European Journal of Ophthalmology / Vol. 13 no. 5, 2003 / pp. 433-438

Genetic polymorphism of HLA DR in a Scottish population of patients with pars planitis

K.H. GREINER[1], D.W.L. WILSON[2], J. THOMSON[2], D.J. KILMARTIN[1], S.J. URBANIAK[2], J.V. FORRESTER[1]

[1]Department of Ophthalmology, Grampian University Hospitals
[2]Tissue Typing Laboratory, Aberdeen and Northeast Scotland Transfusion Service, Aberdeen, Scotland - UK

PURPOSE. Human leucocyte antigen (HLA) class II influences the immunological susceptibility for a variety of diseases including many types of non-infectious intraocular inflammation. Previous studies on North American patients with pars planitis, a subtype of intermediate uveitis, reported an increased prevalence of HLA DR15 in this population. In contrast, two European studies could not find an association between HLA DR2 or its allelic subtype DR15 and various forms of intermediate uveitis. We therefore investigated the genotype frequency of HLA DR alleles in a Scottish population of patients with typical pars planitis.

METHODS. Twenty patients with pars planitis were identified from the uveitis database of Grampian Universitiy Hospitals. Only patients with bilateral vitritis and snowbanks in at least one eye in the absence of systemic disease were included in the study. Fifteen patients and 34 healthy controls underwent HLA DR genotyping for all DRB genes using PCR sequence specific primers.

RESULTS. HLA DR15 was found in 13% of patients with pars planitis and in 24% of controls. There was no statistically significant difference between these two groups. Furthermore, the frequencies of HLA DR 1, 3-14, and 16 did not differ significantly between patients and controls.

CONCLUSIONS. There appears to be no association between the occurrence of pars planitis and the HLA DR15 or other known HLA DR genotypes in Scottish patients. However, the small sample size limits the power of this study. (Eur J Ophthalmol 2003; 13: 433-8)

KEY WORDS. Pars planitis, Uveitis, HLA DR, Genotyping

Accepted: January 27, 2003

INTRODUCTION

The gene products of the human leucocyte antigen (HLA) complex play a pivotal role for the regulation of the immune response. HLA molecules and their associated peptides interact with the antigen-specific T-cell receptor on T-cell precursors in the thymus during the selection process to shape the T-cell repertoire of individuals (1). In adults, HLA molecules influence the immune response to exogenous antigens by their peptide binding preferences which determine the antigen-derived peptides displayed to T-cells (2). It its very likely that their peptide binding preferences also influence immune responses to self proteins.

Several eye diseases are associated with HLA antigens. Diabetic retinopathy (HLA DQB1*0201/0302) (3), ocular cicatricial pemphigioid (HLA B12) (4), optic neuritis (HLA DR2) (5, 6) and many forms of uveitis are

This study was presented at the ARVO 2002 Meeting in Fort Lauderdale, USA

1120-6721/433-06$03.00/0

some examples. The associations between birdshot chorioretinopathy and HLA A29.2 (7, 8), Behcet syndrome and HLA B*5101 (9), and some forms of anterior uveitis and HLA B27 are well known (10, 11).

Pars planitis is a subgroup of intermediate uveitis and characterized by vitritis, snowbank exudates, vitreous condensation over the inferior peripheral retina and pars plana, usually in both eyes. Peripheral retinal vasculitis is also frequently found (12). The cause and pathogenesis of pars planitis are unknown particularly with regard to the characteristic formation of the large "snowbanks" which appear to be accumulations of exsudative material involving choroid, retina and vitreous gel in the region of the vitreous base. Pars planitis has been reported to both precede and follow the diagnosis of multiple sclerosis (13-15). The link between multiple sclerosis and HLA DR2 has been well established (16-18). These associations stimulated the interest for studying the link between pars planitis and HLA DR2 or its subtypes DR15 and DR16.

