British Journal of Ophthalmology 1994; 78: 298-301

ORIGINAL ARTICLES - Laboratory science

Genetic and clinical determinants for the T cell mediated immune response against the cornea specific protein BCP 54

M J Jager, H J Völker-Dieben, L de Waal, F G Kok, L Broersma, R van der Gaag

Abstract

T cell mediated immune responses against the cornea specific protein BCP 54 have been observed in patients with uveitis, Fuchs' heterochromic cyclitis, and corneal disease. The pathophysiological role of this anti-BCP 54 response in corneal disease is not known. In order to ascertain whether the presence of such an immune response is related to the corneal disease itself or related to genetic influences, the anti-BCP 54 response was determined in 104 patients with severe corneal disease, using a monocyte migration inhibition assay. The results were compared with the presence of a variety of ocular parameters as well as with the distribution of HLA antigens in these patients. While only 7% of healthy controls responded to BCP 54, 37% of the patients showed a positive response (p=0·002); in particular, patients with previous graft rejection, non-herpetic keratitis, and bullous keratopathy reacted against BCP 54. No relation with known risk factors for corneal transplantation, such as corneal neovascularisation, was observed. No significant association with the presence of any of the HLA antigens was observed. It was concluded that the main inducer of an anti-BCP 54 response is corneal disease itself, and that the presence of corneal disease is able to break the immunological privilege typical of normal corneas.

(*Br J Ophthalmol* 1994; 78: 298-301)

Department of Ophthalmology, Leiden University, Leiden, Netherlands
M J Jager

Department of Ophthalmology, Diaconessenhuis, Leiden, Netherlands
H J Völker-Dieben

Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands
L de Waal

Department of Medical Statistics, Leiden University, Netherlands
F G Kok

Department of Ophthalmo-Immunology, The Netherlands Ophthalmic Research Institute, Amsterdam, Netherlands
L Broersma
R van der Gaag

Correspondence to:
Dr M J Jager, Department of Ophthalmology, AZL, PO Box 9600, 2300 RC Leiden, Netherlands.

Accepted for publication
3 November 1993

A T cell mediated immune response against a cornea specific protein (BCP 54) was observed in patients with inflammation of the anterior segment of the eye due to uveitis, melting of the cornea, and Fuchs' heterochromic cyclitis.[1 2] BCP 54 is the main soluble protein of the cornea,[3] and has been identified as an aldehyde dehydrogenase.[4 5] An immune response against this protein is rare in individuals without ocular disease (5%).[2]

In a previous study of 55 patients, we observed an increased incidence of the anti-BCP 54 response in patients with corneal disease necessitating a corneal graft.[6] Since only a proportion of patients with identical corneal disease showed a positive cellular immune response, we wondered which factors determined the presence of the immune response against BCP 54, and whether such a response is related to risk factors for corneal transplantation like the degree of corneal vascularisation. Another possibility is that the differences in immune response are genetically determined, since the ability of an individual to generate an immune response is partly controlled by genes of the major histocompatibility complex (MHC). Associations between MHC specificities and the response against specific disease associated proteins or pathogens have been observed. An example from animal experiments is that, following sensitisation with myelin basic protein, only mice with a specific MHC type developed experimental allergic encephalitis owing to the presence of a T cell mediated immune response against the myelin basic protein.[7] In humans, some associations have been proposed as well - for example, the association between coeliac disease and the presence of DR3 and DR7 and a lower cell mediated immune response against gluten.[8] Another example is the association between HLA-DR4 and an increased cell mediated immune response against *Mycobacterium tuberculosis* in patients with leprosy.[9]

To test the hypothesis that either clinical or genetic factors determined the response against BCP 54, we tested the anti-BCP 54 reactivity in 104 patients with corneal disease necessitating corneal transplantation and related the response to a variety of clinical parameters and to the distribution of the HLA class I and II genes in these individuals.

Patients and methods

PATIENTS

Patients with different corneal diseases were referred to the Department of Ophthalmology of the Academic Medical Centre of the University of Amsterdam (n=56), or to the Department of Ophthalmology of the Diaconessenhuis in Leiden (n=48). Age, sex, type of corneal disease (14 with herpes keratitis, four with an inherited dystrophy, 14 with a non-herpetic keratitis, 19 with keratoconus, six with a primary endothelial decompensation, 11 with a previous non-immunological graft failure, 10 with a previously rejected graft, 20 with secondary endothelial decompensation, and six with trauma), degree of vascularisation of the recipient cornea, the number of previous corneal transplants, blood

transfusions, and pregnancies were registered. The presence of anti-leucocyte (anti-HLA) antibodies was determined in 86 out of 104 patients.

