HLA-DR4 May Determine Expression of Actinic Prurigo in British Patients

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Human leukocyte antigen (HLA) associations have been reported in Amerindian patients with actinic prurigo. To determine if similar associations are present in the British Caucasoid population with actinic prurigo, 26 patients underwent serological typing for HLA Class I and II antigens. DNA analysis by both sequence-specific priming and group-specific amplification with single-stranded oligonucleotide probe hybridization was used to confirm the DR and DQ typing and to perform DR4 subtyping. All patients were DR4 positive, and 25 of 26 patients were DQ7 positive. DR4 subtyping revealed 12 of 20 patients tested to be DRB1*0407. A nonsignificant association was also found with HLA B55 that is in linkage disequilibrium with DRB1*0407. No HLA associations were found in 25 British Caucasoid patients with polymorphic light eruption. DRB1*0407 is rare in European Caucasoids without actinic prurigo, and HLA-DR4 may have an important role in determining expression of this disease. Key words: photo-sensitivity/photodermatoses/tissue typing. J Invest Dermatol 106:362–364, 1996

Actinic prurigo (AP) is a rare, chronic, cutaneous photosensitivity disorder of generally childhood onset and female predominance. It is characterized by the presence of persistent excoriated papules, nodules, and plaques, occasionally with eczematous change and chelitis, on mainly light-exposed sites, occasionally with shallow, linear, pitted scars [1]. The condition is also found more frequently in native American (Amerindian) populations, where it is often familial [2] and of late onset [3].

AP is generally held in Britain to be distinct from polymorphic light eruption (PLE), although part of the same spectrum of disease, as some patients have overlapping features of both conditions [1]. This remains controversial, however, and others believe AP to be a subgroup of PLE [4]. PLE is a common photodermatosis, of onset generally in the first three decades of life, characterized by the intermittent appearance of erythematous papules, vesicles, and plaques. Involvement of covered areas is unusual. There is generally a clear relation to prior ultraviolet exposure, with onset of lesions within hours, occasionally days, following exposure; the eruption then generally subsides without scarring within days to weeks.

Tissue typing of Colombian Amerindians with AP has demonstrated an apparently increased frequency of human leukocyte antigen (HLA) CW4 [2]; other initially reported associations with B40 and CW3 have later been discounted [2]. Canadian Amerindians also have an increased frequency also of HLA CW4, which although not statistically significant is likely to be relevant in conjunction with the Colombian data, and an increased frequency of HLA A24 [5]. Such associations may well have an important role in determining disease expression and we have therefore studied the HLA status of British Caucasoid patients with AP to establish if similar associations are present. Ethnic variations in HLA disease associations are well recognized, however, and therefore all patients and controls in this study were carefully chosen to be British Caucasoids.

MATERIALS AND METHODS

All Caucasian patients with a clinical diagnosis of actinic prurigo seen in the Department of Photobiology of the St. John’s Institute of Dermatology at St. Thomas’ Hospital over a 1-yr period were studied along with 25 Caucasian patients with PLE. AP was distinguished from PLE by the presence of all or several of the following features: the presence of persistent lesions with prurigo papules or nodules, a tendency to activity in winter, the presence of lesions on covered sites, eczematization of lesions, a tendency to superficial scarring, and associated chelitis or conjunctivitis, although none of these features was diagnostic in isolation. Patients with overlap features of actinic prurigo and polymorphic light eruption, as, for example, with lesions on exposed sites of greater than 2-wk duration but no other associated features of AP, were excluded in order to ensure that the patient groups were distinct. Venesection was performed following verbal consent from the patient.

Serological Typing Serological typing for HLA Class I and II antigens was performed in all patients by means of a standard lymphotoxicity assay, with well-characterized antisera for HLA-A, -B, -C, -DR, and -DQ. The results were compared with those of 292 normal, previously tissue-typed Caucasian control patients.

