Oligonucleotide Typing Reveals Association of Type I Psoriasis with the HLA-DRB1*0701/2, -DQA1*0201, -DQB1*0303 Extended Haplotype

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Although the pathogenesis of psoriasis is still a matter of debate, there are several lines of evidence supporting the concept of this disease being immunologically mediated with T cells playing a crucial role. Because a considerable portion of the cellular infiltrate in psoriasis consists of activated T-helper cells, expression of HLA class II antigens might be of particular importance for the understanding of its pathogenesis. Therefore, we investigated the HLA type of patients with type I (early onset, positive family history) and type II (late onset, no family history) psoriasis by means of serology (n = 89) and genotyping using sequence-specific oligonucleotide probes (n = 64). Serologic analysis of class I documented the association of type I psoriasis with HLA-Cw6, -B13, and -B57, whereas type II psoriasis showed a weaker correlation with HLA-Cw2 and -B27. Genotyping using SSO for class II detected the elevation of the HLA-DRB1*0701/2 allele frequency from 13% in normal population to 36% in type I, but only to 15% in type II psoriatics. Moreover, positive correlations with type I psoriasis were detected for HLA-DQA1*0201 and HLA-DQB1*0303. The HLA-DRB1*0701/2, -DQA1*0201, -DQB1*0303 extended haplotype was found exclusively in type I psoriasis. This is the first report documenting the association of distinct HLA class II alleles with type I psoriasis as detected on the DNA level, an approach both more specific and more sensitive when compared to serology. *J Invest Dermatol 100:749–752, 1993*

Psoriasis is a chronic hyperproliferative inflammatory disease affecting about 2% of Caucasians. Among the characteristic histologic features are epidermal hyperproliferation and infiltration of both dermis and epidermis by inflammatory cells including neutrophils, lymphocytes, macrophages, and mast cells [1].

Although the pathogenesis of psoriasis is still a matter of discussion, its association with distinct human leukocyte antigens (HLAs) is one feature psoriasis has in common with most autoimmune diseases studied so far [2–4] and thus supports the concept of psoriasis being a T-cell–mediated autoimmune disease [5,6]. Based on this HLA association two types of psoriasis can be differentiated: type I, manifesting itself early in life and frequently affecting other family members, is associated with HLA-Cw6 (73.8% versus 20.4% in normals), -B13 (34.3% versus 12.3%), and -Bw57 (29.7% versus 6.2%). Type II with a significantly later onset and lack of positive family history shows a weaker correlation with HLA-Cw2 (27.3% versus 5.5%) and -B27 (25.8% versus 10.1%) [7,8].

The primary physiologic role of HLAs in the activity of the mature immune system is sampling of peptides derived from the extracellular and intracellular protein pools by class II and class I molecules, respectively, for display in a multivalent form on the surface of the cell [9,10]. These complexes are then available for interaction with the clonally distributed $\alpha\beta$ receptors of CD4+ and CD8+ T cells, allowing the initiation of antigen-specific T-cell immune responses. HLA class II molecules are encoded in the HLA-D region on the short arm of chromosome 6 [11]. This region is subdivided into three subregions, HLA-DR, -DQ, and -DP. All subregions contain one functional expressed A gene (DRA1, DQA1, and DPA, respectively). HLA-DQ and -DP have also one B gene (DQB1 and DPB1), whereas several B genes are contained within DR (B1, B3, B4, and B5). All genes encoding class II molecules with the exception of DRA1 and DPA1 are highly polymorphic with the variability localized to the second exon encoding the amino-terminal extracellular domain [12,13].

Because the majority of T cells in the psoriatic infiltrate belongs to the CD4+ subset [14,15] and thus recognizes antigens in the context of class II molecules, data concerning association of these molecules with both types of psoriasis might be of particular importance for understanding the pathogenesis of this disease. We therefore analyzed the HLA types of patients with chronic stable psoriasis by means of serology (n = 89) and hybridization with specific oligonucleotides (n = 64). Our results document a strong correlation of type I but not type II psoriasis with HLA-DRB1*0701/2, HLA-DQA1*0201, and HLA-DQB1*0303.

