Cellular autoimmunity to retinal specific antigens in patients with Behçet's disease

J H Yamamoto, M Minami, G Inaba, K Masuda, M Mochizuki

Abstract

The notion that autoimmune mechanisms play a role in the pathogenesis of certain uveitic conditions in humans is supported by the observation that lymphocytes from such patients respond in culture against retinal specific antigens which are uveitogenic in animals. A large proportion of uveitis patients with Behçet's disease are reported to respond well to S antigen, to interphotoreceptor retinoid binding protein (IRBP) and to several of their uveitogenic peptides, in particular, the S antigen derived peptide M. Patients with Behçet's disease without ocular involvement were reported not to differ in their responses to S antigen from the responses in the control group, yet 35% of them responded to IRBP and approximately two thirds of them responded to the peptides (peptide M, peptide N, R-4, or R-14). The responses were inhibited by monoclonal antibodies to CD4 and to class II MHC HLA-DR molecules. The presence of lymphocyte responses to retinal antigens in patients with Behçet's disease without uveitis might indicate a preclinical stage of ocular involvement. Thus, these data support the idea that autoimmunity to retinal specific antigens may play a role in the ocular inflammation in Behçet's disease.

(*Br J Ophthalmol* 1993; 77: 584-589)

Department of
Ophthalmology, School
of Medicine, University
of Tokyo
J H Yamamoto
K Masuda

Department of
Parasitology, School of
Medicine, Yokohama
City University
M Minami

Second Department of
Internal Medicine,
School of Medicine,
Teikyo University
G Inaba

Department of
Ophthalmology, School
of Medicine, Kurume
University, 67 Asahi-
machi, Kurume 830,
Japan
M Mochizuki

Correspondence to:
Dr M Mochizuki.

Accepted for publication
6 April 1993

Behçet's disease, a systemic disorder, is the leading cause of endogenous uveitis in Japan.¹ Uveitis is the most serious clinical finding and is responsible for 10-15% of the acquired blindness in Japan.¹ The mechanisms of the ocular inflammation are still controversial.

Several lines of evidence support a significant role for autoreactive T lymphocytes in mediating ocular inflammation. Immunisation with retinal S antigen or interphotoreceptor retinoid binding protein (IRBP) causes an experimental autoimmune uveoretinitis (EAU) that resembles some human uveitic conditions.²⁻⁵ In vivo or in vitro sensitised, S antigen or IRBP specific T cells transferred to naive animals induce EAU.^{6,7}

Furthermore, cellular immune responses to the antigens are demonstrated in patients with certain types of uveitis.⁸⁻¹² We previously studied the cellular immune responses in patients with Behçet's disease, sarcoidosis, and Vogt-Koyanagi-Harada's disease.¹³ The previous study found that patients with Behçet's disease with uveitis exhibited the highest and the most frequent positive responses to S antigen and IRBP, as well as to peptide M, a main uveitogenic site of S antigen.¹³ However, lymphocyte responses to retinal antigens in patients with Behçet's disease without uveitis were not evaluated. Therefore, the present study was aimed at analysing the cellular immune responses to the retinal antigens in patients with Behçet's disease without uveitis to investigate the role of cellular immune responses to uveitogenic retinal antigens in the pathophysiology of uveitis.

Patients and methods

PATIENTS

Peripheral venous blood samples were obtained from patients with Behçet's disease with and without uveitis (Table 1). Patients with uveitis were from the Department of Ophthalmology of Tokyo University Hospital; patients without uveitis were from the Department of Medicine and Physical Therapy of Tokyo University Hospital, and from the Second Department of Internal Medicine and Department of Dermatology of Teikyo University Hospital. Patients with Behçet's disease met the diagnostic criteria for complete or incomplete type set by the Behçet's Disease Research Committee of the Ministry of Health and Welfare of Japan.¹⁴ All patients with uveitis had the posterior segment involved with or without active uveitis. The presence of retinal infiltrates, perivasculitis, snowbanking, vitreous haze, or cystoid macular oedema was accepted as evidence of ocular activity. Twenty four of 43 patients with Behçet's disease with uveitis (60%) had the active form of the disease when tested. Patients were tested irrespective of their current medical therapy (usually consisting of cyclosporine, prednisone, colchicine, and/or cyclophosphamide) or of the level of uveitis activity. Thirty six control subjects were selected from either non-research staff or from clinic patients not being seen for a uveitic condition, and in whom a retinal or choroidal disorder had been ruled out. Patients with Behçet's disease with uveitis and control subjects were tested in the period between October 1988 and November 1991, while patients with Behçet's disease without uveitis were tested in 1991. Only patients who gave informed consent were enrolled in the study.