HLA class II molecules are heterodimeric membrane proteins from the DR, DP and DQ locus of chromosome 6. HLA DR molecules consist of a nonpolymorphic alpha chain and highly polymorphic beta chains (19). Nomenclature to define DR molecules, therefore, takes into account only the beta chain coded in the DRB locus. Evidence that expression of HLA class II alleles directly influences autoimmunity has been obtained in mice transgenic for HLA class II alleles (20, 21). Interestingly, in Goodpastures syndrome with a positive association with HLA DR15, there is a dominant negative association with DR7 and DR1 demonstrating the variability of HLA DR effects (22).

Two studies with well-defined patient populations in the USA (Caucasian or white patients) reported an association between HLA DR15 and pars planitis based on genotype analysis (23, 24). In another paper by Malinowski et al (25), HLA DR2 expression was linked to an increased risk for pars planitis. Tang et al (26) described an association between HLA DR15 and intermediate uveitis. However, these two latter studies involved ethnicly mixed patient populations and used serologic or microlymphocytotoxicity tests for HLA-typing.

In contrast to these investigations in North America, two European studies could not detect any association between HLA DR2/DR15 and intermediate uveitis including pars planitis (27, 28). However, in both studies, the ethnic origin of the patient population was not well defined. Furthermore, both studies performed lymphocytotoxicity tests for HLA typing. To provide data compatible with the results of the US studies we, thus, determined frequencies of all previously identified HLA DR genotypes within a well-defined population of patients with pars planitis in Scotland.

PATIENTS AND METHODS

A review of the uveitis data base of the Grampian University Hospitals in Aberdeen was conducted and 20 consecutive Scottish (Caucasian) patients with pars planitis identified from the period 1996-2000. The patients underwent a full ophthalmological examination and ultrasound biomicroscopy to identify and quantify the level of pars plana exsudates (29). The Bioscore (degree of vitreous opacification Grade 0-5 modified from Nussenblatt et al (30) as described in (31)) was determined to quantify the inflammatory activity. The inclusion criteria for this study were: vitritis, snowbanks in at least one eye and vitreous cells or debris in the fellow eye. The subclassification of patients into groups with mild and severe disease was based on the occurence of complications (evidence of macular edema).

Patients with a concomittant or anamnestic systemic disease were excluded from the study. For this purpose, patients underwent a comprehensive physical examination, a chest radiograph and laboratory tests to exclude systemic autoimmune disorders, sarcoidosis, tuberculosis, syphilis and acute infectious diseases. Serologic test for Lyme disease was performed in patients with a history of deer tick exposure. Based on clinical histories, patients with multiple sclerosis or with a first-degree relative with multiple sclerosis were excluded from the study. Fifteen patients fulfilling the above criteria were included in the study. The control group was composed of 34 age and sex matched randomly selected healthy blood donors from the same ethnic background (Scotland).

Following informed consent, 10 ml of peripheral blood were taken from patients and controls for HLA DR typing. DNA was extracted using the salt extraction method (32). HLA DR typing was performed using polymerase chain reaction sequence specific primer sets (PCR-SSP) (Allset and Classic PCR-SSP, Dynal Ltd, Liverpool) based

on the method by Olerup and Zetterquest (33). We assayed all alleles of the HLA DR beta chain so far identified. HLA DR types were assigned based on the PCR-SSP patterns using WHO nomenclature (34).

Statistical analysis was based on the Fisher's exact test with Bonferroni adjustments for multiple tests. Significance was attributed when the p value was less than 0,05. Estimations of type II error and power were performed with the NCSS / PASS 2000 software (NSCC, Kaysville, Utah, USA).

RESULTS

Fifteen patients were enrolled into the study. The clinical characteristics of the study population are summarized in Table I. Four patients had evidence of macular edema. In three patients, a cataract was documented.

