are still too many missing pieces in the puzzle for us to obtain a sufficiently complete picture of experimental autoimmune thyroiditis. The picture becomes more complete, however, although also more ramified, when it is viewed in relation to what we know of autoimmune thyroiditis in humans.

II. AUTOIMMUNE THYROIDITIS IN HUMANS

The incidence of circulatory thyroidal auto-antibodies in humans has been studied almost simultaneously by two groups of investigators. One was Roitt and Doniach's group in England [27]. They began with the high gamma globulin values in the serum of patients with Hashimoto's struma lymphomatosa. They thought that this increase in gamma globulin, seen in relation to the histological picture of the illness, might be an expression of an immunological response. Roitt and his coworkers observed that serum from Hashimoto patients gave positive precipitation reactions with human thyroid extracts or with purified human thyroglobulin, but no reaction occurred with extracts from other organs. The antibody was thus specific to the organ.

The other group consisted of Witebsky and his coworkers in the U.S. [44]. Their starting point was experimental autoimmune thyroiditis and the similariity in the histological respect of this state and chronic thyroiditis in humans. Using the "tanned cell" hemagglutination technique, they demonstrated thyroid-specific antibodies in patients suffering from chronic thyroiditis.

From a physical-chemical standpoint, the relatively few thyroid auto-antibodies which have been investigated have an electrophoretic mobility resembling a gamma globulin (γ_{88}). They appear to have a sedimentation constant of 7 S in analytical ultracentrifuging. It is still possible that individual antibody molecules belong to the 18 S globulins [34].

We know that the 7 S group of antibodies globulins is able to pass freely through the placenta. In connection with this, thyroid antibody can also be demonstrated in the serum of newly-born children of mothers with autoimmune thyroiditis.

In some of these children the antibody is found in somewhat lower titer than in the mother [17]. The difference in antibody titer can be explained by the fact that not all the antibody molecules belong to the 7 S group, but some belong to the 18 S group. The latter group does not pass through the placenta. I myself have studied cord blood from the child of a woman with thyroid antibodies. The antibody had the same titer in mother and child. Thirty-five days after birth the antibody was still in the child but the titer had fallen 1 to 2 degrees [22]. It is also known that thyroid antibody can diffuse freely inward in the body liquids of polyarthritis patients [23].

While the antibodies found in experimental autoimmune thyroiditis are directed as far as we know now against only thyroglobulin, it seems that in human illness there are many thyroid-specific antigens which are effective. In addition to antibodies against thyroblobulin, there can also be found antibodies against another antigen in the colloid [1]. This antigen, called CA2, is not further characterized for the time being. Antibodies against an antigen in the thyroid epithelium's cytoplasma can also occur. This antigen appears to belong to the cells' microsome fraction.

The thyroid antibodies can be demonstrated by various serological techniques. Boyden's hemagglutination technique is the most sensitive method for demonstrating antibodies to thyroglobulin. Here we use tannin-treated erythrocytes as carriers of the antigen. This reaction is extremely sensitive, but makes great demand on the accuracy and insight of the tester. We also have other indirect agglutination techniques available. For example, bentonite or latex particles can be used as carriers of thyroglobulin, although the latex reaction is somewhat less sensitive than the hemagglutination method [43].

Precipitation techniques can also be used to demonstrate thyroglobulin antibody. The precipitation can be conducted either in the "classic" manner in a test tube, or after diffusion in a gel, for example, by the Oudin or Ouchterlony method, or by immunoelectrophoresis. Generally speaking, precipitation techniques are somewhat less sensitive than agglutination techniques, and this is also the case in regard to thyroglobulin antibodies. By and large, the higher the titers of these antibodies found in hemagglutination techniques the greater is the chance that they will give positive precipitation reactions. There are many exceptions to this rule, however. I have seen a number of instances where antibodies with moderate hemagglutination titer around 1000 gave positive

precipitation reaction, but antibodies with agglutination titer of 1:20,000 gave no precipitate in the gel diffusion technique [24].

The complement-fixing technique can be used to demonstrate antibodies against the intracellular thyroid-specific antigen [2], while human thyroglobulin antibodies do not react. For the complement-fixing reaction it is necessary to use extracts of thyroid tissue from patients with thyro-toxicosis as antigen. Extracts of normal tissue show some activity but not enough to be of practical use.

When the "antiglobulin consumption" technique is used, antibodies against a cell-connected thyroid-specific antigen are found in thyroiditis sera [21]. We do not yet know to what extent this antigen is identical with the antigen which reacts in the complement-fixing reaction.