MATERIALS AND METHODS

Patients and HLA-Typing of Class I Antigens Eighty-nine patients with chronic stable psoriasis from the Department of Dermatology, University of Ulm, and the Dermatology Clinic Bad Bentheim, FRG, were assigned to either type I or type II psoriasis. Sixty patients with positive family history and onset not later than at the age of 30 were grouped to type I psoriasis; 29 patients with a

Manuscript received October 2, 1992; accepted for publication January 29, 1993.

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Abbreviations: HLA, human leukocyte antigen; PCR, polymerase chain reaction; SSO, sequence specific oligonucleotides.

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Table I. Frequencies[a] of DRB1, DQA1, and DQB1 Alleles Differing Significantly in Chromosomes of Patients with Type I or Type II Psoriasis and Controls [16]

HLA Allele	Control[b]	Type I[c]	Type II[d]
DHRB1*0701/02	35 (13.2)	34 (36.2)[e]	5 (14.7)
DQA1*0201	35 (13.2)	35 (37.2)[e]	5 (14.7)
DQB1*0303	15 (5.7)	20 (21.3)[e]	0

[a] Each column gives the absolute numbers and, in brackets, the percentage.
[b] 265 chromosomes tested.
[c] 94 chromosomes tested.
[d] 34 chromosomes tested.
[e] Statistically significant ($p < 0.0001$).

negative family history and onset later than age of 40 were regarded as suffering from type II psoriasis. Data obtained by Begovich *et al* [16] served as control. The genetic characteristics of the population studied by Begovich *et al* are comparable to the population in Northern Europe [17]. Analysis of HLA class I molecules was done by means of standard serologic methods [18].

Polymerase Chain Reaction and Gel Electrophoresis Polymerase chain reaction (PCR) was essentially done as previously described [19] with the following modifications. For generic amplification of 286-bp fragments of DRB second exon AMP1 5′-TTCGCCGCTGCACTGTGAAGCTCTC and AMP3 5′-CCGAATCCTTCGTGTCCCCACAGCCG were used as primers. After 30 cycles of PCR with 2.5 units of Thermos aquaticus DNA polymerase (Cetus/Perkin Elmer) products were checked for single-band amplimers on ethidium bromide–stained minigels. After heat denaturation of the DNA, aliquots were spotted manually on nylon membranes and immobilized by ultraviolet irradiation (120 mJ, Stratalinker, Stratagene).

Sequence-Specific Oligonucleotides and Hybridization Conditions The oligonucleotides were made on an Applied Biosystems 380B DNA synthesizer and have been described elsewhere [20]. Each sequence-specific oligonucleotide was 3′-end labeled by terminal polynucleotidyl transferase (Boehringer Mannheim) with dideoxy-digoxigenin. Filters were pre-hybridized in a solution that contains 3 M tetramethylammonium chloride, 100 μg/ml salmon sperm DNA, 50 mM Tris-HCl, 0.1% sodium dodecylsulfate, 5 × Denhards solution and 2 mM ethylenediaminetetraacetic acid for 2 h, then 3–5 pM labeled sequence-specific oligonucleotides probes were added and hybridization continued for at least 3 h. After removal of the hybridization solution, filters were washed under stringent conditions in a buffer containing 3 M tetramethylammonium chloride, 50 mM Tris-HCl, and 0.2% sodium dodecylsulfate. Hybridized oligonucleotides were detected using a digoxygenin-specific monoclonal antibody conjugated to alkaline phosphatase (Boehringer Mannheim) and binding was visualized with chemiluminescence substrate (ECL, Amersham) by autoradiography.

Statistical Evaluation Absolute numbers of chromosomes positive for the alleles under investigation were determined and percentages were calculated. For those alleles where clear differences were observed compared to the control population chi square tests of homogeneity were performed. In cases with a p value less than 0.0001 the observed difference was rated as statistically significant (Table I).

RESULTS

Patient Groups Based on the anamnestic data, 60 of the 89 patients were grouped to type I psoriasis whereas 29 patients were regarded as type II psoriatics (data not shown). Although classification was done according to patient history, subsequent HLA class I typing showed the well-established pattern (see below), thus underlining the validity of these informations for the classification of psoriasis.

