A New HLA Extended Haplotype Containing the A*2910 Allele in Birdshot Retinochoroidopathy: Susceptibility Narrowed to the HLA Molecule Itself

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PURPOSE. Birdshot retinochoroidopathy (BSRC) is a rare posterior uveitis characterized by distinctive, multiple, hypopigmented choroidal and retinal lesions. Most, if not all, patients are white and share the major histocompatibility antigen HLA-A29. Furthermore, the A*2902 subtype is closely associated with BSRC, and only a very few patients share the A*2901 subtype. Surprisingly, although A*2901 and A*2902 differ only by a single mutation (D102H), studies of microsatellites located near HLAA have shown that two strong A*2901 and A*2902 extended haplotypes are observed in patients and control subjects. The present study analyzes the HLA-A extended haplotype of two patients who were HLA-A*2910 carriers.

METHODS. Among 180 patients who fulfilled internationally defined criteria for the diagnosis of BSRC and who were HLA-A29 subtyped, two patients were found to be HLA-A*2910 carriers. These patients were tested for the microsatellite alleles MOGa, -b, -c, and -e (of the myelin oligodendrocyte glycoprotein [MOG] gene) and D6S265, D6S510, RF, C5_a_4_5, and D6S105.

RESULTS. Although A*2902 and A*2910 differed by only a single mutation, (E177K) a new A*2910 extended haplotype was found to be distinct from the A*2901 and A*2902 extended haplotypes previously described in patients and control subjects. Among all studied microsatellite markers, no allele was shared by these extended haplotypes.

CONCLUSIONS. These results suggest that susceptibility to BSRC is linked to the histocompatibility HLA-A29 molecule itself, although the development of the disease also involves inherited or probably acquired factors not linked to the major histocompatibility complex. (Invest Ophthalmol Vis Sci. 2010;51:2525–2528) DOI:10.1167/iovs.09-4329

Birshot retinochoroidopathy (BSRC) is a rare posterior uveitis characterized by distinctive, multiple, hypopigmented choroidal and retinal lesions that were first identified by Ryan and Maumenee1 and are strongly associated with human leukocyte antigen HLA-A29. Criteria for the diagnosis of BSRC are based solely on clinical factors2 and an absolute requirement is the presence of “birdshot lesions” in both eyes. They are hypopigmented, at the level of the choroid, usually round or oval, most often one quarter to one half optic disc diameter in size, and clustered around the optic disc, nearly always with involvement of the inferior and nasal peripapillary area. Blurred vision and floaters are the most prevalent visual sympotoms. Patients may also report dyschromatopsia and poor contrast sensitivity. Macular edema is the most common cause of decline in visual acuity and ~10% of patients are legally blind.3

In a review, Shah et al.,4 with two exceptions (including a case report of a black woman in whom sarcoidosis was not adequately ruled out), reported that the patients with BSRC were white (337 patients, 14 articles), and 95.7% of 488 patients (36 articles) were HLA-A29 positive. The relative risk of BSRC among HLA-A29-positive individuals has been estimated to be 50 to 224, and most investigators recognize the presence of the HLA-A29 allele as a necessary criterion for BSRC diagnosis for research purposes. HLA-A29 is present in as many as 7% of whites and is subdivided in more than 20 subtypes—mostly A*2902 in whites and A*2901 in Asians. Even Asians who live in the same country as whites in Europe or in the United States appear to be exempt from BSRC. Moreover, African Americans living in North America and carrying A*2902 at a frequency of 3.57% appear to be immune from the disease. We found that gene sequences from all patients and healthy individuals sharing the A*2901 or A*2902 subtype were identical.5–7 Furthermore, the study of polymorphisms in the HLA-A region in patients and control subjects shown no differences but surprisingly defined two strong A*2901 and A*2902 extended haplotypes.7

We report in this study the discovery of two white patients exhibiting a clinical pattern typical of BSRC and sharing the recently described A*2910 subtype.8 In the second part of this study, we analyzed polymorphisms in the HLA-A region of these two patients. The microsatellite alleles tested were RF (GAA repeats, 250 kb to the telomeric side), MOGa and -b (CA repeats in intron 2 of the myelin oligodendrocyte glycoprotein [MOG] gene, located 400 kb to the telomeric side of the HLA-A locus), MOGe (CA repeats upstream of the MOG gene), MOGe (TAAA repeats in exon 8 of the MOG gene), D6S10 (CA-GA repeats, 27 kb to the centromeric side), and D6S265 (CA repeats, 100–70 kb to the centromeric side). The more remote microsatellite alleles C5_a_4_5 (TTTA repeats, 200 kb to the...
Materials and Methods

Patients and Healthy Subjects

We are the French reference laboratory in a BSRC study (http://www.orphanet.net). One hundred eighty patients with BSRC and 18 healthy HLA-A29-bearing subjects were analyzed for HLA-A29 subtypes. All patients were Caucasians. Two ophthalmologists independently confirmed that patients met internationally defined criteria for the diagnosis of BSRC.2 Healthy subjects were blood donors or volunteers. HLA-A*29 genotyping was performed in compliance with French law and in accordance with the Declaration of Helsinki for research involving human subjects.

