Correlation of the HLA-A1,B8 haplotypes with circulating autoantibodies in a family with increased incidence of autoimmune disease

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Abstract. Two cases of Addison’s disease, two cases of scleroderma, three cases of primary hypothyroidism possibly due to Hashimoto’s thyroiditis, three cases of diabetes mellitus, and two cases of ovarian failure and secondary amenorrhoea were diagnosed in a single family. In 44 members of four generations of the family including all the deceased, we have performed HLA typing and measurement of circulating autoantibodies. All diseased patients were older than 12 years, all possessed HLA-B8 antigen, and all but two showed specific autoantibodies in their serum. In contrast, none of the family members without HLA-B8 developed any of the autoimmune diseases in spite of the fact that in 13 of them some circulating autoantibodies were demonstrable in the serum. It is concluded that genetic factors play an essential role in the development of autoimmune in the studied family. The individuals acquire circulating autoantibodies as they develop the disease. Environmental factors play a secondary role as evident from the age dependence. HLA typing can become an important diagnostic tool in identifying the individuals at a risk of autoimmune disease. Detection of circulating serum autoantibodies alone correlated poorly with the autoimmune disease.

Increased coincidence of idiopathic hypothyroidism and Addison’s disease became known as the Schmidt’s syndrome (Schmidt 1926). However, a number of endocrine and other diseases presumably due to autoimmune pathogenic mechanisms have been shown to occur with increased frequency in the same individual or in members of the same family. These include diseases such as: chronic lymphocytic (Hashimoto’s) thyroiditis, Graves’ disease, Addison’s disease, hypocalcaemia of hypoparathyroidism, hypopituitarism, primary gonadal failure, autoimmune oophoritis and orchitis, insulin dependent diabetes mellitus, myasthenia gravis, idiopathic thrombocytopenic purpura (ITP), pernicious anaemia, vitiligo, alopecia, lipodystrophy, Sjögren’s syndrome, systemic lupus erythematosus, scleroderma, candidiasis, retinitis pigmentosa, congenital nerve deafness, IgA deficiency with malabsorption and steatorrhoea, dermatitis herpetiformis, active chronic hepatitis, and graft-vs-host disease (Frey et al. 1973; Irvine 1975; Volpe 1977; Segal & Weintraub 1976; Edwards et al. 1976; Wilson et al. 1978; Van Thiel et al. 1977; Lawley et al. 1977; Nye & Evans 1977). The incidence of malignancy also seems to be increased in the affected families. Recently it has been recognized that some of the above listed diseases occur in individuals who possess specific HLA antigens. Thus, in insulin dependent diabetes mellitus, there is an increased frequency of HLA-B8, W15, DR3, and DR4 (Svejgaard et al. 1975; Nerup 1978). Graves’ disease is associated with B8 in caucasians.

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and B35 in orientals (Volpe 1978; Farid et al. 1977). Association of Hashimoto’s thyroiditis with B8 and DR3 was also increased (Molus & Farid 1978). The incidence of B8 was shown to be increased also in patients with ITP (Goebel et al. 1977). Addison’s disease is quite consistently associated with HLA B8 (Irvine 1980). The individuals with IgA deficiency with malabsorption, thyroiditis and diabetes mellitus were shown to possess the HLA antigens A2, B8, and DR3 (Van Thiel et al. 1977). Patients with systemic lupus erythematosus and Sjögren’s syndrome seem to have increased frequency of B8, W15, A1, and DR3 antigens (Hochberg 1977; McDevitt & Bodner 1974). Thus, determination of the critical HLA group can serve as a genetic marker which identifies individuals at a high risk of developing the autoimmune disease.

We have studied 44 members of one family where there was an increased incidence of Hashimoto’s thyroiditis and hypothyroidism, Addison’s disease, scleroderma, and diabetes. The results of the study suggest that the individuals at the highest risk of developing an autoimmune disease are those possessing HLA B8 antigen, plus circulating autoantibodies in the serum.

