CLINICAL STUDY

Does the shared epitope genotype influence either the susceptibility to or the phenotype of corneal melting?

M. McKIBBIN, B. CLARK, J.D. ISAACS, A.J. MORRELL, B. GRIFFITHS, A.W. MORGAN, H.C. GOOI

Abstract

Purpose **To investigate the role of the shared epitope alleles in determining susceptibility to and the phenotype of corneal melting in patients with rheumatoid arthritis (RA).**
Methods **The HLA class 1 and 2 genotype was determined for 17 patients with rheumatoid-associated corneal melting by the phototyping method. HLA-DR4 subtyping was performed by PCR sequence-based typing. The frequency of all the shared epitope alleles and, in particular, of the higher-risk *0401 and *0404 alleles, was compared with healthy controls and unrelated RA patients, with and without extra-articular manifestations. A comparison was also made between the shared epitope genotype of the corneal melt patients and local, ocular disease characteristics.**
Results **Thirteen (76%) patients with corneal melt possessed at least one shared epitope allele and 5 (29%) possessed two alleles. The dominant alleles were variants of the DR4 family, notably the *0401, *0404 and *0408 alleles. Both the allele frequency and a double dose of shared epitope alleles were more common in the three RA patient groups than in the healthy, control group ($p < 0.005$). Although the frequency of the higher-risk alleles was similar in the three RA patient group, a trend existed for a double dose of higher-risk alleles to be more common in the patients with either corneal melt or other extra-articular manifestations ($p > 0.2$). No association was found between the number or type of shared epitope alleles and any of the ocular disease characteristics studied.**
Conclusions **The results of this study suggest that the shared epitope alleles do not influence the ocular disease phenotype of corneal melt in RA patients. Shared epitope determination of RA patients may help to identify those susceptible to either corneal melt or other extra-articular disease. RA patients with a double dose of higher-risk alleles may have an increased risk of corneal melt.**

M. McKibbin
A.J. Morrell
Department of Ophthalmology
St James's University Hospital
Leeds, UK

J.D. Isaacs
B. Griffiths
A.W. Morgan
Department of Rheumatology
St James's University Hospital
Leeds, UK

B. Clark
H.C. Gooi
Department of Tissue Typing
St James's University Hospital
Leeds, UK

Mr Martin McKibbin ✉
Eye Clinic
St James's University Hospital
Beckett Street
Leeds LS9 7TF, UK
e-mail: martinmckibbin@cswebmail.com

Funded by a grant from the Northern and Yorkshire Regional Health Authority

Presented at the Oxford Ophthalmological Congress, July 2000

Received: 11 September 2000
Accepted in revised form: 5 February 2001

Key words Corneal melt, Disease susceptibility, Shared epitope, Ulcerative keratitis

Part of the genetic susceptibility to rheumatoid arthritis (RA) is conferred by possession of a common sequence in the third hypervariable region of the HLA-DRB1 gene, known as the shared epitope.[1–3] Class 2 genes are normally expressed only on cells with immune function, such as antigen presenting cells and activated T cells. The shared epitope sequence forms part of at least 14 different DRB1 alleles, including some, but not all, of the DRB1*01, *04, *10 and *14 subtypes.[3] Although the alleles containing the shared epitope sequence are present in the healthy population, their prevalence is greatly increased in RA patients from all ethnic groups and they are found in approximately 70% of Western RA patients.[2]

In addition to increasing the susceptibility to RA, the shared epitope alleles may also influence the prognosis for RA patients and identify disease subtypes with distinct clinical profiles. For example, some HLA-DR4 variants are associated with nodular disease, seropositivity, early radiological erosions and extra-articular manifestations.[4–6] However, not all shared epitope alleles are equal in terms of risk and a hierarchy exists, with the DRB1*0401 and *0404 alleles being higher risk and most strongly associated with the aforementioned features of severe disease whereas the DRB1*0101, *0102 and *1001 alleles carry a lower risk.[3,7] As with RA susceptibility, possession of two shared epitope alleles, a double dose, confers greater risk than either of the constituent alleles alone.[5,8] The highest-risk genotype contains either the *0401 or *0404 alleles on each haplotype.[2,6]