All 15 patients had HLA DR genotyping. The frequencies of assayed HLA DR alleles for patients and controls are given in Table II. Two of 15 patients (13%) with pars planitis and eight of 34 controls (24%) were HLA DR15 positive ($p>0.05$). All HLA DR15 positive patients and controls were further subtyped and found to be HLA DR1501. There were no significant differences in the frequencies of the other examined HLA DR alleles between the patient and the control population.

Since the number of patients and controls was small, the chance for a type II error in this study is large. For cross tabulation analysis of HLA DR15 frequencies in patients and controls, a type II error of 0.86 was determined. A type II error of this size does not allow definitive conclusions about the association of pars planitis with HLA DR15. Two previous studies reported a positive association of HLA DR15 and pars planitis (23, 24). Therefore, we compared the frequency of HLA DR15 observed in our patient population with those in these studies. The frequency of HLA DR15 in our patient population (13.3%) was significantly lower ($p<0.05$) than a frequency of 46.9% in patients observed by Raja et al (23) and a frequency of 64.3% in patients as reported by Oruc et al (24). An estimation of type II errors of these comparisons yielded values of 0.5 and 0.16, respectively.

Despite the relatively small sample size, we performed a subclassification of the patient population into groups with mild and severe disease. One of the four patients with severe disease had a HLA DR15 genotype. This frequency did not differ significantly from the HLA DR15 frequency in the healthy control population and in the group of patients with mild disease. The frequency of the other genotypes was also not significantly different between the three groups (healthy controls, patients with mild and severe disease).

TABLE I - CLINICAL CHARACTERISTICS OF THE STUDY POPULATION

Patient	Lens R/L	Macula R/L	Bioscore R/L
01*	clear / clear	ERM / n	0 / 0
02	clear / clear	CMO / n	0 / 0
03	clear / clear	n / n	1 / 1
04	IOL / clear	n / n	1 / 0
05	clear / clear	n / n	0 / 0
06	clear / clear	n / n	0 / 0
07*	clear / clear	n / n	1 / 0
08	clear / clear	CMO / n	1 / 0
09	clear / IOL	n / CMO	0 / 2
10	clear / clear	n / n	0 / 0
11	clear / clear	n / n	1 / 1
12	clear / clear	n / n	0 / 0
13	clear / clear	n / n	0 / 0
14	clear / CAT	n / n	0 / 0
15	clear / clear	n / n	0 / 0

* indicates patients with the HLA DR15 genotype.
n=Normal, CMO=Cystoid macular edema, ERM=Epiretinal membrane

TABLE II - TOTAL NUMBERS OF DETECTED HLA DR ALLELES (HLA DR 11 and 12 are subtypes of HLA DR5, HLA DR 13 and 14 are subtypes of HLA DR6, HLA DR 15 and 16 are subtypes of HLA DR2)

	Patients (n=15)	Controls (n=34)
HLA DR	n	n
01	3	9
03	7	9
04	4	9
07	6	11
08	2	2
09	0	1
10	0	2
11	1	5
12	1	1
13	1	5
14	2	2
15	2	8
16	0	0

Furthermore, we investigated whether the HLA DR15 genotype is correlated with an increased inflammatory activity as determined by the Bioscore. There was no significant difference in the Bioscore between patients with and without the HLA DR15 genotype.

DISCUSSION

The understanding of the pathophysiologic mechanisms of pars planitis is still incomplete. Histopathologic examinations of snowbanks revealed inflammatory cells expressing HLA DR antigens and glial cells, predominantly Müller cells (35, 36). Müller cells themselves can express HLA class II antigens (37). The abundance of HLA class II expressing cells in the inflammatory exsudates in pars planitis suggest an immunomodulatory role of HLA class II for this disease. Boyd et al (38) speculated that an antecedant infection might have initiated the disease through the process of molecular mimicry. The induction and onset of the disease could depend on the HLA type of the patients possibly predisposing them to a molecular mimicry.