Table 1 Characteristics of patients and controls

Clinical entity	Number of cases	Average age (years)	Male/female	Disease duration (months)	Uveitis activity (active/inactive)
Behçet's disease with uveitis	43	41 (24-66)*	35/8	95 (8-540)*	24/19
Behçet's disease without uveitis	17	46 (28-65)	5/12	122 (16-288)	-
Control	36	32 (14-80)	23/13	-	-

*Range.

ANTIGENS

Bovine S antigen was purified by the method of Dorey *et al.*¹⁵ whereas bovine IRBP was isolated as described by Redmond *et al.*¹⁶ S antigen derived peptides were based on the bovine S antigen sequence reported by Shinohara *et al.*¹⁷ The peptides were synthesised in accordance with the method of Donoso and co-workers,¹⁸ on a benzhydrylamine resin using an automated peptide synthesiser (SAM II, Bioscience, Inc, San Rafael, CA, USA). Peptides M and N occupy sequences 303 to 320 (DTNLSSTIIKEGIDKTV) and 281 to 302 (VPLLANNRERRGIALDGKIKHE) of the bovine S antigen, respectively.^{19,20} The IRBP derived peptides were synthesised and purified by Applied Biosystems Inc, Foster City, CA, USA, using the t-BOC chemistry, on a peptide synthesiser 430A. The peptide sequences were derived from the sequence of bovine IRBP as determined and reported by Borst and associates.²¹ This consisted of sequence 1158–1180 (HVDDTDLYLTIPARSVGAADGS) for R-4 and of sequence 1169–1191 (PTARSVGAADGSSWEGVGVVPDV) for R-14.^{22,23}

MONOCLONAL ANTIBODIES

Hybridoma cell lines producing monoclonal antibodies (mAbs) anti-CD4 (OKT4), anti-CD8 (OKT8), anti-HLA-DR (L243), anti-CD2 (TS2/18.1.1), and anti-CD11a (LFA-1) (TS1/22.1.1.13) were obtained from ATCC (Rockville, MA, USA). MAb directed against CD3 (OKT3) was purchased from Becton-Dickinson (Mountain View, CA, USA).

LYMPHOCYTE PROLIFERATION ASSAY

Mononuclear leucocytes from heparinised blood samples were separated by gradient centrifugation (Ficoll-Paque, Pharmacia, Uppsala, Sweden) and cultured in Roswell Park Memorial Institute (RPMI) 1640 medium with HEPES (Gibco, Grand Island, NY, USA), supplemented with penicillin (100 units/ml), streptomycin (100 µg/ml), glutamine (2 mmol/l), and heat-inactivated human AB serum 10% (lot no 29309048, Flow Laboratories, Inc, McLean, VA, USA).

Cultures were set up in triplicate consisting of 2×10^5 cells/well, incubated for 5 days in flat bottom, 96 well microplates, at 37°C with 100% humidity and 5% carbon dioxide in air. The retinal antigens were added at the concentrations of 4, 20, or 100 µg/ml. Cultures were pulsed for the last 16 hours with tritiated thymidine (³H-TdR, New England Nuclear, Boston, MA; 2 Ci/mmol, 0.5 µCi per 20 µl/well) and the incorporated radioactivity was counted by a liquid scintillation counter. Several antigens were tested simultaneously. However, not all antigens could be tested on every patient or control owing to the small amount of blood available and the number of mononuclear cells that could be recovered.

