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HLA Antigen Expression in Autoimmune Endocrinopathies

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Summary

The HLA allelic frequency was determined in three groups of autoimmune endocrinopathies: A) 30 patients with autoimmune thyroiditis, B) 20 patients with polyglandular activation of autoimmunity, and C) 10 patients with the autoimmune polyglandular syndrome type II. The groups were defined by the clinical state and serological parameters. Healthy blood donors of Caucasian population from the US database of HLA frequencies served as the controls. In group A, a higher occurrence of HLA-A24 (21.7 %) was found as compared to group B (5.0 %) and to the controls (8.5 %), of HLA-B27 (15.0 %) and of HLA-DR-11 (20 %) as compared to the controls (4.2 % and 8.5 %). In group B, a higher occurrence of HLA-A3 (25.0 %) was found as compared to group A (10 %) and to the controls (11.8 %), and of HLA-B8 (22.5 %) as compared to group A (8.3 %) and to the controls (8.6 %). In this group the occurrence of HLA-DR3 (30.0 %) was higher as compared to group A (10.0 %) and to the controls (9.8 %) and of HLA-B8 (30.0 %) as compared to group A (8.3 %) and to the controls (8.6 %). Genetic markers indicate a similarity of groups B and C. Patients in these groups could be at different stages of the same disease, however, some distinctions between them lead us to consider the possibility whether different epigenetic factors could extend the difference between these groups in the course of clinical development.

Key words

HLA • Autoimmune thyroiditis • Autoimmune polyglandular syndrome type II • Polyglandular activation of autoimmunity

Introduction

Autoimmune endocrinopathies belong to organ specific autoimmune diseases with an incidence of about 7 % and as for other autoimmune diseases, they are more frequently encountered in women probably due to the assumed hormonal influences (Šterzl and Zamrazil 1999). Autoimmune endocrinopathies can appear not only in their isolated form, but may also be ascribed to groups of endocrinopathies belonging to the so-called autoimmune polyglandular syndrome (APS). APS is classified into three types: APS type I, APS type II and APS type III (Muir *et al.* 1994). A relatively frequent finding in autoimmune endocrinopathies such as the Hashimoto thyroiditis (AT) is the occurrence of autoantibodies against other endocrine organs without signs of functional impairment leading to clinical manifestations (Laureti *et al.* 1998b). This group of autoimmune endocrinopathies has been designated by our group as "polyglandular activation of autoimunity" (PAA). In patients with

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autoimmune thyroid diseases we have encountered most frequently the simultaneous occurrence of antibodies against steroid-producing cells in ovaries combined with antibodies against layers of the adrenal cortex (Šterzl *et al.* 1996).

The onset of autoimmune endocrinopathies, as well as of other autoimmune diseases, is multifactorial in character and the factors that participate in the onset of autoimmune endocrinopathies include genetic predisposition, external etiological factors and disorders of the regulation in the microenvironment of target organs. As a matter of fact, the genetic predisposition is of great importance. Genetic susceptibility to the development of an autoimmune disease is a complex situation involving many different genes (polygenetic nature) and their products interacting with each other and external stimuli (Heward and Gough 1997). The only exception is the APS type I caused by the mutation in the AIRE gene, the first demonstration of a monogenetic autoimmune disease (Peterson *et al.* 1998). The most important genetic factor seems to be the polymorphism of the major histocompatibility complex, MHC (HLA in man) (Wucherpfennig and Strominger 1995). Several endocrine autoimmune components of APS II (such as Addison´s disease, thyroid diseases, type I diabetes mellitus or premature ovarian failure) share a common genetic background (Badenhoop *et al.* 1995, Huang *et al.* 1996) with one critical region in the HLA chromosomal locus (Peterson *et al.* 2000)

Up to now, it is not completely clear whether the different clinical manifestations of autoimmune endocrinopathies depend primarily on external factors and on the level of the disorder in regulatory mechanisms (e.g. TH1/TH2) (Hrdá *et al.* 2003), or on the differences in genetic predisposition, e.g. association with different antigens of the HLA locus. The question is, whether there is also a difference on the genetic level between the three groups under study, differentiated by serological, clinical and functional parameters.

Three groups of autoimmune endocrinopathies, namely autoimmune thyroiditis, APS type II and PAA, were compared in our study in the field of antigen expression HLA-A, HLA-B and HLA-DR.

Methods

Patients

Participants of this study were selected from patients of the Institute of Endocrinology in Prague. Based on the detection of organ-specific autoantibodies and the clinical history three groups of patients were chosen:

Group A – 30 patients with autoimmune thyroiditis
Group B – 20 patients with PAA
Group C – 10 patients with APS type II (Addison's disease with autoimmune thyroiditis and/or IDDM)

The mean age of patients in group A was 45.6 years, in group B 43.9 years and in group C 42.4 years. Group A consisted of 27 women and 3 men, group B of 19 women and 1 man, and group C of 8 women and 2 men.