We studied the genetic polymorphism of HLA DR in a Scottish population of patients with pars planitis using a high-resolution screening method for detecting allelic subtypes. We did not find an association between the assayed HLA DR alleles and neither the occurrence nor the severity of pars planitis. Due to strict inclusion criteria, the sample size in our study was relatively small. The small number of patients and controls increased the chance for a type II error and reduced the power of this study. However, our results are well in accordance with two studies on the expression of HLA DR 2 or its subtype DR15 in mixed populations from Europe involving patients with pars planitis or intermediate uveitis (27, 28).

The importance of patient selection is emphasized by the fact that HLA frequencies are known to vary greatly in different geographic areas, races and ethnic groups. Interestingly, the same disease (type I diabetes) has been associated with different HLA types in Africans and Caucasians (39). The reported frequencies for HLA DR15 range between 14,5% in Iran (40) and 43% in Cameroon (39). The HLA DR15 frequency for Caucasians is approximately 20-28% (23, 24, 26). The HLA DR15 prevalence in our control population from Scotland (23,5%) is well in accordance with these data.

Two genotype studies on Caucasian populations of patients with pars planitis from Maryland and Missouri (23, 24) reported a link between HLA DR15 and pars planitis. A direct comparison of the patient populations in these reports with the patient population in our study revealed significant differences of the HLA DR15 frequencies. There could be several reasons for this discrepancy. The study performed by Oruc et al (24) was compromised by the small sample size as compared to the number of initially identified patients with pars planitis. Notably, out of 110 initially selected patients with pars planitis only 28 patients could be enrolled into the study. This selection might have introduced a bias into the patient recruitment. In the largest study on pars planitis involving 32 patients, Raja et al (23) reported only a modest increase of the risk for pars planitis (OR = 2.86) in patients with HLA DR15. In this study, 46.9% of patients with pars planitis exhibited the HLA DR15 genotype as compared to 23.6% in controls. Although the published p-value for comparing both frequencies was 0.004, an application of the Bonferroni adjustment for multiple tests (n=14 in this paper) would have resulted in a p-value of 0.056.

Interestingly, 68% of the patients in the study by Raja et al (23) had developed macular edema. This profile of disease severity is in contrast to the profile seen in our patient population (27% macular edema). These data might indicate a different practice of patient referral. Whereas Raja et al recruited patients seen in the Wilmer Ophthalmological Institute, we have studied an unselected patient group from a large region in Scotland which provides a good estimate of the prevalence and severity of pars planitis within the population of that region. Furthermore, 18.9% of the patients studied by Raja et al (23) had multiple sclerosis. Multiple sclerosis has been associated with HLA DR2 (17, 18). Importantly none of our patients nor their first degree relatives had multiple sclerosis. This contrast suggests further genetic differences between the patient populations.

Other HLA DR alleles studied in patients with pars planitis or intermediate uveitis were HLA DR 1, 3-18 and 51-53 (23, 24, 27, 28). Oruc et al (24) found a significantly increased prevalance of HLA DR17, which is a subtype of HLA DR3, and HLA DR51 in patients with pars planitis. Our negative results for these HLA DR alleles are in accordance with the other papers (23, 27, 28).

Despite discrepancies concerning an association of pars planitis with HLA DR15, all studies published so far identified a large subgroup of patients without HLA DR15. Thus, it remains to be elucidated whether environmental risk factors such as the typical repertoire of infections within a geographic region might trigger the inflammatory reaction seen in pars planitis.

Reprint requests to:
Kathrin H.Greiner, MD
Department of Ophthalmology
Grampian University Hospitals
Medical School, Foresterhill
Aberdeen AB25 2ZD, Scotland, UK
k.greiner@abdn.ac.uk

ACKNOWLEDGEMENTS

The authors thank Peter Hufnagl for advice with the statistical analysis.