Materials and Methods

Clinical data. Forty-four members of the family in four generations have been examined. All individuals were caucasiats. The pedigree is illustrated in Fig. 1. In some of the individuals, the HLA was inferred from the types found in their offspring. These presumed HLA types are in brackets. All individuals were examined clinically and by laboratory studies which included complete blood count, multi-channel serum biochemical profile, fasting and 1- and 2-h postprandial serum glucose, a.m. serum cortisol, T₄, thyroid stimulating hormone (TSH), and T₃ resin uptake. In patients with hypogonadism, plasma oestradiol, luteinizing hormone (LH), and follicle stimulating hormone (FSH) were also measured. All hormonal assays were performed in the same reputable reference laboratory using specific radioimmunoassays.

Circulating autoantibodies. Antithyroglobulin and antimicrosomal antibodies were measured by tanned red cell technique (Wellcome Thymne Kits). Immunofluorescence with undiluted serum was used in the detection of human pancreatic islet cell antibodies, and adrenal antibodies. Antinuclear antibodies, gastric parietal cell antibodies, smooth muscle, and mitochondrial antibodies were measured by immunofluorescence with sera diluted to 1:10. All positive reactions were titered to end point.

HLA typing. HLA typing was performed by the microdroplet lymphocyte cytotoxicity assay as described elsewhere (Terasaki & Park 1980). Antisera detecting HLA-A and B antigens were used. One hundred and twenty-two sera recognizing at least 19 antigens from the A series and 19 from the B series, from the NIH Serum Bank and the UCLA Tissue Typing Laboratory were utilized for characterization. Forty-four members of the family in four generations were typed.

Results

Clinical findings. Two members of the family suffered from Addison’s disease. The first patient, III-19 in the pedigree in Fig. 1, was 18 years old. Hypocortisolism was diagnosed in his case at the age of 14 years when the patient became weak, hypotensive, hyperpigmented, anorectic, and his a.m. serum cortisol was measured to be below 2μg/100 ml. The second patient, III-2, age 31 years, carried the diagnosis of hypocortisolism since age 28 years when he was admitted to the hospital in adrenocortical crisis. Both patients are currently on replacement therapy with prednisone. The second patient was the proband in the present study.

Diagnosis of primary hypothyroidism was made in three members of the family on the basis of low serum T₄, T₃ resin uptake, and elevated TSH. All 3 patients also demonstrated slightly enlarged thyroid gland which was of a firm consistency. The diagnosis of hypothyroidism was established in one patient who also suffered from Addison’s disease (III-2), and the diagnosis of hypothyroidism preceded the diagnosis of Addison’s disease by one year. The second patient (II-2 in Fig. 1) was treated for hypothyroidism several years prior to the diagnosis of scleroderma. The third patient (III-4) also suffered from amenorrhoea with elevated serum gonadotrophins, which did not resolve after the patient was made euthyroid by adequate thyroid replacement therapy.

Three members of the family suffered from diabetes mellitus with fasting hyperglycaemia, and one developed glucose intolerance. In II-5 the diagnosis of insulin dependent diabetes was made shortly after the diagnosis of scleroderma, at age 37 years. Two other patients had insulin dependent diabetes mellitus with severe complications, with the onset of the disease at age 20 years (II-9) and 25 years (II-12). Patient II-9 was deceased at age
Pedigree of the studied family. The generations are labelled by roman, the individuals by arabic numbers. As indicated, circles are for females, squares for males; diseased individuals have the left half of their symbol blackened; hatched right half of the symbol indicates the presence of circulating autoantibodies. The diamond stands for a number of individuals not included in this study and for number of stillbirths or early neonatal death when associated with a black dot. The HLA type is to the lower right of the symbol of the corresponding individual. The interrupted line suggests possible linkage. HLA haplotypes of some individuals were inferred from the haplotypes of their offspring, and they are in brackets.

40 years from myocardial infarction after he developed severe peripheral neuritis and renal failure. Diabetes of the second patient (II-12) also resulted in renal failure, treated by haemodialysis at the time of the study. She was also previously treated for cerebrovascular thrombosis.