Necrotising keratitis or corneal melting occurs most commonly in association with a systemic vasculitis, especially RA. Both the central or paracentral and the peripheral forms of corneal melting are characterised by ulceration and rapidly progressive loss of the

corneal stroma. The pathogenesis of corneal melting is poorly understood but a variety of factors are common to both anatomical forms, including keratoconjunctivitis sicca, lid margin disease, inflammatory cell infiltrate, a local vasculitis and an underlying systemic disease association.[9,10] Corneal melting affects a minority of patients with RA. It typically occurs in seropositive patients with other extra-articular manifestations and end-stage joint disease, but often many years after the original diagnosis. The onset of corneal melting is an ominous sign, with the eye acting as a marker for the other manifestations of systemic vasculitis and for reduced life expectancy.[10]

To date, it has not been possible to predict which patients are at risk of corneal melting. Both ocular surface disease and RA are common, but only a minority of patients with both problems develop corneal melting. Given the association of the shared epitope with RA and, in particular, with the extra-articular manifestations of RA, our objective was to determine the presence of the shared epitope alleles in patients with rheumatoid corneal melt. By comparing the shared epitope frequency with that in other RA patients, we planned to establish whether shared epitope determination might help to identify RA patients who are most at risk of corneal melting and whether patients with corneal melting represent a distinct subgroup of patients with extra-articular disease. In addition, using the clinical features of the ocular disease, we planned to investigate whether the shared epitope alleles may determine the ocular disease phenotype.

Patients and methods

After ethics committee approval had been obtained, patients in the Yorkshire Region with a diagnosis of rheumatoid-associated sterile corneal melting were invited to take part in the study. Clinical data, including the anatomical site of the melt, the number of eyes affected and the presence of a prior contact lens cornea (peripheral corneal thinning with an intact epithelium) was determined from case note review, patient interview and slit-lamp examination.

DNA was extracted from peripheral blood lymphocytes as previously described.[11] At a concentration of 0.1 μg/μl, the DNA was subjected to polymerase chain reaction (PCR) amplification in a GeneAmp PCR 9600 (Perkin Elmer) or PTC-100 machine. The products were analysed by electrophoresis on agarose gels and visualised by ethidium bromide staining. A molecular weight marker ranging from 100 bp to 600 bp was used to determine the fragment sizes. HLA class 1 and class 2 typing of the corneal melt patients was performed at the molecular level using the phototyping technique, a PCR sequence-specific priming method which allows the simultaneous determination of HLA-A, -B, -C, -DR and -DQ specificities in a patient sample.[12] HLA-DR4 subtyping was performed by the method of PCR sequence-based typing on an Amersham-Pharmacia ALF Express II system using the HLA-DRB plus reagents (Amersham-Pharmacia) according to the manufacturer's instructions.

At present, the shared epitope sequence has been found in the following alleles: DRB*0101, *0102, *0401, *0404, *0405, *0408, *0409, *0410, *0413, *0416, *0419, *0421, *1001 and *1402. The alleles DRB*0101 and *0102 were not distinguished by the phototyping method and are referred to collectively as *0101/2. The shared epitope genotype and the allele frequency of all shared epitope alleles and of the *0401 and *0404 alleles (number of times the test allele occurs in the population divided by the total number of alleles) was determined for the patients with rheumatoid corneal melting. To investigate an association with susceptibility to corneal melting, a comparison was made with three groups of unrelated and ethnically matched United Kingdom subjects without corneal melt, namely healthy blood donor controls, RA patients without extra-articular disease features and RA patients with extra-articular features.[13] (The following were included as extra-articular disease features: subcutaneous nodules, vasculitis, pulmonary fibrosis and peripheral neuropathy.) For statistical analysis, the frequency and possession of a double dose of either the shared epitope alleles or the higher-risk *0401 and *0404 alleles was compared for the control and patient groups. To assess the role of the shared epitope alleles in determining the ocular phenotype, a comparison was also made between the shared epitope genotype of corneal melt patients and ocular disease characteristics, namely the presence of central or peripheral disease, contact lens cornea prior to the acute melt and unilateral or bilateral disease.

Statistical analysis was performed using the SPSS 9.0 Windows package, using 2×2 contingency tables and the Pearson chi-square test for comparison.

Results

Seventeen rheumatoid factor positive, Caucasian RA patients were recruited into the study, of whom 10 were female. Fourteen patients had peripheral corneal melting, of whom 5 had bilateral disease. One of the two patients with central or paracentral melting had evidence of bilateral disease. Another patient initially presented with a paracentral melt and then developed a peripheral melt in the same eye.