REFERENCES

1. Sebzda E, Mariathasan S, Ohteki T, Jones R, Bachmann MF, Ohashi PS. Selection of the T cell repertoire. Ann Rev Immunol 1999; 17: 829-74.
2. Buus S, Sette A, Colon SM, Miles C, Grey HM. The relation between major histocompatibility complex (MHC) restriction and the capacity of Ia to bind immunogenic peptides. Science 1987; 235: 1353-8.
3. Agardh D, Gaur LK, Agardh E, Landin-Olsson M, Agardh CD, Lernmark A. HLA-DQB1*0201/0302 is associated with severe retinopathy in patients with IDDM. Diabetologia 1996; 39: 1313-7.
4. Mondino BJ, Brown SI, Rabin BS. HLA antigens in ocular cicatricial pemphigoid. Arch Ophthalmol 1979; 97: 479.
5. Francis DA, Compston DA, Batchelor JR, McDonald WI. A reassessment of the risk of multiple sclerosis developing in patients with optic neuritis after extended follow-up. J Neurol Neurosurg Psychiatry 1987; 50: 758-65.
6. Morrissey SP, Borruat FX, Miller DH, et al. Bilateral simultaneous optic neuropathy in adults: clinical, imaging, serological, and genetic studies. J Neurol Neurosurg Psychiatry 1995; 58: 70-4.
7. Nussenblatt RB, Mittal KK, Ryan S, Green WR, Maumenee AE. Birdshot retinochoroidopathy associated with HLA-A29 antigen and immune responsiveness to retinal S-antigen. Am J Ophthalmol 1982; 94: 147-58.
8. Tabary T, Lehoang P, Betuel H, et al. Susceptibility to birdshot chorioretinopathy is restricted to the HLA-A29.2 subtype. Tissue Antigens 1990; 36: 177-9.
9. Brewerton DA, Albert E. Rheumatology. Behçets disease. In: Dausset J, Svejgaard A, eds. HLA and Disease. Baltimore: Williams & Wilkins, 1977; 99-102.
10. Brewerton DA, Caffrey M, Nicholls A, Walters D, James DC. Acute anterior uveitis and HL-A 27. Lancet 1973; 2: 994-6.
11. Lyons JL, Rosenbaum JT. Uveitis associated with inflammatory bowel disease compared with uveitis associated with spondyloarthropathy. Arch Ophthalmol 1997; 115: 61-4.
12. Malinowski SM, Pulido JS, Folk JC. Long-term visual outcome and complications associated with pars planitis. Ophthalmology 1993; 100: 818-24
13. Berger BC, Leopold IH. The incidence of uveitis in multiple sclerosis. Am J Ophthalmol 1966; 62: 540-5.
14. Giles CL. Peripheral uveitis in patients with multiple sclerosis. Am J Ophthalmol 1970; 70: 17-9.
15. Nissenblatt MJ, Masciulli L, Yarian DL, Duvoisin R. Pars planitis - a demyelinating disease? Arch Ophthalmol 1981; 99: 697.
16. Chester GH, Blach RK, Cleary PE. Inflammation in the region of the vitreous base. Pars planitis. Trans Ophthalmol Soc U K 1976; 96: 151-7.
17. Duquette P, Decary F, Pleines J, Boivin D, Lamoureux G, Cosgrove JB, Lapierre Y. Clinical sub-groups of multiple sclerosis in relation to HLA: DR alleles as possible markers of disease progression. Can J Neurol Sci 1985; 12: 106-10.
18. Kellar-Wood HF, Wood NW, Holmans P, Clayton D, Robertson N, Compston DA. Multiple sclerosi and the HLA-D region: linkage and association studies. J Neuroimmunol 1995; 58: 183-90.
19. Davey MP, Rosenbaum JT. The human leukocyte antigen complex and chronic ocular inflammatory disorders. Am J Ophthalmol 2000; 129: 235-43.
20. Rosloniec EF, Brand DD, Myers LK, et al. Induction of autoimmune arthritis in HLA-DR4 (DRB1*0401) transgenic mice by immunization with human and bovine type II collagen. J Immunol 1998; 160: 2573-8.
21. Taneja V, David CS. HLA transgenic mice as humanized mouse models of disease and immunity. J Clin Invest 1998; 101: 921-6.