CONTROLS

The control population for the BCP 54 test consisted of 20 healthy people without ocular problems, varying in age from 22 to 56 (mean 33) years, and 10 people with cataract, varying in age from 53 to 94 (mean 75).

HLA TYPING

HLA typing for the antigens of the A, B, and C loci was performed for 100 patients using the standard two stage microlymphocytotoxicity technique.[10] HLA-DR and DQ typing was performed for 73 patients using the two colour fluorescence method.[11] The antigen frequencies were calculated by direct counting. Frequencies were compared with the standard panel of the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service. Statistical significance between the relative frequencies of HLA antigens in patients with a positive or negative cellular response to BCP 54 and controls was determined by the χ^2 test.

ANTI-HLA ANTIBODIES

The presence of anti-HLA antibodies was determined in a standard two stage microlymphocytotoxicity technique, using a panel of either 21 or 53 cells.[10]

ISOLATION OF BCP 54

Corneal epithelium was scraped from bovine corneas. The epithelium was homogenised and centrifuged. BCP 54 was subsequently purified from the supernatant by anion exchange chromatography and showed only one band when tested by sodium dodecyl sulphate polyacrylamide gel electrophoresis.[12]

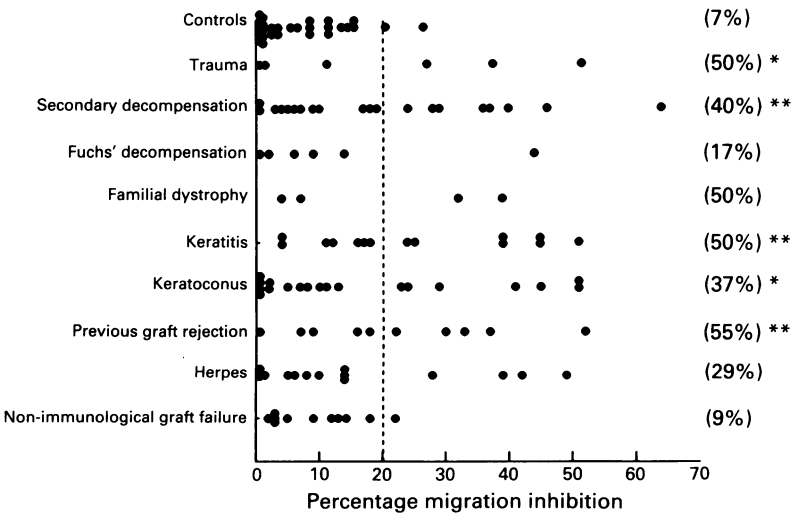

*Figure 1 Percentage migration inhibition is shown as a dot for each patient in the different disease groups and for a group of 30 healthy controls. The percentage of positive responders in each group is also shown. The number of stars indicates the level of significance as determined by the χ^2 test comparing patients with the controls: *=$p<0{\cdot}05$, **=$p<0{\cdot}01$. The broken line indicates the difference between negative (to the left) and positive responders.*

THE TWO STEP MONOCYTE MIGRATION INHIBITION ASSAY

The T cell mediated immune response against BCP 54 was determined with a two step migration inhibition assay, using peripheral blood mononuclear cells (for extensive description see Van der Gaag *et al*[2]). In short, the cells were incubated with 25 μg/ml concanavalin A to test the general mitogenic responsiveness of the cells, or with 50 μg/ml purified BCP 54, or with medium alone as a control for spontaneous migration inhibition factor (MIF) production. Samples not responding to concanavalin A or samples which induced spontaneous MIF were not analysed for the BCP 54 response. After incubation for 20 hours, cell free supernatants were harvested by centrifugation and assayed in the second step of the test.