Secondary amenorrhoea occurred in 2 females (II-11 and III-4 in the pedigree in Fig. 1) at the ages of 27 and 24 years, respectively. In both cases low plasma oestradiol levels were associated with elevated serum LH and FSH, which was compatible with premature ovarian failure.
Two female patients suffered from scleroderma, and in both of them clinical diagnosis was made at age 37 years and was confirmed by biopsy. One of the patients (II-5) had prominent skin disease and the scleroderma was associated with diabetes and hypothyroidism. In the second patient (II-2) the skin was clinically normal but she had prominent visceral symptoms such as dysphagia and decreased intestinal motility. There were no other associated conditions in this case.

It is notable that 9 members of three adult generations of the studied family suffered from malignancy which involved stomach or bowel carcinoma in 5, cancer of the pancreas and thyroid in one each, and cancer of the bladder and lung in the remaining 2. It is also noteworthy that in the last three generations of the family there was a total of 11 stillbirths and 4 deaths in the neonates. In the I generation, there were 12 additional uncompleted pregnancies (not illustrated).

**Correlation of HLA antigens and the disease entities.** All of the above mentioned diseases occurred in members of the family who possessed HLA-A1,B8 haplotypes. There were 17 individuals with these particular HLA haplotypes out of the total of 44 in whom the typing was performed. In 5 more individuals, the A1,B8 haplotypes were inferred. There were three different sources of A1,B8 haplotypes related to the disease states. Six diseased individuals were the descendants of I-8, and their A1,B8 haplotypes were inherited from the husband of I-8. The A1,B8 haplotypes of III-2 were derived from the husband of II-4. The patient II-2, suffering from scleroderma, possessed the third type of A1,B8 haplotype.

The diagnosis of the critical autoimmune disease was made in most of the family members in the third decade of their lives and only occasionally in the second decade. In both patients with scleroderma, the diagnosis was made in fourth decade, at age 37 years. All patients with the A1,B8 haplotypes who were free of disease at the time of examination were 12 years old or younger (Table 1). None of the other members of the family who did not possess the HLA-A1,B8 became ill from any autoimmune disease.

**Table 1.**

Members of the family possessing HLA B8 antigen: correlation with the disease state and circulating autoantibodies.

<table>
<thead>
<tr>
<th>Patient*</th>
<th>Age</th>
<th>Sex</th>
<th>Disease state</th>
<th>Age at time of DX</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thyroid</td>
</tr>
<tr>
<td>II-2</td>
<td>57</td>
<td>F</td>
<td>Scleroderma</td>
<td>37</td>
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<tr>
<td>II-5</td>
<td>50</td>
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<td>Scleroderma, IDDM, hypothyroidism</td>
<td>37</td>
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</tr>
<tr>
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<td>40</td>
<td>M</td>
<td>IDDM</td>
<td>20</td>
<td>-</td>
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<tr>
<td>II-11</td>
<td>36</td>
<td>F</td>
<td>Hypogonadism</td>
<td>27</td>
<td>-</td>
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<tr>
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<td>F</td>
<td>IDDM</td>
<td>25</td>
<td>+</td>
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<tr>
<td>III-2</td>
<td>31</td>
<td>M</td>
<td>Addison's disease, hypothyroidism</td>
<td>28</td>
<td>+</td>
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<tr>
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<td>F</td>
<td>Hypothyroidism, hypogonadism</td>
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<td>+</td>
</tr>
<tr>
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<td>21</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III-19</td>
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<td>M</td>
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<td>14</td>
<td>-</td>
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<tr>
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<td>M</td>
<td>-</td>
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<td>M</td>
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</table>

* Refers to the pedigree in Fig. 1. IDDM: Insulin dependent diabetes mellitus. M: male. F: female.
Correlation of autoimmune disease, the presence of circulating autoantibodies, and HLA haplotypes. Twenty-one patients out of 44 had demonstrable circulating antibodies in their serum. The types of antibodies and the titers are apparent from Table 2. Strikingly, all individuals possessing HLA-A1,B8 haplotypes and positive autoantibodies in the serum developed some autoimmune disease. In two patients only (II-9 and II-11), one with diabetes, one with ovarian failure, was the testing for circulating autoantibodies in the serum negative. Only 2 patients without clinically obvious disease, but possessing HLA-A1,B8, had positive autoantibodies which were directed against the thyroid in III-24 and against the nuclear antigen in III-22. Thirteen members of the family who did not possess the A1,B8 haplotypes were found to have some circulating autoantibodies in the serum. In contrast to the A1,B8 positive group of patients, none of those individuals developed an autoimmune disease.