HLA Typing

HLA class I (HLA-A, -B, and -C) typing was performed by polymerase chain reaction with sequence-specific oligonucleotides (PCR-SSO) with a commercial kit and device (Labtype*SSO; One Lambda, Canoga Park, CA) on genomic DNA extracted from peripheral blood. HLA-A29 subtyping was assessed by polymerase chain reaction with sequence-specific primers (PCR-SSP) with a commercial kit (SSP HLA-A*29; Olerup, Saltsjöbaden, Sweden).

Microsatellite Markers

Names, distances from the HLA gene, localization, and analysis of the microsatellite markers studied as well as PCR primers and conditions are depicted elsewhere.7 Briefly, genomic DNA was amplified by PCR with Taq DNA polymerase (ampliTaq DNA polymerase; Applied Biosystems, Inc. [ABI], Foster City, CA), amplified products were denatured for 7 minutes at 96°C and chilled on ice before being electrophoresed on 6% acrylamide gels with urea at 7 M followed by silver staining (Promega, Madison, WI). A 10-bp DNA ladder from 30 to 330 bp and a 25-bp DNA ladder from 25 to 500 bp (Invitrogen, Carlsbad, CA) were used to estimate allele sizes.

Results

Clinical Data and HLA Typing

Among 180 patients who fulfilled internationally defined criteria for the diagnosis of BSRC and who were HLA-A29 subtyped, two patients were found to be HLA-A*2910 carriers. In contrast, the HLA-A*2910 allele was not found among 18 healthy HLA-A*29 control subjects.

The first patient, patient 1, a 61-year-old white woman, was 53 years of age at the onset of the disease. Typical birdshot lesions were observed (Fig. 1). Her visual acuity was count-fingers in the right eye and 0.7 in the left eye. In addition to typical birdshot lesions, an optic neuropathy with a central scotoma was diagnosed in the right eye. Before her care in our center, she was treated by oral corticosteroid from 1999 to 2001 and by intravenous immunoglobulins from 2001 to 2004. The patient was subsequently not treated. HLA typing was A*29, 68 B*08,60 Cw*10, 12.

The second patient, patient 2, was a 71-year-old white woman who was 68 years of age at the onset of the disease. Typical birdshot lesions (Fig. 2) were present, and fluorescein angiography showed leakage from the retinal veins. Her visual acuity was 0.9 in the right eye and 1.0 in the left eye. She was treated by repeated subtenon injections of triamcinolone. Her HLA typing was A*01, 29 B*08,14 Cw*07, 08.

Microsatellite Markers

Table 1 shows the allele sizes of the different microsatellite markers for both A*2910-bearing patients and the results for A*2901 and A*2902 from our prior study7: The number of studied haplotypes was 6 in A*2902 healthy subjects, 12 in A*2902 BSRC patients, 11 in A*2901 healthy subjects, and 3 in A*2901 BSRC patients. Haplotypes from A*2902 patients and A*2902 healthy subjects shared the same allele sizes for D6S265, D6S510, MOGa, MOGb, MOGc, and MOGe markers. For the C5_4_5 marker the most frequent allele was present in all haplotypes from BSRC A*2902 patients and in half of A*2902 haplotypes in healthy subjects. For the RF marker the most frequent allele was present in half of the haplotypes from A*2902 BSRC patients and in one haplotype of the A*2902 healthy subjects. All studied A*2901 haplotypes shared the same alleles at each locus. For locus C5_4_5, D6S265, D6S510, RF, MOGb, MOGc, and D6S105, the two A*2910 haplotypes shared one common allele. For locus MOGa and MOGe, they shared two common distinct alleles.

Discussion

The strong association with HLA-A29, beneficial effects of immunosuppressive agents such as cyclosporine in BSRC, similar features with experimental autoimmune uveitis, make BSRC likely to be an autoimmune illness. HLA-A29 is subdivided into the telomeric side) and D6S105 (CA repeats, 1500–2500 kb to the centromeric side) were also studied.

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Discussion

The strong association with HLA-A29, beneficial effects of immunosuppressive agents such as cyclosporine in BSRC, similar features with experimental autoimmune uveitis, make BSRC likely to be an autoimmune illness. HLA-A29 is subdivided into
21 subtypes, but A*2901 and A*2902 are by far the most frequent alleles. BSRC is not restricted to the A*2902 subtype; about 10 A*2901-bearing patients have been identified (Refs. 7, 10, 11). To our knowledge, this is the first study to report BSRC patients with A*2910. This allele is characterized by a nucleotide substitution (adenine for guanine) in exon 3 at position 258, and this subtype therefore differs from A*2902 by only a single mutation (E177K) located at the exterior part of the T-cell receptor-binding site and should be functionally inactive. Comparative HLA class I sequence analysis demonstrates that Lys177 is absent in all HLA-A and HLA-B locus (http://www.anthonyonlans.org.uk). HLA-A consensus at this position is Glu, HLA-B consensus at this position is Asp, which both are acid amino-acyls. Of note, whereas the consensus for HLA-C for this position is Glu, more than 36 HLA-C alleles have Lys177. From family analysis and sequence comparisons, the HLA-A*2910 allele has arisen from intragenic recombination with HLA-C. HLA-C antigens are underrepresented at the cell membrane, probably because the HLA-C groove does not catch peptides with efficiency. Therefore, HLA-C alleles were difficult to identify by microlymphocytotoxicity (LCT) before the era of molecular biology. The HLA-A*2910 allele coded by the HLA-A*2910 allele was clearly identified by standard LCT, implying that the peptide-binding groove remained fully active.