Confirmation of Serology The HLA Class II serological results were confirmed by the more discriminatory method of genotyping in 20 AP patients. The typing for DRB was by polymerase chain reaction (PCR)/sequence-specific priming after the method of Olverup et al [6], the primers and probes obtained as kits from the British Society of Histocompatibility and Immunogenetics, and that for DQ by PCR/sequence-specific priming after the method of Bunce and colleagues [7], from whom the primers were obtained; DNA was extracted in each instance by the salt method [8]. These

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Abbreviation: AP, actinic prurigo.
results were then compared with those of a separate group of 177 normal Caucasian controls typed at the DRB and DQA and B subregions by group-specific amplification using PCR followed by single-stranded oligonucleotide probe hybridization [9].

**DR4 Subtyping** Although DR4 is serologically homogeneous, it may be further defined by molecular techniques into at least 14 different subtypes. Subtyping was performed by PCR/single-stranded oligonucleotide probe hybridization using the method of Lanocialby et al [10], with the modification that probes were labeled with fluorescein, and detection of hybridization was by the ECL system (RNP 3000; Amersham International, Little Chalfon, Bucks, U.K.). Essentially, the oligonucleotide probes [10] were 3' end labeled by deoxynucleotidyl transferase with fluorescein-dUTP. Hybridization took place using the reagents provided with stringent washes [10] and detected with an anti-fluorescein horse radish peroxidase conjugate (supplied). Bound peroxidase was detected through the enzymatic reduction of peroxidase to luminol (supplied in the presence of an enhancer and the resultant light emission detected using closely opposed XAR5 film (Kodak). The results were confirmed by PCR/sequence-specific priming [11], 20 consecutive unrelated DR4 subjects of unknown subtype and 5 DR4 homozygous cell lines being simultaneously analyzed as controls; these cell lines were chosen to include Dw4 (DRB1*0401/1; Priess), Dw10 (DRB1*0402/2; PV90), Dw14 (DRB1*0404/4; MTF), Dw15 (DRB1*0405/5; SJAH), and Dw13, specifically selected as DRB1*0407/7; JHAF.

**Statistical Analysis** Fisher’s exact test was used to calculate the significance of any HLA associations noted in AP, a correction factor of 100 being used for the number of antigens tested (HLA-A 22, -B 40, -C 8, -DR 15, -DRA 4, -DQA 7), and the corrected probability being quoted as p c. The 2 x 2 table was used to calculate chi-square for independence with Yates correction.

**RESULTS**

Twenty-six AP patients were studied, 21 female and 5 male with ages ranging from 7 to 50 y, the average age of disease onset being 14 y. Twenty-five PLE patients were also studied, 21 female and 4 male with ages ranging from 7 to 43 y the average age of disease onset being 23 y. The age of the healthy controls ranged from 5 to 60 years.

**Class I Typing** No significant Class I association was found, although 6 of 26 patients were HLA-B55 compared with 11 of 292 controls (χ² = 14, Fp = 0.0011, Fpc = 0.11); there were also very slight increases in HLA-Cw4 and -Cw3 not approaching statistical significance. No patient was HLA-A24. No distortion of antigen frequency was present in the PLE group.

**Class II Typing** All 26 AP patients were HLA-DR4 (Table I) in contrast to only 70 of 177 controls (χ² = 31, Fp = 4.46 E-10 [0.00000000045], Fpc = 4.46 E-7 [0.00000045]). Ten patients were homozygous for DR4. Twenty-five of 26 patients were DQ7 compared with 53 of 177 controls (χ² = 39, Fp = 4.7 E-11, Fpc = 4.7 E-9 [0.000000047]). Only 9 of 25 PLE patients were HLA-DR4 (χ² = NS).

**DR4 Subtyping** Twelve of 20 AP patients tested were subtype DRB1*0407 (Table I) but none of 20 controls (χ² = 14.4, Fp = 0.000023, Fpc = 0.002), who showed instead the subtype frequency distribution expected of British Caucasoids [11,12]. One of the homozygous patients (Patient 20) was DR4 Dw13 (DRB1*0403 or 0407) and DR4 Dw14 (DRB1*0404 or 0408) but no further subtype assignment was possible with the available technology; this patient has not been included as DRB1*0407.