The diagnosis of autoimmune thyroiditis was based on ultrasound findings and positivity of antibodies against thyroid peroxidase (TPO) and/or thyroglobulin (Tg). Patients were monitored at the Institute of Endocrinology for more than 10 years.

The PAA group comprised patients with autoimmune thyroiditis and serological finding of positivity for other organ-specific autoantibodies (Table 1). Patients with APS type II were selected on the basis of anamnestic data about adrenocortical insufficiency, positivity of autoantibodies against 21 hydroxylase (21-OH), TPO and/or thyroglobulin and/or positivity of antibodies against glutamic acid decarboxylase (GAD). During the study all patients were on corticosteroid replacement therapy.

Healthy blood donors of Caucasian origin from the US database of HLA frequencies served as the controls.

Organ specific autoantibodies

Autoantibodies against TPO and Tg were detected in sera by the ELISA method (kit Autostat II, Cogent Diagnostics Ltd., UK).

Autoantibodies against antigens of the adrenals, ovaries and islet cells were determined by the method of indirect immunofluorescence on monkey tissues (Binding Site, UK). The sections were incubated with patient sera for 30 min and, after thorough washing, they were incubated with fluorescein-conjugated antibody against human immunoglobulin (Hu IgG/FITC, Binding Site UK). The evaluation was performed under a fluorescence microscope at 490 nm.

Autoantibodies against GAD, specific islet antigen 2 (IA2) and 21-OH were detected by the RIA method, Solupharm, Czech Republic.

HLA analysis

Genomic DNA was isolated from peripheral blood by a modified method according to (Miller *et al.*

1988). HLA typing in loci HLA-A, HLA-B and HLA-DR was carried out by the method of allele specific PCR.

Statistical evaluation

Allelic frequencies were compared between individual groups and to the controls by two-sided Fisher's exact test.

Results

Comparison of group A to groups B and C and to the controls

In group A a significantly higher occurrence of HLA-A24 (21.7 %) was found as compared to group B (5.0 %, p=0.024) and to the controls (8.5 %, p=0.007). Furthermore, a significantly higher occurrence of HLA-B27 (15.0 %) and HLA-DR11 (20 %) was found in group A as compared to the controls (4.2 %, p=0.01) (8.5 %, p=0.013).

Comparison of group B to groups A and C and to the controls

In group B a significantly higher occurrence of HLA-A3 (25.0 %) was found as compared to group A (10 %, p=0.043) and to controls (11.8 %, p=0.047). Furthermore, a significantly higher occurrence of HLA-B8 (22.5 %) was found in group B as compared to group A (8.3 %, p=0.045) and to controls (8.6 %, p=0.012).

Comparison of group C to groups A and B and to the controls

In group C a significantly higher occurrence of HLA-DR3 (30.0 %) was found as compared to group A (10.0 %, p=0.040) and the controls (9.8 %, p=0.047). Furthermore, a significantly higher occurrence of HLA-B8 (30.0 %) was found in group C as compared to group A (8.3 %, p=0.024) and to the controls (8.6 %, p=0.007).

Figures 1-3 show the allelic frequencies of HLA-A (Fig. 1), HLA-B (Fig. 2) and HLA-DR (Fig. 3) in all groups and controls.

Table 1. Autoantibodies in the PAA group

	Indirect immunofluorescence						RIA		
	Autoantibodies against adrenals				anti	anti	anti	anti	anti
Patient	z.glomerularis	z.fasciculata	z.reticularis	medulla	ovary	islet cells	21-OH	GAD	IA2
1	+	-	+	-	+	+	-	-	-
2	-	-	+	-	+	-	-	-	-
3	+	-	+	-	-	-	-	-	-
4	-	-	-	-	+	-	-	-	-
5	+	-	+	+	+	+	-	-	-
6	-	-	-	-	+	-	-	-	-
7	+	-	+	+	-	-	-	-	-
8	-	-	-	-	+	-	-	-	-
9	+	+	+	+	-	-	-	-	-
10	-	-	-	-	+	-	-	-	-
11	+	+	+	+	+	-	-	-	-
12	+	-	+	-	+	+	-	-	-
13	+	-	-	-	+	-	-	-	-
14	+	-	+	-	+	-	-	-	-
15	+	-	+	-	+	+	-	-	-
16	-	-	-	-	+	-	-	-	-
17	+	+	+	-	+	-	-	-	-
18	+	-	+	-	+	-	-	-	-
19	+	+	+	+	+	-	-	-	-
20	+	+	+	+	+	-	-	-	-