HLA Class I Typing by Serology Analysis for class I molecules was done serologically (n = 89) and revealed a frequency of 83% for Cw6 in type I psoriatics (data not shown). B13 was found in 18% and B57 in 55%. In type II psoriasis, we observed 38% positive for Cw6; the percentages for B13 and B57 were 17% and 7%, respectively. These frequencies are much closer to those seen in normals, where the respective numbers are 16.5%, 5.7%, and 5.7%.

HLA-DRB1*0701/02 Is Associated with Type I but Not Type II Psoriasis Expression of HLA class II molecules was examined by the use of sequence-specific oligonucleotides in 64 patients, 47 type I and 17 type II psoriatics. Twenty DRB1 alleles were found in the patients analyzed (DRB1*0101/02, -*1501, -*1502, -*1601, -*0301, -*0401, -*0402, -*0403, -*0404, -*0408, -*1101, -*1103, -*1104, -*1201, -*1301, -*1302, -*1303, -*1401, -*0701/02, -*0801).

The allele most frequently detected was HLA-DRB1*0701/02. This allele was present in 28 patients (60%) with type I psoriasis; six of them were homozygous. In contrast, only five type II psoriatics (29%) were positive for this allele, none of them being homozygous. At the chromosomal level, the numbers were 34 (36%) in type I and five (15%) in type II psoriatics compared to 13% in the control population (Table I). These differences in frequency are statistically significant ($p < 0.0001$) as confirmed by the chi-square test of homogeneity. All other DRB1 alleles tested showed no significant difference.

HLA-DQA1*0201 as Well as -DQB1*0303 Are Significantly Increased in Type I Psoriasis Seven DQA1 (DQA1*0101, -*0102, -*0103, -*0201, -*0301, -0401*, -*0501), and 11 DQB1 (DQB1*201, -*301, -*302, -*303, -*501, -*502, -*503, -*601, -*602, -*603, -*604) alleles were detectable in our patients. The only statistically significant difference observed was the elevation of HLA-DQA1*0201 and -DQB1*0303 in type I psoriasis. Twenty-nine type I psoriatics were DQA1*0201 positive, representing 62% of the patients with type I psoriasis, whereas only five type II psoriatics (29%) had this allele. The corresponding numbers at the DNA level were 35 (37%) and five (15%), respectively. In the control population, 13% of the chromosomes were found to be positive for this allele (Table I). Some alleles, e.g., DQA1*0103, were found to be considerably more frequent in type II compared to type I psoriasis. However, in the case of DQA1*0103 the p value was 0.0058 and the increase therefore not considered statistically significant.

The most pronounced correlation of a DQB1 allele with either type of psoriasis was noted for DQB1*0303, which was present in 18 individuals, all of them type I psoriatics representing 38% of all patients in this group. The genetic frequency was calculated to 21%. This value, too, reached significance when compared to the frequency of 5.7% observed in the control population (Table I).

Extended Haplotype HLA-DRB1*0701/2, -DQA1*0201, -DQB1*0303 Is Exclusively Found in Type I Psoriasis Besides the association of individual alleles with both types of psoriasis we also investigated the frequencies of important extended haplotypes in either type. Due to linkage disequilibrium in HLA, DRB1*0701 is associated with DQA1*0201. These two alleles form an extended haplotype with DQB1*0201 or, more rarely, with DQB1*0303. Interestingly, the rare extended haplotype consisting of alleles HLA-DRB1*0701/2, DQA1*0201, and DQB1*0303 was found on 20 chromosomes in 18 individuals with type I psoriasis; thus it was present in 38% of type I psoriatics. In contrast, this haplotype was not detectable in type II psoriatics. On the other hand, HLA-DRB1*0701/2 association with HLA-

Table II. Percentage of Patients with Type I or Type II Psoriasis Exhibiting One of Two Extended Haplotypes Containing HLA-DRB1*0701/2

	HLA-DRB1*0701/2 and HLA-DQB1*0201	HLA-DRB1*0701/2 and HLA-DQB1*0303
ype I	10.6	38.3
ype II	23.5	0.0

DQB1*0201 was found in 24% of type II psoriatics and 11% of type I psoriatics (Table II).