In the patients with corneal melt, the frequency of most of the HLA class 1 and 2 genes was similar to that in the United Kingdom population and so no new HLA class 1 and 2 disease associations were found (data not shown). For the shared epitope alleles, 13 patients (76%) possessed at least one allele. The dominant alleles were variants of the DR4 family, with the *0401 allele occurring in 7 patients and the *0404 and *0408 alleles each occurring in 2 patients respectively. Five patients (29%) possessed two shared epitope alleles and 3 of these had a higher-risk *0401 and/or *0404 combination. The

Table 1. *The shared epitope genotype of the 17 RA patients with corneal melt and the anatomical site of the melt, number of eyes affected and the presence of a contact lens cornea prior to the acute melting process*

Site of ulceration	Affected eyes	Prior contact lens cornea	Shared epitope alleles
Peripheral	R	–	–
Peripheral	R and L	R and L	–
Peripheral	L	L	0401
Peripheral	R	–	0101/2
Peripheral	R	R	1001
Peripheral	R	R	0401, 0401
Peripheral	R and L	–	0408
Peripheral	L	–	0401
Peripheral	R and L	–	0101/2, 0401
Peripheral	L	–	0404
Peripheral	R and L	R	0401, 0408
Peripheral	L	–	0401, 0401
Peripheral	R and L	R and L	–
Peripheral	L	–	0401
(Para)central then peripheral	R	R	–
(Para)central	R	–	0101/2
(Para)central	R and L	–	0404, 0404

R, right eye; L, left eye.

combined allele frequency of the higher-risk alleles *0401 and *0404 was 35%. The shared epitope genotype of the corneal melt patients is given in Table 1.

In each of the RA patient groups, both the overall frequency and a double dose of shared epitope alleles was greater than in the healthy, blood donor control group ($p < 0.002$), but there was no significant difference between the three patient groups (Table 2). Although the overall frequency of the higher-risk alleles was similar in the three RA patient groups, there was a non-significant trend for a double dose of higher-risk alleles to be more common in the patients with either corneal melt or extra-articular manifestations ($p > 0.2$). The frequency of the higher-risk alleles is shown in Table 3.

We were unable to demonstrate an association between possession of one or two shared epitope alleles, particularly the higher-risk alleles, and any of the ocular disease characteristics chosen as potential markers of ocular disease phenotype or severity (Table 1).

Discussion

Class 2 genes are normally expressed only on cells with immune function, such as B cells, monocytes, macrophages, dendritic cells and activated T cells.[14] The class 2 region contains three main loci, namely HLA-DR, -DQ and -DP. Every class 2 antigen is a heterodimer, containing an α and β glycoprotein chain. The HLA class 2 gene, DRB1, encodes the DRβ glycoprotein and is highly polymorphic. The shared epitope is contained in certain alleles of the DRB1 gene. Molecular typing resolves allelic variation at the DNA level and allows for further differentiation of the DRB1 subtypes, identifying each of the alleles which contains the shared epitope sequence. As HLA genes are co-dominant, each individual can express two allelles at each class 2 locus.

In this study, the frequency of shared epitope alleles in a small group of patients with rheumatoid-associated corneal melting or necrotising keratitis was determined. The shared epitope frequency, and particularly the presence of a double dose, was greater in the corneal melt patients than in the healthy control group (Table 2). In double dosage, the higher-risk alleles, *0401 and *0404, were more common in the patients with corneal melt or with extra-articular manifestations than in those with uncomplicated RA, although this failed to reach statistical significance (Table 3). No association between the shared epitope genotype, particularly possession of the higher-risk alleles, and the ocular disease phenotype was present.

Several studies have shown that the shared epitope alleles increase the susceptibility to RA, and that individuals with a double dose of alleles are at greater risk than those with a single copy.[2,4,7,8] Other studies have shown that the shared epitope alleles influence the prognosis for RA patients.[3,5,6] It is not clear why the shared epitope alleles affect disease susceptibility and severity. Part of their influence may be explained by the location of the shared epitope sequence in the peptide-binding site of the HLA-DR molecule.[4,7] Here they may modify the binding of arthritogenic peptides, T cell receptors or both. Rheumatoid factor production is

Table 2. *Frequency of all the shared epitope alleles in the control and patient groups*

Patient group	*n*	No SE alleles	Single SE allele	Double dose of SE alleles	SE allele frequency
Blood donor controls	91	41 (45%)	45 (50%)	5 (5%)	30%
RA without EAM	69	10 (15%)	41 (59%)	18 (26%)*	56%
RA with EAM	30	6 (20%)	13 (43%)	11 (37%)*	57%
RA with corneal melt	17	4 (24%)	8 (47%)	5 (29%)*	53%

SE, shared epitope; RA, rheumatoid arthritis; EAM, extra-articular manifestations.
*$p < 0.005$.