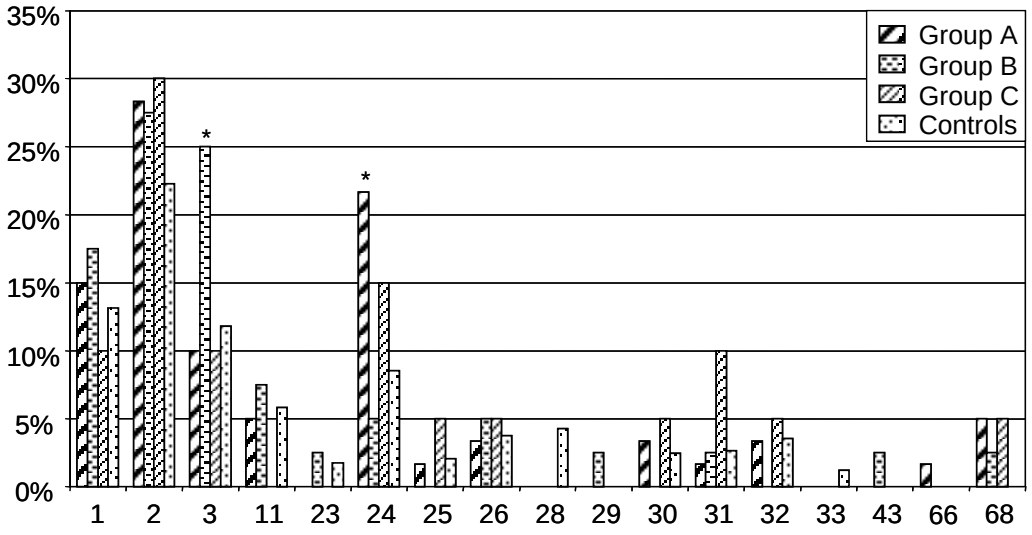

Fig. 1. Allelic frequencies of HLA-A; asterisks indicate significant differences ($p<0.05$) from other groups

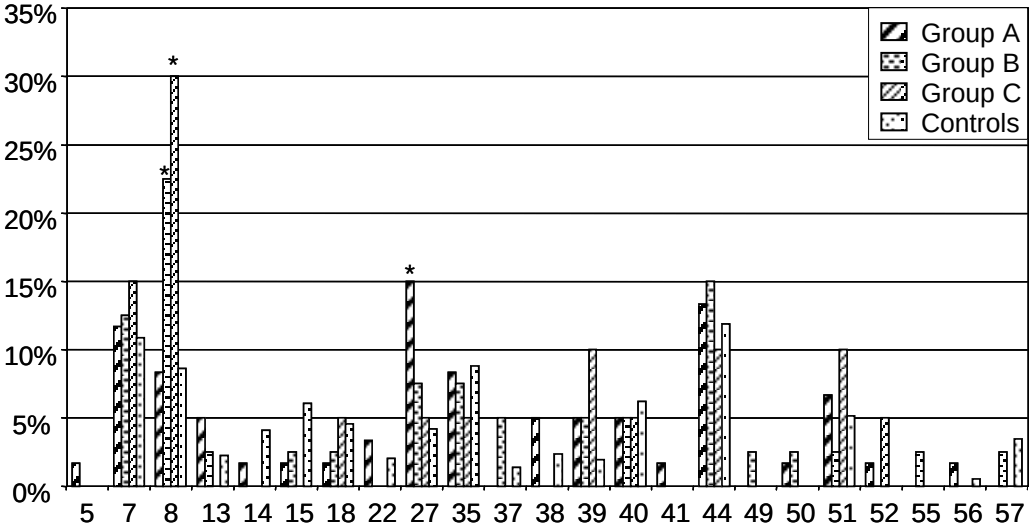

Fig. 2. Allelic frequencies of HLA-B; for other legend see Fig. 1.