The immunofluorescence technique can be used to demonstrate antibodies against all the thyroid-specific antigens mentioned. Antibodies against C2A2 at present can be demonstrated only by this technique [1]. The advantage of the fluorescence method is primarily that we can observe directly in the microscope the localization of the antigen-antibody reaction. Both thyroglobulin and C2A antibodies impart fluorescence to the colloid, but probably form differently. Antibodies against the intracellular antigen impart fluorescence to the thyroid epithelium's cytoplasma.

It is of some interest that Balfour and his coworkers [1], using a fluorescing antiglobulin reagent, have achieved a positive "direct antiglobulin reaction" in the colloid in sections of thyroid tissue from patients operated on for thyroid disorders. This seems to indicate that a fixing of gamma globulin, probably antibodies against the colloid, has occurred in vivo.

It is of great theoretical interest, also, that in sera from Hashimoto patients the tissue culture technique demonstrated a factor which acts cytotoxically on the thyroid epithelium. This factor seems to be a gamma globulin, and there are strong reasons to believe that the cytotoxic and the complement-fixing antibodies represent the same immunization system [25].

Thyroid-specific antibodies can also be demonstrated in passive cutaneous anaphylaxis in guinea pigs.

The immunization response in patients with autoimmune thyroiditis can also be demonstrated by other than a

serological technique. Buchanan and his coworkers [6] found that thyroid extracts gave positive cutaneous reactions in many of these patients. In most cases the reaction had the character of Arthus' phenomenon, but in one of the patients it was of the delayed type.

In the years which have elapsed since thyroid antibodies were first discovered in patients with chronic thyroiditis, it has been found that some antibodies can occur in other thyroid illnesses.

The frequency of antibody occurrence in the various illnesses varies from laboratory to laboratory. There are many reasons for this. First of all, the frequency naturally depends on what technique was used and whether one or several methods were employed. Even if the same technique in principle is used, modifications in the conduct of the test which are seemingly trifling can cause significant deviation. In addition to this methodological respect, the criteria used in clinical diagnosis can vary. The classification of thyroiditis types thus is difficult. For example, the classification of chronic thyroiditis used by Roitt and Doniach's group differs from that used by Witebsky's group.

Struma lymphomatosa Hashimoto is the illness in which circulatory thyroid antibodies are most frequently found, and in which the titers average highest. Roitt and Doniach [26] find antibodies in fully 98% of these patients, but most authors give lower frequencies [24, 43]. The titer decreases gradually under heavy doses of cortisone. If cortisone is stopped the titers again increase [4]. After an operation the titer usually decreases.

A few patients with Riedel's struma have also been studied. Here, too, most of the patients appeared to have antibodies in their serum [22, 43].

In acute and subacute thyroiditis, thyroiditis antibodies are rarer and are usually of moderate to low titer [22, 26, 43].

The thyroid illness which from an immunological viewpoint evinces the greatest similarity to Hashimoto's disease is idiopathic myxedema. The antibody frequency is somewhat lower, to be sure, and the titer values are somewhat lower on average. Most authors indicate that antibodies were found in about [illegible] of the patients [24, 26, 43]. Because of similarities in the immunological relationship, many believe that myxedema is a result of a diffuse thyroiditis. More recent

Table 1. Occurrence of Hemagglutinating Thyroid Antibodies in Oslo Blood Donors Aged 18-65 Years

Kjønn og aldersgruppe (1)		Antall undersøkte (2)		Positive i alt (3) Antall (4)		Prosent (5)		Titer > 1.000	Titer > 10.000
♀	50 – 65 år (6)	121		15		12,4			
	34 – 49 år	57	269	6	26	10,5	9,7	14 (5,2%)	2 (0,7%)
	18 – 33 år	91		5		5,5			
♂	50 – 65 år	121		4		3,3			
	34 – 49 år	74	297	4	10	5,4	3,4	1 (0,3%)	1 (0,3%)
	18 – 33 år	102		2		2,0			

1) Sex and age group
2) Number studied
3) Positive total
4) Number
5) Percent
6) Years

pathological and anatomical investigations support this theory [32]. Antibody determination with incipient and light cases of myxedema probably has the greatest clinical diagnostic importance. The antibody diagnosis is not hampered if the patient has used iodine-containing preparations. Negative antibody reactions are found especially in old myxedema cases. The destruction of the organ can have progressed so far that the antigen's stimulus has fallen off.