We studied some of the polymorphisms available in the HLA-A region of these two patients by allele size measurement of microsatellite markers amplified by PCR. The results, compared with previous data, are summarized in Table 1. It is noteworthy that the two A*2910 patients did not share the ancestral haplotype HLA-A29, -B44, or -Cw16 and were HLA-A heterozygous (second HLA-A alleles were A68 and A1 for patients 1 and 2, respectively). Every microsatellite markers studied shared a common allele, so that the A*2910 extended haplotype can be assumed. Even if the A*2910 extended haplotype cannot be defined at the MOGa and MOGb loci unambiguously because the two patients shared the same two alleles, the A*2910 extended haplotype clearly differs from the previously defined A*2901 and A*2902 extended haplotypes. Although A*2910 and A*2902 differ by only one mutation (E177K) and A*2902 and A*2901 by one mutation (D102H), their surrounding microsatellite polymorphisms appear to be fully different.

The pathophysiological mechanism of BSRC is still unknown. The inner retina may be involved, but the lesions are believed to be located in the choroid. The S-antigens and interphotoreceptor retinoid-binding protein are located in the outer retina, and portions of the S-antigen have been reported to bind to the HLA molecule in vitro. Whether retinal antigens are likely to be the inciting factors in BSRC has not been determined. However, these antigens could play a role later, as downstream events or the immune response to these antigens may reflect a bystander phenomenon in which peptides released by inflammation become autoantigens playing a role in spreading the autoimmune response. Although the direct role of natural killer (NK) cells in BSRC pathogenesis is not clear, genetic data indicate that killer cell immunoglobulin-like receptors (KIR)-HLA combination are likely to influence both cytotoxic T lymphocytes and NK cell responses toward autoimmunity in BSRC patients. The fact that BSRC is so uncommon while as much as 7% of the white population is HLA-A29 positive remains unexplained. Finding patients with the A*2910 subtype in addition to the previously described A*2901-

| Allele Sizes of the Different Microsatellite Markers for the A29 Subtypes |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | C5_4_5          | D6S265          | D6S510          | RF              | MOGa            | MOGb            | MOGe            | MOGe            |
| A*2901           | 307             | 190             | 170             | 128             | 160             | 132             | 218             | 124             |
| A*2902           | 299†            | 126             | 190             | 327†            | 126             | 160             | 122             | 222             |
| A*2910 No. 1    | 315307          | 12126           | 194190          | 164             | 126130          | 162180          | 132134          | 218230          |
| A*2910 No. 2    | 315303          | 120122          | 194178          | 164276          | 126130          | 162138          | 218230          | 116140          |

Sizes are expressed in base pairs. Shown in parentheses are distances in megabases from the HLA-A locus to the marker (negative values imply centromeric locations in relation to HLA-A). A*2901 and A*2902 results are from both healthy and BSRC subjects studied previously. A*2910 No. 1 and No. 2 are two A*2910 patients.

† The most frequent allele if not 100%. Slash separates the two allele sizes for patients 1 and 2, when necessary.
and A*2902-bearing patients, should rule out a different presentation of a putative peptide by each subtype.

We still cannot explain the selection pressure leading to similar A*2901, A*2902, and A*2910 alleles with haplotypes that are so different. The fact that no allele of any studied microsatellite markers in A*2910 subjects was shared with A*2901 and A*2902 patients reinforced the idea that susceptibility to BSRC relies on the HLA-A29 molecule itself.

Most Caucasians express the A*2902 subtype, whereas Asians express the A*2901 subtype. A*2910 has been identified in a 8-year-old Turkish girl with leukemia when a family-related stem cell donor was being sought. Its frequency in major ethnic groups remains unknown. As previously discussed: (1) HLA-A*2901 extended haplotypes from white patients and Asian healthy subjects seem identical; (2) even though living in the same country as whites in Europe or the United States, Asians seem to be exempt from BSRC; (3) both the A*2901 and A*2902 extended haplotypes seem to be identical in patients with BSRC or in healthy individuals; and (4) the fact that a new distinct A*2910 extended haplotype has been herein described, altogether reinforce the necessity for another factor to trigger the autoimmune reactivity in HLA-A29 subjects, which is not genetically linked to the major histocompatibility complex, including KIR. An acquired triggering element on that genetic background also cannot be ruled out.

References

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