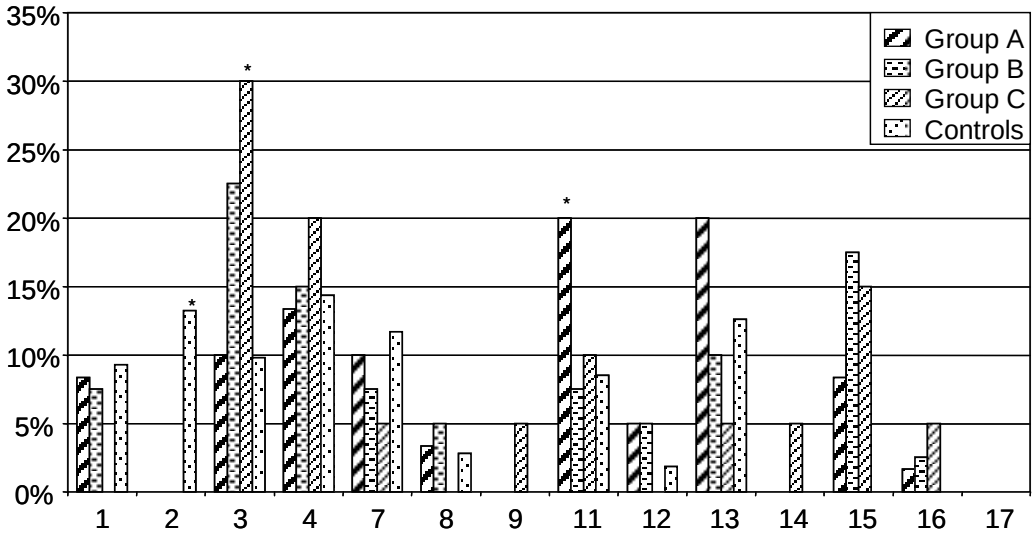

Fig. 3. Allelic frequencies of HLA-DR; for other legend see Fig. 1.

Discussion

Genetic predisposition is an important etiological factor for the onset of an autoimmune disease; however, it is not necessarily sufficient for the clinical development of the disease (Kono and Theofilopoulos 1996). For this development, epigenetic external factors are also important, e.g. viral or bacterial infections (Gianani and Sarvetnick 1996).

It is known that the highest risk HLA genotype for the APS type II and the isolated form of Addison's disease consists of the genotype DR3/4, DQ2/DQ8 with DRB1*0404 (Robles *et al.* 2002). In the HLA class I region, the HLA-B8 allele and gene polymorphisms are associated with APS type II and the isolated form of the Addison's disease (Gambelunghe *et al.* 1999). Our project was focused on the question, whether the group of patients, which do not show the development of clinical signs or exhibit them only in the very late stages (PAA), is genetically identical or different from the group with apparent clinical signs (APS type II), or if different epigenetic factors play a role in the different clinical development. We have chosen the expression of HLA system antigens (HLA-A, HLA-B, HLA-DR) as a marker of the genetic predisposition.

Our results have indicated differences in the HLA system between the isolated autoimmune thyroiditis and both groups with signs of "polyglandular involvement", i.e. patients with either PAA or APS type II. HLA-DR3 allele is considered to be an important risk factor for the development of polyglandular involvement (Betterle *et al.* 1996) and we found no significant difference between PAA and the APS type II in the allelic frequency of HLA-DR3. Further, we found no difference between PAA and the APS type II in the frequency of allele HLA-B8 which was described to be associated with APS type II (Weetman *et al.* 1991). Our study demonstrated that the two groups with the development of polyglandular involvement, both with clinical signs and with subclinical progress, belong to a group with a very close genetic predisposition bound to antigens of the HLA system, where the difference in the clinical development probably depends on the epigenetic factors.

The presence of autoantibodies against 21 hydroxylase appeared as an important factor, differentiating the groups of PAA and APS type II. The group of patients with PAA had no positive autoantibodies against 21 hydroxylase, but the autoantibodies against adrenals detected by indirect immunofluorescence were positive. All patients with APS type II had autoantibodies against 21 hydroxylase present. These results confirm that 21 hydroxylase is the main antigen of the adrenal cortex (Rees Smith and Furmaniak 1995*)* and that the presence of autoantibodies against 21 hydroxylase is highly specific for autoimmune adrenal insufficiency (Laureti *et al.* 1998a*).* The increased risk of progression into clinically apparent Addison's disease in adults was described in patients with high titers of autoantibodies against adrenals and 21 hydroxylase in association with HLA-DR3 (Betterle *et al.* 1997).

We suppose that epigenetic mechanisms hitherto not fully understood can activate the expression of target antigens such as 21 hydroxylase in genetically predisposed individuals. Hence the presence of autoantibodies against 21 hydroxylase appears as an important prognostic factor for the development of more severe impairment of functional cells and for the development of clinically manifest polyglandular involvement.

It can thus be concluded that the expression of HLA antigens indicates a genetic distinction between isolated autoimmune thyroiditis and both groups with signs of "polyglandular involvement" (PAA and APS type II). These two groups with signs of "polyglandular involvement" exhibit only very slight genetic differences in the area of HLA antigens and the different clinical development depends probably on epigenetic factors. An important finding, which differentiates these two groups, apparently involves the presence of autoantibodies against 21 hydroxylase, which is closely associated with the development of Addison's disease and with the groups of polyglandular activation of autoimmunity which represents an important serological prognostic marker of the clinical development of the APS type II.

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