Discussion

In 44 members of four generations of one family we have observed two cases of Addison’s disease, two cases of scleroderma, three cases of hypothyroidism due most probably to Hashimoto’s thyroiditis, three cases of insulin dependent diabetes mellitus, and two cases of hypergonadotrophic hypogonadism. The prevalence of Addison’s disease in young males (age group 0 to 24 years) is less than 0.5 per 100,000 (Mason et al. 1968). Although its true incidence is unknown, scleroderma is even less frequently encountered than...
Addison's disease. Familial scleroderma is extremely rare, and has been a subject of few case reports (Barnett 1974). Thus the frequency of at least those two diseases in our family is certainly not fortuitous. Natural history of the studied family seemed to be: increased intrauterine and perinatal mortality, a period relatively free of disease during the first two decades of life, increased incidence of autoimmune and collagen disease in the third and fourth decades, and increased risk of malignancy in the aging groups. It is likely that immune mechanisms are involved in all of these conditions.

All studied family members affected by autoimmune diseases possessed HLA haplotypes A1,B8. B8 has been shown previously to be associated with autoimmune and collagen disease as described in the Introduction. The co-existence of A1 antigen can be a result of the linkage disequilibrium. However, it can also have special meaning in this family showing high frequency of malignancy besides the autoimmune disease. Association of both autoimmunity (Irvine 1980) and malignant disease (Svejgaard et al. 1975) with A1 antigen has also been described previously. To our knowledge, positive correlation of HLA-A1,B8 with scleroderma or with hypergonadotrophic hypogonadism has not been previously reported. The significance of such correlation has to be confirmed in a larger series.

The HLA-A1,B8 haplotypes in the studied family were of five different origins: from the husbands of I-8, II-4, II-9 and III-5, and from one of the parents of II-2. However, II-9 possessed her own A1,B8 haplotypes which were also transferred to two of her children. The fact that A1,B8 haplotypes in the diseased individuals were of different origins further underlines the importance of genetic predisposition that is not tied to a single proband. This can be responsible for inconsistent patterns of inheritance in a number of human disease states.

The presence of circulating autoantibodies in the serum is one of the hallmarks of autoimmune disease. In our study, circulating autoantibodies were demonstrated altogether in 21 of 44 tested individuals, or in almost 50%. However, their presence was poorly correlated with the incidence of overt disease and its severity, and this seems to be a general experience. What seemed to be a critical factor for development of an autoimmune disease, though, was the combination of HLA-A1,B8 haplotypes and the presence in serum of autoantibodies. Previously, Thomsen et al. (1975) pointed out the coincidence of HLA-B8, adrenal antibodies and clinical manifestations of Addison's disease. Our observations suggest the importance of the combination of HLA-B8 and the circulating autoantibodies for other autoimmune diseases as well.

Of the 17 directly typed HLA-B8 positive individuals, 8 developed an autoimmune disease and, even more convincingly, all but one member of the family with HLA-B8, who were 18 years old or older, became clinically ill. Only 2 of the 8 diseased patients did not demonstrate any circulating autoantibodies while the 6 remaining patients did. Of the 2 negative patients, one suffered from hypogonadism where the critical antiovary antibodies were not tested, and the other one from insulin dependent diabetes mellitus. In contrast, in the 10 HLA-B8 positive members of the family free of disease, the presence of circulating autoantibodies could be demonstrated in 2 only.

In a striking contrast to the presence of a disease in individuals combining HLA-B8 and positive circulating autoantibodies, none of the individuals with circulating autoantibodies but lacking the HLA-B8 antigen developed a disease.

In addition to HLA-B8 and the presence of circulating autoantibodies, age seemed to be another critical factor for the development of autoimmune disease. The youngest patient who ever developed the disease was 12 years old, in most of them the disease became manifested in the second or third decade, and the scleroderma in the 2 patients was diagnosed at age 37 years. Such course suggests the importance of environmental factors in addition to the genetic determinants. These factors have not been identified in the present study; they can include both hormonal and immunologic influences.

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References


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