For the second step of the MIF test, human monocytoid cells (U937) were mixed with Seaplaque agarose. One μl droplets were pipetted in flat bottom wells of a microtitre tray and 100 μl of cell free supernatant, obtained in the first step, was added to each well (triplicate). After incubation for 20 hours the areas of monocyte migration out of the agarose droplets were measured and the results, expressed as % migration inhibition (MI) were calculated as follows:

$$\%\,MI=\left(1-\frac{\text{mean area in test supernatant}}{\text{mean area in control supernatant}}\right)\times 100$$

Control supernatants came from tubes containing mononuclear cells in culture medium alone. A mean migration inhibition of 5% was calculated from the values from healthy individuals and an MI of 20% or more was taken as a positive reaction. There was no direct effect of concanavalin A or BCP 54 alone on the migration of U937 cells.

STATISTICAL ANALYSIS

For determination of an association between the anti-BCP 54 immune response and individual or ocular parameters, two logistic regression analyses were applied using SPSS-PC on a personal computer. The logistic regression analysis was used to test the simultaneous relation of the clinical variables (age of the recipient, sex, pregnancies, blood transfusions, presence of leucocyte antibodies, type of corneal disease, corneal vascularisation, number of previous transplants, type of intraocular lens) on the outcome of the anti-BCP 54 response. Using a forward selection procedure, the computer selected the variable(s) that contributed substantially to the prediction. Since sex and pregnancy are confounded, two separate logistic analyses were performed with each of these two variables; also, individual cross tabulations of various parameters were studied.

Any discrepancy between the outcome of the logistic regression and the individual χ^2 analyses can be explained by the participation of a lower number of patients in the logistic regression: only patients with no missing data (mainly data about anti-HLA antibodies) for any of the parameters tested were used in the logistic regression analyses (85 patients).

Table 1 Presence of cellular immune response against BCP 54 in comparison with individual parameters

	Cellular immune response against BCP 54		
	No	*Positive (%)*	*p Value*
Female	60	47	0·01
Male	44	23	
Number of pregnancies*:			
None	18	78	0·004
At least one	41	32	
Number of blood transfusions†:			
None	88	39	0·33
One	6	33	
More than one	8	12	
HLA antibodies:			
<5% panel reactivity	76	36	0·78
>5% panel reactivity	10	40	
Age (years):			
0–19	3	66	0·41
20–29	16	38	
30–39	11	55	
40–49	9	33	
50–59	10	10	
60–69	16	25	
70–79	21	38	
80–100	18	44	

*Unknown for one patient; †unknown for three patients.

Results

CLINICAL PARAMETERS

A positive MIF test against BCP 54 was seen more often in patients with corneal disease than in controls not suffering from corneal disease (37% of 104 patients versus 7% of 30 controls, p=0·002). The incidence of a cellular immune response against BCP 54 varied from 9% to 50% per group of patients with different corneal diseases (Fig 1). On the whole, patients with previous non-immunological graft failure had the lowest frequency of positive responses (9%).

A logistic regression analysis was used to test the simultaneous relation of the clinical variables on the outcome of the anti-BCP 54 response. An analysis with inclusion of pregnancies (while not including sex since these two factors are confounded) showed that the variable pregnancy could be used as a predictor (p=0·03) for the probability of a positive anti-BCP 54 response. Analysis of individual cross tabulations of the various predictors versus the anti-BCP 54 response (Table 1) showed a significantly higher response rate in women (p=0·01), and an association with the absence of pregnancy (p=0·004) (Table 1).

Analysis of individual cross tabulations of the various ocular parameters did not show any significances (Table 2).

HLA ANTIGENS

Anti-BCP 54 responders versus non-responders

We compared the distribution of the different HLA genes in patients with and without an immune response against BCP 54. A comparison of the frequencies showed an increased presence of HLA-A25 (p=0·02) and B18 (p=0·04) in responders compared with non-responders (Table 3). These results are not significant after correction for the number of comparisons. No significant differences in the frequencies of any of the HLA-Cw, DR, DR52/53, or DQ antigens were observed (data not shown).