**DISCUSSION**

We have shown complete concordance between AP and the Class II antigen HLA-DR4 in the 26 patients studied, clearly a highly significant association. Further analysis has also revealed a strong association with the DR4 subtype DRB1*0407. In addition, we have shown a strong association with the Class II antigen HLA-DQ7 and a weaker, not statistically significant, association with the Class I antigen HLA-B55. No significant distortion in antigen frequency was detected at the HLA-A24, -Cw4, and -Cw3 loci, however, and, therefore, our data do not repeat the previous findings of Class I associations in Americans with AP [2,5]. The weak association found by us with HLA-B55 is likely to be related to its linkage with DRB1*0407. The strong association with DQ7,
found in 25 of our 26 patients may well be for the same reason, as DQ7 is also in linkage with DRB1*0407 in the British population [12,13]. It remains possible that DQ7 is of primary importance, but this is relatively unlikely as no increase was observed in HLA-DR5, which is associated with DQ7 in British Caucasoids. DRB1*0407 is uncommon in European Caucasoids, making up 4.4–6.7% of DR4-positive individuals depending on the group studied [12,13]. Thus, its markedly increased incidence in our patients with AP seems likely to be of pathogenic significance.

HLA-DR4 has previously been found to be associated with a number of autoimmune diseases, notably rheumatoid arthritis [13] and a number of skin diseases particularly pemphigus vulgaris in Jewish patients, vitiligo, drug-induced lupus erythematosus, and herpes gestationis (DR4/DR3 heterozygotes) [14,15]. The precise significance of such HLA associations in the pathogenesis of disease has not been elucidated, but it has been postulated that different individuals vary in their response to different antigens and that this variation is determined genetically and linked to the major histocompatibility complex. HLA restriction may thus determine binding of particular disease-related peptides, or alternatively allow specific T-cell repertoire. Since AP has features suggesting it may be a T-cell-mediated immunological disorder [1], and since the DR4 group of alleles, varying considerably in the third hypervariable region which is believed to interact with the T-cell receptor, have distinct T-cell specificities, class II restriction in AP may conceivably determine the T-cell response. On the other hand, and perhaps more likely, since direct comparison between the 3-dimensional crystal structure of HLA-DR1 (DRB1*0101) [16] and that of DRB1*0407 indicates that the positions that make DRB1*0407 unique from other DR4 alleles (positions 74 and 86) are intimately involved in peptide binding, the HLA type in AP may instead determine the response to a peptide antigen, conceivably one induced by ultraviolet irradiation, to initiate the characteristic AP cutaneous response.

DRB1*0407 is more common in Amerindians than in Caucasoids, which may possibly help explain the greater prevalence of AP in that population; since DRB1*0407 is associated with DQ8 and not DQ7 in Amerindians [17]. Class II typing is now awaited with interest in that ethnic group and may reveal such a link. Serological DR4 typing has already been performed in Colombian Amerindians, showing an initial increase in DR4, which was not, however, statistically significant nor later confirmed [2]. Newer methods may now conceivably reveal such an association. Of considerable interest, no HLA associations were found in PLE patients, confirming previous findings in Canadian Caucasoids and suggesting that PLE is distinct from AP [18]. The HLA Class II antigen DR4 thus appears to be strongly associated with AP in British Caucasoid patients and may therefore have a causal role in the pathogenesis of the disease by determining or modifying the response in the disease to a light-induced antigen. In contrast, the absence of an HLA association in PLE suggests that, at least in British Caucasoids, this distinguishes the clinical responses in the two patient groups and may thus act as a marker to distinguish the two conditions. Study of patients with overlap features of AP/PLE will now be of interest to delineate more clearly the role of HLA genes in expression of the two diseases. Further study of Amerindian patients is also needed to determine whether the HLA association in AP explains the greater prevalence of the disease in those populations.

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