DISCUSSION

Although the pathogenesis of psoriasis is still a matter of debate, there is accumulating evidence for the concept of psoriasis being an autoimmune disease with T cells playing an important role: besides macrophages, they account for the majority of the mononuclear infiltrate [14,21]. Most of them belong to the CD4+ subset [15] and express activation markers [14,15,22]. A number of proteins abnormally expressed on the surface of keratinocytes in psoriatic epidermis is known to be inducible by cytokine secretion of T cells [23,24]. Finally, several therapeutic approaches using T-cell-suppressive agents have been proved to be effective [25-27]. Thus, if the hypothesis of the T-cell-mediated autoimmune pathogenesis was true, data on the expression of HLA class II molecules are crucial to understand the pathomechanisms underlying this disease, because it is class II rather than class I molecules that present peptides to CD4+ cells.

The PCR made it possible to identify nucleotide sequence variations directly at the genomic level using sequence-specific oligonucleotide probes for DNA hybridization [28,29]. Genotyping revealed a significantly higher degree of class II polymorphism than defined by other methods [30]. Thus, PCR-based oligonucleotide typing is a powerful method of defining HLA polymorphism, which proves superior to other techniques. This is particularly true in comparison to serologic approaches to define class II polymorphism.

Attempts to correlate HLA type and susceptibility to psoriasis often focused on the association with class I molecules [7,8,31], although an increased frequency of HLA-DR7 has been noted as early as 1979 [32] and since then has repeatedly been confirmed [33]. Sakkas *et al* were able to define HLA-DR7a as an important susceptibility factor for psoriasis and psoriatic arthritis [34]. Our data, too, document this HLA-DR7 correlation and extend it insofar as we were able to show that this correlation holds true only for type I but not for type II psoriasis (Table I). Consistent with our findings are preliminary data by Henseler (Henseler, personal communication). Interestingly, he found 100% of type I psoriatics positive for HLA-DR7, whereas only 33% of the type II psoriatics expressed this molecule. In contrast, we observed HLA-DRB1*0701/2 only in 60% of patients with type I psoriasis. In case of type II psoriasis, our numbers equal those of Henseler. One explanation might be the inferior specificity of serologic phenotyping versus genotyping, particularly in the case of class II typing.

Besides HLA-DRB1*0701/02, alleles HLA-DQA1*0201 and HLA-DQB1*0303 showed a significantly higher correlation with type I psoriasis (Table I). Not only the association of distinct HLA class II alleles with type I psoriasis is remarkable, but also the association of these HLA class II alleles with each other. Yunis *et al* identified two extended haplotypes involving HLA-DRB1*0701/2: this allele was found to be associated either with DQw2 and the DQB1*0201 allele or with DQw9 and the DQB1*0303 allele [35], the latter haplotype being less frequent [36]. Due to linkage disequilibrium in HLA, DRB1*0701/2 is associated with DQA1*0201, which therefore is part of both haplotypes. To our knowledge, only the former type has been found to show association with certain diseases [37]. Thus, it is interesting to note that in type I psoriatics the rare haplotype consisting of HLA-DRB1*0701/2 and HLA-DQB1*0303 was found to be more than three times as frequent as the HLA-DRB1*0701/2, -DQB1*0201 haplotype. In contrast, this rare haplotype does not appear at all in type II psoriasis (Table II). These data might point towards HLA-DQB as a major susceptibility antigen for type I psoriasis.

Only rarely, a clear increase in the frequency of a class II allele was observed in type II psoriasis versus type I and the control population. One example is DQA1*0103. The p value was 0.058 for this increase and thus did not reach statistical significance. Thus, a possible association of type II psoriasis with certain class II molecules should not be excluded on the basis of these data. However, our data make it extremely unlikely that type I and type II psoriasis show associations with similar HLA class II molecules. Therefore, if antigens are involved in their respective pathogenesis, exclusive sets of antigens might exist for both types to trigger a T-helper-cell-mediated autoimmune reaction.

To summarize, this is the first report demonstrating the correlation of several HLA-DR and -DQ molecules with type I psoriasis at the genomic level. Association with class II molecules further supports the concept of type I psoriasis being a T-cell-mediated autoimmune disease.

The expert technical assistance of G. Deehsle is gratefully acknowledged. We thank S. F. Goldmann for HLA-A, -B, and -C typing. W.-H. Boehncke is supported by grant F01/92 of the University of Ulm.

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