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Association between *Eco*RI fragment-length polymorphism of the immunoglobulin lambda variable 8 (IGLV8) gene family with rheumatoid arthritis and systemic lupus erythematosus

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Abstract

The human immunoglobulin lambda variable 8 (IGLV8) subgroup is a gene family containing three members, one of them included in a monomorphic 3.7-kb *Eco*RI genomic fragment located at the major lambda variable locus on chromosome 22q11.1 (gene IGLV8a, EMBL accession No. Z73650) at 100% frequency in the normal urban population. The second is a polymorphic RFLP allele included in a 6.0-kb *Eco*RI fragment at 10% frequency, and the third is located in a monomorphic 8.0-kb *Eco*RI fragment at 100% frequency, the last being translocated to chromosome 8q11.2 and considered to be an orphan gene. Our Southern blot-*Eco*RI-RFLP studies in normal individuals and in patients with rheumatoid arthritis (RA) or with systemic lupus erythematosus (SLE), using a specific probe for the IGLV8 gene family (probe pVL8, EMBL accession No. X75424), have revealed the two monomorphic genomic fragments containing the IGLV8 genes, i.e., the 3.7-kb fragment from chromosome 22q11.1 and the 8.0-kb fragment from 8q11.2, both occurring at 100% frequency (103 normal individuals, 48 RA and 28 SLE patients analyzed), but absence of the 6.0-kb IGLV8 polymorphic RFLP allele in all RA or SLE patients. As expected, the frequency of the 6.0-kb allele among the normal individuals was 10%. These findings suggest an association between the absence of the 6.0-kb *Eco*RI fragment and rheumatoid arthritis and systemic lupus erythematosus.

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- Rheumatoid arthritis
- Systemic lupus erythematosus
- Human V-lambda 8 genes
- Polymorphism of V-lambda genes
- RFLP-disease association

The human immunoglobulin lambda light chains contribute about 40% to the functional serum antibodies, indicating the important role played by these chains in the antibody response (1). The immunoglobulin lambda variable (IGLV) locus extends about 800 kb on chromosome 22q11.1-11.2 and has been mapped and fully sequenced by contig methodology locating all the known functional V-lambda genes and pseudogenes (2-5).

We know the positions of about 70 germline V-lambda sequences of which 30-33 are functional V-lambda genes distributed into 11 IGLV gene families (IGLV1-

IGLV11) (2-5).

The V-lambda genes are associated with *Eco*RI site-flanked genomic fragments detectable in Southern blots of genomic DNA samples (2,6,7).

An orphan gene belonging to the IGLV8 family translocated from the major IGLV locus on chromosome 22q11.1 to chromosome 8q11.2 was recently described, and there is evidence that its expression is associated with the manifestation of autoimmune diseases (8-10).

Rheumatoid arthritis (RA) is a heterogeneous systemic rheumatic disease of unknown etiology affecting synovial membranes of the joints, with both environmental and genetic factors contributing to its manifestation (11).

Systemic lupus erythematosus (SLE) is a disease also of unknown etiology characterized by inflammation in many different organ systems associated with the production of antibodies reactive with nuclear (DNA and RNA), cytoplasmic and cell membrane antigenic determinants (12).

We evaluated the polymorphism of the IGLV8 gene family in patients with RA or with SLE and normal individuals from an urban Brazilian population in the Ribeirão Preto region, São Paulo State, southeastern Brazil, by Southern hybridization.

A total of 48 RA (36 women and 12 men) and 56 SLE patients (52 women and 4 men) fulfilling the American College of Rheumatology criteria for RA (13) and 103 normal individuals (71 women and 32 men) were studied. The study was approved by the Ethics Committee of Hospital das Clínicas de Ribeirão Preto, USP (No. 4953/98).

Among the 103 normal individuals analyzed (71 women and 32 men) we demonstrated the monomorphic 8.0- and 3.7-kb *Eco*RI fragments at 100% frequency and the 6.0-kb *Eco*RI fragment at 10% frequency (for both male and female groups) corroborating our previous observations (6) (Figure 1A).

In contrast, we were not able to demonstrate the polymorphic 6.0-kb *Eco*RI fragment in RA and SLE patients (Figure 1B and C). The absence of the 6.0-kb allele in the tested population of autoimmune patients is significantly different from the 10% incidence in the normal group as evaluated by statistical analysis by the chi-square test ($\alpha = 0.05$, $P = 0.0136$).

There are some known IGLV genes whose *Eco*RI fragments migrate between 5.7 and 6.5 kb and others whose *Eco*RI fragments migrate between 7.5 and 8.6 kb (2).

Our restriction fragment-length polymorphism (RFLP) studies of the different IGLV gene families in the Brazilian population, including the same normal individuals analyzed here, revealed that these fragments are highly polymorphic, including deletions (data not shown).

The present results clearly show that the 8.0-kb *Eco*RI band is present in 100% of the individuals analyzed in a monomorphic fashion.

The IGLV7a gene (EMBL accession No. Z73659) migrates in an *Eco*RI fragment of 7.9 kb, but it has only 66% sequence identity with the pVL8 probe. Considering that our Southern membranes were submitted to high-stringency washes, it is unlikely that they remained cross-hybridized with the IGLV7a gene after the washes.