Table 3. *Frequency of the higher risk *0401 and *0404 alleles in the corneal melt and RA groups*

Patient group	*n*	No higher risk alleles	Single higher-risk allele	Double dose of higher-risk alleles	Allele frequency of higher-risk alleles
RA without EAM	69	22 (32%)	41 (59%)	6 (9%)	38%
RA with EAM	30	14 (46%)	11 (37%)	5 (17%)*	35%
RA with corneal melt	17	8 (48%)	6 (35%)	3 (17%)*	35%

RA, rheumatoid arthritis; EAM, extra-articular manifestations.
*$p > 0.2$.

closely related to the presence of certain shared epitope alleles, especially *0401.[4] Alternatively, sequence homology also exists between *0401 and proteins of Epstein-Barr virus and *E. coli*, suggesting a basis for molecular mimicry and autoimmunity.[6] Linkage disequilibrium of HLA-DQ alleles and other non-HLA genes with DR4 alleles may also explain the association with RA.[4,6]

The current model of RA suggests that it occurs in genetically suspectible individuals exposed to unknown environmental triggers. Some patients produce rheumatoid factor, an IgM antibody directed against their own IgG.[15] Production of rheumatoid factor leads to immune complex formation and deposition in vessels, which results in complement activation and a microvasculitis. Several features of the cornea may predispose it to involvement in vasculitic disease.[16] The peripheral cornea derives its nutrients from a vascular network which extends approximately 0.5 mm into the clear cornea. This vascular supply and the tight arrangement of collagen fibres serves to facilitate immune complex deposition.[16] Additionally an inflammatory response could be initiated or perpetuated through the presentation of antigen and an HLA molecule to specific T cells by antigen presenting cells, such as Langerhans and dendritic cells. These cells, which express HLA class 2 antigens, are present at low levels in the peripheral corneal epithelium and stroma but their density and class 2 antigen expression are increased in inflammatory corneal disease.[14,16]

Corneal melting is rare and usually affects only a minority of patients with rheumatoid vasculitis.[17,18] The process is typically a late feature, occurring almost exclusively in rheumatoid factor positive patients with widespread extra-articular disease. Without systemic immunosuppression, the disease often progresses to corneal perforation and is associated with a reduced life expectancy. Limbal vasculitis with immune complex deposition, inflammatory cell infiltrate and collagenase production mirror the changes in the rheumatoid synovium.[19] Given these features, we hypothesised that patients with corneal melting may represent a separate subgroup of RA patients, characterised by an increased frequency of a double dose of the shared epitope alleles. However, the overall frequency and a double dose of the shared epitope alleles was similar in the corneal melt and both the RA control groups and, in this study, the shared epitope frequency was therefore not related to the presence of extra-articular disease
(Table 2). Although the combined allele frequency of 35% for the high-risk alleles *0401 and *0404 in the corneal melt patients was similar to that in the two RA control groups and in other published series, a trend existed for a double dose of higher-risk alleles to be more common in the patients with either corneal melt or other extra-articular manifestations.[6,7] These findings do not support our original hypothesis and suggest instead that corneal melting is another manifestation of extra-articular RA. In turn, this would suggest that of the three potential triggers of corneal melting in RA, namely systemic immune-mediated inflammation, epithelial instability due to keratoconjunctivitis sicca and local ocular infection, the most likely is systemic immune-mediated inflammation.[9] Shared epitope determination of RA patients may help to identify specifically the minority at risk of corneal melting.

The shared epitone genes appear to influence the phenotype of rheumatoid disease, affecting not only the propensity to extra-articular disease but also the severity of the joint disease. The high-risk alleles, *0401 and *0404, are associated with early radiological erosions and possession of these alleles is used as a justification for early, aggressive immunosuppression.[3,6,20] In corneal melting there is an excess of the matrix metalloproteinases, MMP-1, MMP-2 and MMP-9, relative to local tissue inhibitors and this might be the result of enhanced antigen or receptor binding by the shared epitope sequence.[21,22] By comparing the shared epitope genotype of the patients with their ocular disease characteristics, we tried to establish an association. As the study was retrospective, the anatomical site of the corneal melting, presence of a contact lens cornea and the number of eyes involved were used for comparison. We were unable to show an association with these ocular features. It is interesting that 9 of the 23 eyes had a contact lens cornea prior to their rapidly progressive melt. The contact lens cornea is another ocular manifestation of RA but has not previously been associated with corneal melting. This figure is higher than we had expected and this feature may represent a chronic or self-limiting form of the melting process.