22. Phelps RG, Jones V, Turner AN, Rees AJ. Properties of HLA class II molecules divergently associated with Goodpasture's disease. Int Immunol 2000; 12: 1135-43.
23. Raja SC, Jabs DA, Dunn JP, et al. Pars planitis: clinical features and class II HLA associations. Ophthalmology 1999; 106: 594-9
24. Oruc S, Duffy BF, Mohanakumar T, Kaplan HJ. The association of HLA class II with pars planitis. Am J Ophthalmol 2001; 131: 657-9
25. Malinowski SM, Pulido JS, Goeken NE, Brown CK, Folk JC. The association of HLA B8, B51, DR2, and multiple sclerosis in pars planitis. Ophthalmology 1993; 100: 1199-205
26. Tang WM, Pulido JS, Eckels DD, Han DP, Mieler WF, Pierce K. Association of HLA-DR15 and intermediateuveitis. Am J Ophthalmol 1997; 124: 70-5.
27. Arocker-Mettinger E, Georgiew L, Grabner G, Mayr WR. Intermediate uveitis: do HLA antigens play a role? Dev Ophthalmol 1992; 23: 15-9.
28. Martin T, Weber M, Schmitt C, et al. Association of intermediate uveitis with HLA-A28: definition of a new systemic syndrome? Graefes Arch Clin Exp Ophthalmol 1995; 233: 269-74.
29. Greiner KH, Kilmartin DJ, Forrester JV, Atta HR. Grading of pars planitis by ultrasound biomicroscopy - echographic and clinical study. Eur J Ultrasound 2002; 15: 139-44.
30. Nussenblatt RB, Palestine AG, Chan CC, Roberge F. Standardization of vitreal inflammatory activity in intermediate and posterior uveitis. Ophthalmology 1985; 92: 467-71.
31. Forrester JV, Okada AA, Benezra D, Ohno S. Posterior segment intraocular inflammation guidelines. The Hague: Pubs Kugler Publications, 1998; 176-7.
32. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16: 1215.
33. Olerup O, Zetterquest H. HLA-DRB1*01 subtyping by allele specific PCR amplification: a sensitive, specific and rapid technique. Tissue Antigens 1991; 37: 197-204.
34. Robinson J, Malik A, Parham P, Bodmer JG, Marsh SG. IMGT/HLA database - a sequence database for the human major histocompatibility complex. Tissue Antigens 2000; 55: 280-7.
35. Green WR, Kincaid MC, Michels RG, Pederson JE, Kenyon KR, Maumenee AE. Pars planitis. Trans Ophthalmol Soc UK 1981; 101: 361-7.
36. Wetzig RP, Chan CC, Nussenblatt RB, Palestine AG, Mazur DO, Mittal KK. Clinical and immunopathological studies of pars planitis in a family. Br J Ophthalmol 1988; 72: 5-10.
37. Roberge FG, Caspi RR, Chan CC, Kuwabara T, Nussenblatt RB. Long-term culture of Muller cells from adult rats in the presence of activated lymphocytes/monocytes products. Curr Eye Res 1985; 4: 975-82.
38. Boyd SR, Young S, Lightman S. Immunopathology of the noninfectious posterior and intermediate uveitides. Surv Ophthalmol 2001; 46: 209-33.
39. Mbanya JC, Pani LN, Mbanya DN, Sobngwi E, Ngogang J. Reduced insulin secretion in offspring of African type 2 diabetic parents. Diabetes Care 2000; 23: 1761-5.
40. Amirzargar A, Mytilineos J, Farjadian SH, et al. Human leukozeyte antigen class II allele frequencies and haplotype associations in Iranian normal population. Hum Immunol 2001; 62: 1234-8.