The IGLV9S1 gene is a sequence-tagged site for the IGLV locus and migrates in a 5.6-kb *Eco*RI fragment. Our previous RFLP studies showed that it is present in 100% of individuals from different populations (7,14). The 6.0-kb *Eco*RI band detected with the pVL8 probe was present in 10% of the normal individuals and absent in the RA or SLE patients analyzed.

Further studies are being done in our laboratory to survey the expression of the IGLV8 allele present in the 6.0-kb *Eco*RI fragment and to evaluate a possible functional significance with regard to the clinical status of the RA or SLE patients.

We have polymorphism and/or sequence

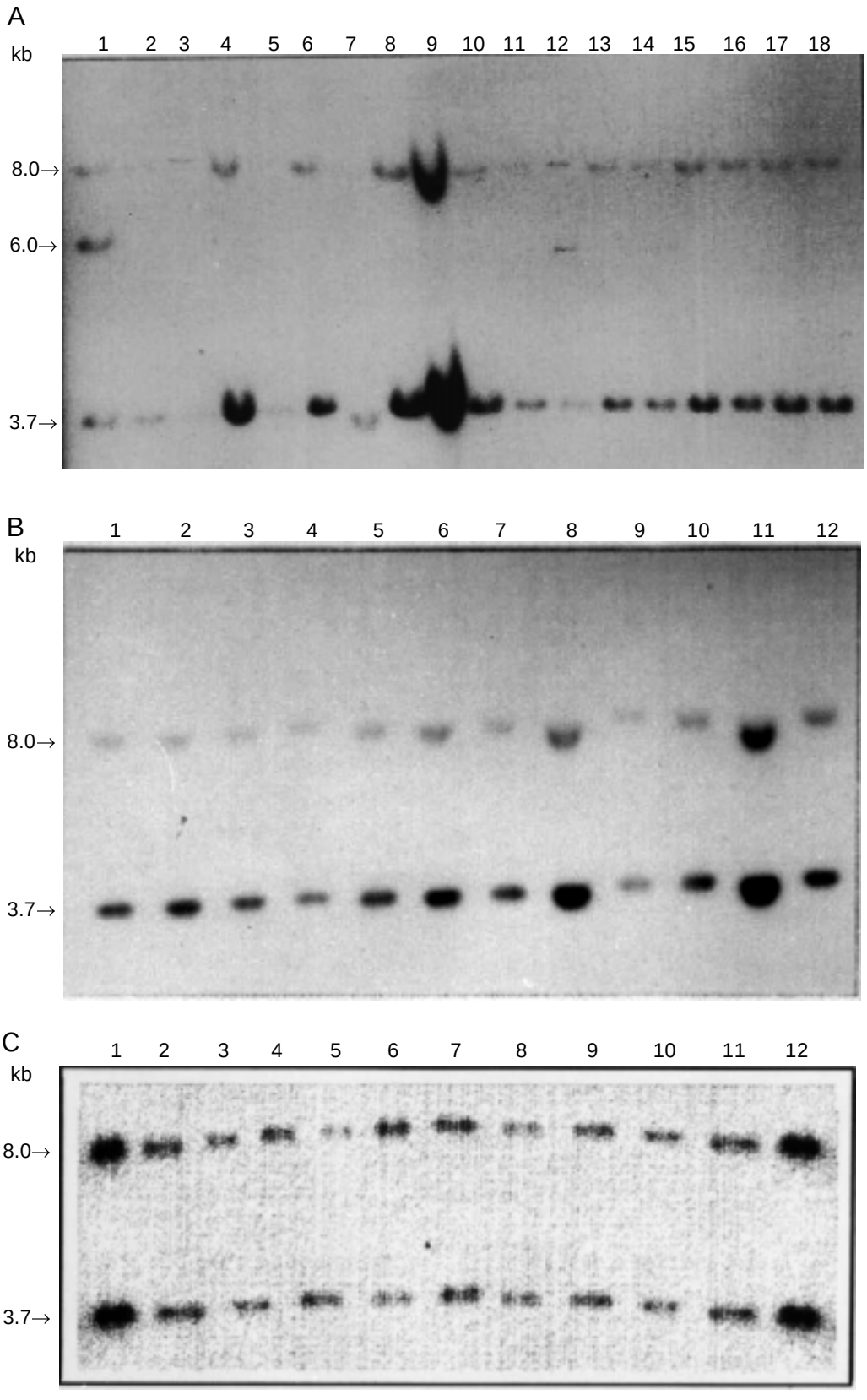

Figure 1 - Southern blots of genomic DNA samples digested with *Eco*RI and hybridized with the pVL8 probe. All samples from the normal individuals presented the 8.0- and 3.7-kb monomorphic fragments and the 6.0-kb polymorphic fragment (10% frequency) (A). The samples from rheumatoid arthritis (B) and systemic lupus erythematosus (C) patients presented only the 8.0- and 3.7-kb monomorphic fragments. Hybond N+ membranes (Amersham Pharmacia Biotech, Little Chalfont, England) were washed under high-stringency conditions (typical results).

homology evidence suggesting that we can detect the members of the IGLV8 gene family and establish an association between the absence of the 6.0-kb *Eco*RI IGLV8 fragment RFLP allele and rheumatoid arthritis or systemic lupus erythematosus.

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