Table 2 Cellular immune response against BCP 54 in comparison with clinical ocular parameters

	Cellular immune response against BCP 54		
	No	*Positive (%)*	*p Value*
Vascularisation of recipient cornea:			
None	40	38	
One quadrant	19	42	0·75
Two quadrants	16	25	
More	29	38	
Number of previous transplants on this eye:			
None	81	40	
One	18	28	0·47
More than one	5	20	
Type of intraocular lens:			
Aphakic	11	27	
Iris fixated	24	42	0·87
Posterior chamber	3	33	
Phakic	66	36	

Responders versus control panel

The HLA frequencies in positive responders and a (standard) control panel (made up of healthy individuals without ocular disease and not tested for anti-BCP 54 response) were compared to determine whether the HLA distribution of individuals responding to BCP 54 was different from the distribution in a normal population from the same area (with the same genetic background). Our results show an increase in positively reacting patients with HLA-A25 (p=0·05) and B47 (p=0·01) (Table 3). However, these test results no longer show statistical significance after correction for the number of comparisons (Bonferroni correction). No other differences were observed (data not shown).

Discussion

The healthy eye is considered to be immunologically privileged, which means that antigens and even transplants placed in the anterior chamber or in the centre of the cornea do not induce the development of a delayed type (T cell mediated) immune response, although a humoral immune response is possible.[13 14] This is illustrated by the classic experiments of Maumenee,[15] in which he showed that following a corneal transplantation in rabbits, subsequent skin transplants were still rejected in a primary fashion. Reactivity in vitro in the MIF test corresponds to an in vivo delayed type hypersensitivity response.[16] In spite of this ocular immunological privilege, patients with ocular disease sometimes show a positive T cell mediated immune response against a cornea specific protein, BCP 54.[2] Our results indicate

Table 3 Cell mediated immune response against BCP 54 and distribution of HLA antigens in patients (64 responders and 36 non-responders against BCP 54) and in a control panel (n=3528)

	Cellular immune response against BCP 54			Standard panel (n=3528)	
	Frequency (%)				
HLA antigen	*Present (n=64)*	*Absent (n=36)*	*p Value*	*Frequency (%)*	*p Value*
A25	8·3	0	0·02	1·9	0·05
B18	13·9	3·1	0·04	7·0	NS
B47	5·6	0	NS	0·6	0·01

that, in addition to the presence of anterior chamber inflammation as observed in heterochromia of Fuchs', corneal disease is associated with sensitisation of T cells against the cornea specific protein BCP 54. It is as yet unknown whether the delayed type hypersensitivity against BCP 54 leads to an increased severity of corneal disease, or is only an epiphenomenon. However, our data show that the immunological privilege of the eye can be disturbed by the presence of corneal disease. It may be that specific epithelial or endothelial problems are the sensitising incidents, but whether such problems occurred is difficult to determine retrospectively. Support for this idea may be found in a previous study[3] which showed that patients undergoing corneal transplantation and graft rejection could convert from non-responders to responders against BCP 54 and also the other way around: 1 year after transplantation most eyes had reached a clinically stable situation, and reactivity against BCP 54 had decreased significantly.[17]

In our present study we did not find an association between ocular parameters other than 'corneal disease' and sensitisation against BCP 54. Similarly to the previous reports on smaller groups of patients,[6 17] patients with keratoconus showed a low reactivity, and patients with a keratitis a relatively frequent response. Since anti-herpes immunological reactions are an important immunopathological factor in severe corneal herpes, it is surprising that patients with a herpetic keratitis showed a relatively low responsiveness. This is especially surprising since patients in another group where immune responses play a role – that is, patients who had rejected a previous graft, responded much more frequently against BCP 54 than patients with prior graft failure without rejection. It is difficult to point out a common denominator that determines responsiveness, since in all disease groups at least 50% of the patients was non-responsive. One non-disease related significant indicator was observed: individual analyses suggested that women responded more often than men, and that a lower responsiveness was found after pregnancies. We have no explanation for these observations.

With regard to the genetics, no significant differences were observed in HLA distribution between positive responders and healthy controls or between responding and non-responding patients. The small differences that were observed were no longer significant after correction for the number of antigens studied. It may be that certain combinations of HLA genes do not present the BCP 54 peptides to the immune system, but the size of our group may not have been large enough to observe this. Furthermore, it may be that an anti-BCP 54 immune response is not a stable parameter of an individual, and that associations may therefore remain vague. As already mentioned above, we noticed in our previous studies that patients can be converters, although many individuals do not change their reactivity in spite of, for example, the performance of a corneal transplant.[6 17] It may well be that a total lack of inducibility of an anti-BCP 54 response may be genetically determined, although our data do not show a difference in HLA type between positive and negative anti-BCP 54 responders at a time that significant corneal disease was present in all individuals tested. It is therefore clear that the main factor determining immune responsiveness against BCP 54 is the presence of corneal or anterior chamber disease, and that only experimental studies may be able to give an answer to the question whether such an immune response influences the severity of the disease.