In some patients with thyrotoxicosis, thyroid antibodies are found, in perhaps about half the cases. The titers are generally low, however. An operation shows focal lymphoid infiltration in most of the patients [26]. Blagg [3] studied the frequency of thyroid antibodies in thyrotoxicosis patients who had been treated with radioactive iodine. He found the [illegible] antibody frequency [illegible] who afterward became eu- [illegible] as in untreated [illegible], but in those who became hypothyroidal, antibodies were found with significantly [illegible] frequency. The antibody frequency in postactinic and

postoperative hypothyroidism is on almost the same level as in primary hypothyrosis.

A hyperthyroidal phase can occasionally occur during an autoimmune thyroiditis [5]. To differentiate this condition from a regular thyrotoxicosis, and also to select the treatment, antibody diagnosis obviously is of great value. If antibodies are present, then invariably there is increased probability that a secondary hyperthyreosis will occur after operative or radiological treatment.

The differential diagnosis between cancer of the thyroid and a chronic thyroiditis with secondary fibrosis can often be very difficult. The question is whether antibody diagnosis can be of aid in this connection. Thyroid antibodies can sometimes be demonstrated in cancer, too. This should not be surprising. It is known that in histological investigation of cancer of the thyroid, zones of lymphoid infiltrations can also be found, but antibody titers in cancer are usually low. If a high-titer antibody is present, this indicates a more likely diagnosis of thyroiditis. Unfortunately this cannot be relied upon absolutely. I myself have found very high titer antibodies in a patient with lymphogranulomatosis in the thyroid [22].

In non-toxic struma some investigators have found positive antibody reactions to be no more frequent than in normal material [16, 43]. Other investigators found antibodies with somewhat higher frequency [26].

A naturally occurring question is how often thyroid antibodies are found in presumptively healthy humans. Blood specimens from blood donors in Oslo were used to throw light on this question [22, 23]. These blood donors are subjected to a medical screening upon registration and persons with obvious endocrine troubles are refused. The occurrence of thyroid antibodies in this material is studied with the "tanned cell" hemagglutination technique. In this method, titer values below 1000 are called low or moderate. As Table 1 shows, antibodies were found with significantly greater frequency in women than in men. The difference is especially pronounced for titer values above 1000. It also seems that in women the antibody frequency increases with age. The numbers in the separate age groups are relatively small, however, and the difference is not statistically significant. Among the men there is no clearcut difference among the three age groups.

This relatively high frequency of thyroid antibodies

in presumptively healthy humans will perhaps astonish many clinicians, but the findings are really not so surprising. For several decades pathologists have known from autopsy materials that focal thyroiditis in macroscopically normal organs is not a rare phenomenon. This has been corroborated in more recent material. Williams and Doniach [41] found in their test subjects focal thyroiditis with over 10 foci per cm^2 in 22% of the adult females and 6% of the adult males. The ratio of the sexes here is almost the same as that for blood donors with antibodies. It is also worth noting that Williams and Doniach found no instances of focal thyroiditis among the 112 persons in their general series who were below 20 years of age. A study made by Goudie and his co-workers [10] is also highly interesting. They studied thyroid tissue in autopsies of individuals who had participated in research on complement-fixing thyroid antibodies while they were still alive. They found focal thyroiditis in all the 8 patients with antibodies, and only 9 of the 61 patients had a negative reaction.

The clinically diagnosable autoimmune thyroiditis is also far more frequent in women than in men.

In my opinion, these serological, pathological-anatomic, and clinical data all together indicate that the blood donors who have thyroid antibody in their serum actually have an autoimmune thyroiditis, but it is so mild that it cannot be de monstrated clinically. It is only when the thyroiditis attains a fairly serious degree that it is usually possible to diagnose it, either by abnormal palpation or by pathological thyroid functioning.

There is especial interest in the study of the question of whether there is an increased incidence of thyroid antibodies in <u>other diseases</u> where autoimmune mechanisms are thought to play a part. Demonstration of a reaction of this type would be important to an understanding of the mechanism of autoimmune thyroiditis. As stated, there is a marked difference in occurrence of thyroid antibodies between the sexes in presumptively healthy humans. It is therefore necessary to evaluate male and female patients separately if the incidence of thyroid antibodies in other illnesses is to be analyzed. In the case of women, it is perhaps desirable to subdivide them into age groups, too. In many of the published investigations this type of subdivision has not been done.