In this study we were unable to demonstrate a strong association between shared epitope genotype and either susceptibility to or the phenotype of corneal melting in RA patients. As corneal melting in the context of systemic vasculitis is extremely rare, the number of patients in this study is small and we cannot exclude the possibility that an association would be found in a larger study.[17] The results, however, support the view that corneal melting is simply another, albeit rare, extra-

articular manifestation of RA. Shared epitope determination of RA patients may help to predict disease characteristics. Although it does not help to identify specifically those patients at risk of corneal melting, RA patients with ocular surface disease and a double dose of high-risk alleles are likely to be at greatest risk of corneal melting.

References

1. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis: an approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 1987;30:1205–13.
2. Nepom GT. Class 2 antigens and disease susceptibility. Annu Rev Med 1995;46:17–25.
3. Weyand CM, McCarthy TG, Goronzy JJ. Correlation between disease phenotype and genetic heterogeneity in rheumatoid arthritis. J Clin Invest 1995;95:2120–6.
4. Weyand CM, Goronzy JJ. Inherited and non-inherited risk factors in rheumatoid arthritis. Curr Opin Rheumatol 1995;7:206–13.
5. McMahon MJ, Hillarby M, Clarkson RWE, Hollis S, Grennan DM. Major histocompatibility complex variants and articular disease severity in rheumatoid arthritis. Br J Rheum 1993;32:899–902.
6. MacGregor A, Ollier W, Thomson W, Jawaheer D, Silman A. HLA-DRB1*0401/0404 genotype and rheumatoid arthritis: increased association in men, young age at onset and disease severity. J Rheumatol 1995;22:1032–6.
7. Hall FC, Weeks DE, Camilleri JP, Williams LA, Amos N, Darke C, *et al*. Influence of the HLA-DRB1 locus on susceptibility and severity in rheumatoid arthritis. Q J Med 1996;89:821–9.
8. Deighton CM, Cavanagh G, Rigby AS, Lloyd HL, Walker DJ. Both inherited haplotypes are important in the predisposition to rheumatoid arthritis. Br J Rheum 1993;32:893–8.
9. Bernauer W, Ficker LA, Watson PG, Dart JKG. The management of corneal perforations associated with rheumatoid arthritis: an analysis of 32 eyes. Ophthalmology 1995;102:1325–37.
10. Foster CS, Forstot SL, Wilson LA. Mortality rate in rheumatoid arthritis patients developing necrotising scleritis or peripheral ulcerative keratitis. Ophthalmology 1984;91:1253–63.
11. Miller SA, Dykes DD, Polesky HT. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
12. Bunce M, O'Neill CM, Barnardo MC, Krausa P, Browning MJ, Morris PJ, *et al*. Phototyping: comprehensive DNA typing for HLA-A,B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilising sequence specific primers. Tissue Antigens 1995;46:355–67.
13. Griffiths B, Situnayake RD, Clark B, Tennant A, Salmon M, Emery P. Racial origin and its effect on disease expression and HLA-DRB1 types in patients with RA: a matched cross-sectional study. Rheumatology 2000;39:857–64.
14. Taylor CJ, Dyer PA. Histocompatibility antigens. Eye 1995;9:173–9.
15. Weyand CM, Goronzy JJ. Pathogenesis of rheumatoid arthritis. Med Clin North Am 1997;81:29–55
16. Messmer EM, Foster CS. Vasculitic peripheral ulcerative keratitis. Surv Ophthalmol 1999;43:379–96.
17. McKibbin M, Isaacs JD, Morrell AJ. The incidence of corneal melting in association with systemic diease in the Yorkshire Region, 1995–1997. Br J Ophthalmol 1999;83:941–3.
18. Scott DG, Bacon PA, Tribe CR. Systemic rheumatoid vasculitis: a clinical and laboratory study of 50 cases. Medicine 1981;60:288–97.
19. Messmer EM, Foster CS. Destructive corneal and scleral disease associated with rheumatoid arthritis. Cornea 1995;14:408–17.
20. Emery P, Salmon M. Early rheumatoid arthritis: time to aim for remission? Ann Rheum Dis 1995;54:944 7.
21. Riley GP Harrall RL, Watson PG, Cawston TE, Hazelman BL Collagenase (MMP-1) and TIMP-1 in destructive corneal disease associated with rheumatoid arthritis. Eye 1995;9:703–8.
22. Smith VA, Hoh HB, Easty DL. Role of matrix metalloproteinases in peripheral ulcerative keratitis. Br J Ophthalmol 1999;83:1376–83.