M J Jager is the recipient of a fellowship of the Royal Netherlands Academy of the Arts and Sciences.

1 Kruit PJ. Corneal autoimmunity: a clinical and experimental approach. Thesis. Amsterdam: Free University Press, 1987.
2 Van der Gaag R, Broersma L, Rothova A, Baarsma S, Kijlstra A. Immunity to a corneal antigen in Fuchs' heterochromic cyclitis patients. *Invest Ophthalmol Vis Sci* 1989; **30**: 443–8.
3 Silverman B, Alexander RJ, Henley WL. Tissue and species specificity of BCP 54, the major soluble protein of bovine cornea. *Exp Eye Res* 1981; **33**: 19–29.
4 Verjans GMGM, Verhagen C, Hoekzema R, Kijlstra A. Bovine corneal protein 54 kd (BCP 54) shows a strong homology to rat tumor-associated aldehyde dehydrogenase (T-ALDH). *Curr Eye Res* 1990; **9**: 1217–8.
5 Abedinia M, Pain T, Algar EM, Holmes RS. Bovine corneal aldehyde dehydrogenase: the major soluble corneal protein with a possible dual protective role for the eye. *Exp Eye Res* 1990; **51**: 419–26.
6 Jager MJ, Völker-Dieben HJ, Vos A, Broersma L, Kok FG, Van der Gaag R. Cellular and humoral anti-corneal immune response in corneal transplantation. *Arch Ophthalmol* 1991; **109**: 972–7.
7 Zamvil S, Nelson P, Trotter J, Mitchell D, Knobler R, Fritz R, *et al*. T-cell clones specific for myelin basic protein induce chronic relapsing paralysis and demyelination. *Nature* 1985; **317**: 355–8.
8 Scott H, Hirschberg H, Thorsby E. HLA-DR3 and HLA-DR7-restricted T-cell hyporesponsiveness to gluten antigen: a clue to the aetiology of coeliac disease? *Scand J Immunol* 1983; **18**: 163–7.
9 Ottenhoff THM, Torres P, Terencio de las Aguas J, Fernandez R, Eden W van, De Vries RRP, *et al*. Evidence for an HLA-DR4-associated immune-response gene for Mycobacterium tuberculosis. *Lancet* 1986; ii: 310–3.
10 Terasaki PI, Bernoco D, Park MS, Oztur KG, Iwaki Y. Microdroplet testing for HLA-A, B, C and D antigens. *Am J Clin Pathol* 1978; **69**: 213–20.
11 Rood van JJ, Van Leeuwen A, Ploem JS. Simultaneous detection of two cell populations by two colour fluorescence and application to the recognition of B-cell determinants. *Nature* 1976; **262**: 795–7.
12 Kruit PJ, Van der Gaag R, Broersma L, Kijlstra A. Autoimmunity against corneal antigens i. Isolation of a soluble 54 kd corneal epithelium antigen. *Curr Eye Res* 1986; **5**: 313–20.
13 Streilein JW. Anterior chamber associated immune deviation: the privilege of immunity in the eye. *Surv Ophthalmol* 1990; **35**: 67–73.
14 Niederkorn JY. Immune privilege and immune regulation in the eye. *Adv Immunol* 1990; **48**: 191–226.
15 Maumenee AE. The influence of donor-recipient sensitization on corneal grafts. *Am J Ophthalmol* 1951; **34**: 142–52.
16 Van de Plassche-Boers EM, Drexhage HA, Kokje-Kleingeld M, Leezenberg HA. Parameters of T-cell mediated immunity to commensal micro-organisms in patients with chronic purulent rhinosinusitis: a comparison between delayed type hypersensitivity skin test, lymphocyte transformation test and macrophage migration inhibition factor assay. *Clin Exp Immunol* 1986; **66**: 516–24.
17 Jager MJ, Kok FG, Broersma L, Van der Gaag R. Changes in the immune response against BCP 54 following corneal transplantation in man. *Transplant* 1993; **55**: 1379–82.