In chronic rheumatic polyarthritis, thyroid antibodies are found about twice as frequently as in normal subjects. This holds for both men and women [29]. As was the case with

the blood donors, antibodies were found here, too, more frequently among the older than among the younger women.

A striking contrast to the observations on polyarthritis patients is found in patients with Bechterew's disease [23]. Not one of the patients studied could be demonstrated to have thyroid antibody. This observation is no less interesting when it is viewed in relation to the difference in incidence of rheumatoid factors in the two diseases.

Autoimmune thyroiditis also seems to occur relatively frequently in other collagenous diseases besides polyarthritis, including lupus erythematosus disseminatus [14, 40], Sjøgren's syndrome [7], and polymyalgia rheumatica [23].

There are further indications that an immunological relationship exists between pernicious anemia and autoimmune thyroiditis [18]. The serological data are based on pathological-anatomical investigations [10, 41]. It is claimed that there is a relationship between autoimmune thyroiditis and cirrhosis of the liver in women [10, 41]. Skanse and Nilsson [35] studied sera from patients with hyper-gamma globulinemia and found increased incidence of both hemagglutinating and complement-fixing thyroid antibodies.

Wasastjerna [39] has described a patient with the combination of autoimmune hemolytic anemia and Hashimoto's disease. He intimates that a defective etiological factor can be responsible for the two diseases. On the basis of this observation, a direct Coomb's reaction, using a broad-spectrum antiglobulin reagent, was carried out on a total of 295 blood specimens containing thyroid antibodies [22]. Only one of the specimens gave a definitely positive reaction. This specimen was from a patient who gave no clinical suspicion of hemolytic anemia. These observations are evidence against a close immunological relationship between autoimmune hemolytic anemia and thyroiditis.

What then is the <u>mechanism</u> for the production of thyroid auto-antibodies in humans? With the autoimmune diseases it is frequently customary to differentiate between the body's own antigens which are normally isolated from the organism's immunological apparatus, and the antigens which come into close contact with it. The colloid in the thyroid is isolated in the acini, and it has been supposed that it therefore does not contact the immunological apparatus during the period when immunological tolerance is developed. If, later in life and for some reason or other, there is a seepage of colloid out into the surrounding area, the organism will consider that to be equivalent to an antigen foreign to the body, and

there will be immune response. This would lead to an immunological reaction in the thyroid, with severe damage to the acini and emission of more colloid. In this way, we would obtain a type of chain reaction. The hypothesis is based on the relation observed between damage of the basement membrane and high antibody titer in thyroid disease [36]. What primarily causes damage to the basement membrane and emission of thyroid-specific antigen is not known, however. Certain observations which Hjort [11] has published tend to contradict the hypothesis. In 24 out of 29 patients operated on for thyroid disease, thyroglobulin could be demonstrated post-operatively in their serum, but a month later thyroid antibody was found in only one of these patients. This finding hardly precludes the correctness of the theory given, but it does indicate that factors other than colloid emission are important. Experiments with animals have shown how difficult it is to produce auto-antibody after intravenous injection, and the antigen dosage and variations in the antigen's action probably play a part, too. Hjort and Pedersen [17] also found thyroglobulin in the serum of most newborn infants, who theoretically should be immune tolerant. They intimate that autoimmune thyroiditis should be considered a "disturbed tolerance disease." This concept is supported by the frequent occurrence of thyroiditis with other autoimmune diseases. In connection with Hjort and Pedersen's observation, however, it may be mentioned that up to now it has not been possible to produce immunological tolerance to thyroglobulin in animal research [29].

At present, it appears that genetic factors may play the largest part in development of autoimmune thyroiditis. The occurrence of Hashimoto's disease in several members of the same family was first described by Dunning in 1959 [8]. Later, Irvine and his coworkers [19] found the disease in two pairs of uniovular twins. Hall and his coworkers [11, 12], and Volpé and his coworkers [38] made serological studies of families of patients with autoimmune thyroiditis. Their results showed good agreement. If all their findings are collected, it is seen that among the 65 sisters of the probands, thyroid antibodies occurred in 37 of them, i.e., slightly more than half. Of the 28 brothers, 10, or fully one third, had antibodies. Of the total of 53 parents studied, 27 had antibodies. These data are in agreement with inheritance of genetic disposition to autoimmune thyroiditis as a Mendelian dominant trait. The lower frequency of thyroid antibodies in men than in women can perhaps be explained as incomplete penetrance of the trait in males. More data on families are required for a more accurate evaluation of the hereditary process, however.

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