Association of class I, II, and III MHC gene products with systemic lupus erythematosus

Results of a Central European multicenter study

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Summary. Class I, II, and III MHC gene products were examined in 248 Central European SLE patients. The previously reported association with HLA-A1, -B8 and -DR3, and C4AQ0 alleles was confirmed. The frequency of HLA-DR2 was also slightly elevated in SLE patients, while no increase in C4BQ0 alleles was observed. Additional findings were a significantly increased frequency of HLA-B13 and a significant decrease of HLA-B44.

Key words: MHC gene products – Systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) is regarded as a classical autoimmune disease of unknown etiology. Various genetic factors have been implicated in the pathogenesis of this disease, particularly gene products closely associated with or located within the major histocompatibility complex [1].

Since the initial reports by Waters et al. [2] and Grumet et al. [3], several groups have presented varying results with respect to HLA associations. The HLA antigens A1 [4, 5], A2 [6], A10 [7], A11 [8], Bw17 [9, 10], Bw15 [3], Bw17 [12], Bw40 [6], and finally the HLA class II antigens DR2 [13–15] and DR3 [13, 16, 17] have all been reported to show increased frequencies in Caucasian SLE patients. One reason for such divergent results appears to be the relatively small numbers of patients investigated in the majority of these studies. Three reports from the United States and one combined study of European and North American patients have comprised over 100 patients [18–21]. Two of these North American studies revealed significant associations of SLE with HLA-DR2 and HLA-DQ1 [19–21]. Significant associations with HLA-B8 and HLA-DR3, reported from various European centers [4, 16, 17, 22–24], were not found in the North American studies. In two European samples the frequency of HLA-DR2 was found to be increased [4, 25], but this was not confirmed in other European studies [16, 24, 26].

Fielder et al. [27] reported a strong association of null alleles of the complement components C4A and C4B with SLE. These associations were supported by family studies in two reports [28, 29], but were not confirmed in another study by P. Schur (personal communication).

In order to establish in a central European Caucasian population firm associations of SLE with class I, class II, and class III MHC markers, five Central European centers have collaborated in this analysis of 248 patients.

Patients and methods

Between 1983 and 1988 248 Caucasian patients of Central European descent with SLE (according to the 1982 revised ARA criteria for the classification of SLE [30]) were recruited from the outpatient clinics of five centers (Hannover, n = 113; Frankfurt, n = 43; and Freiburg, n = 50 in the FRG; and Bern, n = 16; and Zürich, n = 26 in Switzerland). Overlap syndromes were carefully excluded.

HLA typing. HLA typing of A, B, DR, and DQ antigens was performed using a standard complement-dependent microcytotoxicity assay [31]. HLA-A, -B, and -DR typing for the patients from Bern, Zürich, and partly Freiburg was performed at the Department of Clinical Immunology, Inselspital Bern. HLA typing for all other patients was carried out in the HLA Typing Laboratory of the Medical School Blood Bank at Hannover.
HLA-C typing was also performed to obtain additional information for haplotype determination in future family studies, but because of the well-known lack of accuracy of typing results, the results are not shown here. Frequency data for HLA-A, -B, -DR, and -DQ antigen distributions in European Caucasians from the 1984 HLA workshop [32] were used as controls.

Electrophoretic characterization of C4. C4 electrophoretic variants were determined in neuraminidase-treated ethylenediaminetetraacetate (EDTA) plasma samples using the technique of immunofixation electrophoresis in agarose gels, as previously described [33]. When necessary, distinction between C4A and C4B locus products was achieved by immunoblotting using a monoclonal antibody specific for C4B locus products [34].

Assignment of phenotypes to individuals showing low levels of C4 protein on test gels were confirmed by testing of two independent plasma samples. Assignment of the null alleles AQ0 and BQ0 was made on the basis of differential staining intensities of the electrophoretically separated C4A and C4B products. Within a certain protein range, binding of Coomassie blue is proportional to the concentration of protein [35], therefore, a binding pattern of C4A of double intensity to that at C4B indicates that the individual tested is C4B heterozygous, carrying C4BQ0. This method does not allow the identification of individuals heterozygous for both AQ0 and BQ0. Such individuals can only be identified by family segregation analysis. The nomenclature for alleles of the two C4 loci has been adopted from the 4th International Complement Workshop, Boston, USA [36].

Statistical methods. The \( \chi^2 \)-test was employed for calculation of significant differences; relative risk values (RR) were calculated as described in [37].

Results

HLA antigens

Table 1 shows the frequencies of the HLA-A antigens of 248 SLE patients. A significant increase of HLA-A1 was found in SLE patients, while all other HLA-A locus antigen frequencies did not differ significantly from the controls.

Table 2 demonstrates the frequencies of HLA-B antigens in SLE. A highly significant increase was found for HLA-B8, whereas there was no significant difference for HLA-B7. HLA-B13, previously reported to be elevated in SLE [12, 39], was also significantly increased in our SLE patients; the antigen frequency of HLA-B44 was significantly lower compared with the control sample.

Frequencies of HLA-DR and -DQ antigens are shown in Table 3. The frequency of both HLA-DR3 and HLA-DR2 was significantly increased in SLE. Of the DQ antigens, only HLA-DQ2, which is in strong linkage disequilibrium with HLA-DR3, was significantly increased in SLE.

C4 alleles

Among the C4A alleles, C4AQ0 showed a highly significant increase in SLE patients (19.8% vs 12.0%, \( n=196 \) patients, \( P<0.001 \); Fig. 1). The relative risk (RR) for C4AQ0-positive SLE patients was 1.6. The C4A2 allele frequency was significantly decreased in this group of patients. The distribution of C4B alleles (C4BQ0, C4B1, C4B2, C4B2.9,