The results were expressed as disintegrations per minute (dpm) or as stimulation index [SI (dpm in culture wells with antigen)/(dpm in culture wells without antigen)]. A response was considered positive when the SI was equal or higher than 2.0 (SI ≥ 2.0). A patient with a positive response to a certain antigen was defined as a responder. Data were compared among groups with a one way analysis of variance. Testing for statistical significance was done using Mann-Whitney U test and Kruskal-Wallis H test.

ANTIBODY BLOCKING ASSAY

The mAb was added to the cultures at the initiation of proliferation assays. Optimal concentration of mAb used for blocking assay was determined previously.

$$\% \text{ response} = \frac{(\text{dpm in culture wells with antigen and mAb}) - (\text{dpm in culture wells without antigen or mAb})}{(\text{dpm in culture wells with antigen only}) - (\text{dpm in culture wells without antigen or mAb})} \times 100$$

Results

LYMPHOCYTE PROLIFERATIVE RESPONSES TO BOVINE S ANTIGEN AND BOVINE IRBP

In a preliminary study on the kinetics and on the dose response of the proliferative responses to bovine S antigen and bovine IRBP, peak responses were seen in cultures after 5 days of incubation with S antigen at 4 µg/ml and IRBP at 20 µg/ml. Therefore, these culture conditions were used for the following analysis. As for the lymphocyte proliferative responses, the responses to S antigen (SI) were significantly higher in patients with Behçet's disease with uveitis, mean 8.1 (SE 11.2), than in those without uveitis, 1.6 (0.7) ($p < 0.05$), or in the controls, 1.6 (0.9) ($p < 0.05$) (Fig 1A). For IRBP, the responses were significantly higher in patients with Behçet's disease with uveitis, 5.1 (5.9) compared with the controls, 1.4 (1.4) ($p < 0.05$) (Fig 2A). Responses to IRBP in patients with Behçet's disease without uveitis, 2.3 (1.6), were not as high as those observed in patients with uveitis (Fig 2A).

Based on the incidence of responders, 14 out of 23 (61%) patients with Behçet's disease with uveitis responded to S antigen, while only five of

Figure 1 Lymphocyte proliferative responses to S antigen (A), peptide M (B), and peptide N (C) in patients with Behçet's disease with and without uveitis, and controls. The symbol in the centre of the vertical bars represents the mean of the individual SI for each group, and the vertical bars represent the respective standard error. The broken line represents SI=2.0, the cut off point to define a positive response. Significance level by one way analysis of variance between the responses to S antigen in patients with Behçet's disease with uveitis and the controls, $*p < 0.05$. A significance level, $p < 0.05$, was also observed between the responses to S antigen in patients with uveitis and those without uveitis.

Figure 2 Lymphocyte proliferative responses to IRBP (A), R-4 (B), and R-14 (C) in patients with Behçet's disease with and without uveitis, and controls. The symbol in the centre of the vertical bars represents the mean of the individual SI for each group, and the vertical bars represent the respective standard error. The broken line represents $SI=2.0$, the cut off point to define a positive response. Significance level by a one way analysis of variance between the responses to IRBP in patients with Behçet's disease with uveitis and the controls, $*p<0.05$.

17 (29%) of those without uveitis and seven of 23 (30%) controls responded to it (Table 2). Responders to IRBP were found in 16 of 31 (52%) and in six of 17 (35%) patients with Behçet's disease with and without uveitis, respectively. Four of 28 control subjects (14%) responded to IRBP. A statistical significance was observed between the incidence of responders to IRBP in the group of patients with uveitis compared with the controls ($p<0.01$) (Table 2).

Among the patients and control subjects shown in Table 1, 22 patients with Behçet's disease with uveitis, 17 without uveitis, and 21 control subjects were tested concomitantly with S antigen and IRBP. The group of responders to at least one of the antigens were 14 of 22 patients with Behçet's disease with uveitis (64%), nine of 17 patients without uveitis (53%), and eight of 21 control subjects (38%). In this population, 64% of patients with uveitis responded to both antigens, while only 22% of patients without uveitis, and 28% of control subjects responded to them.

Still considering the population of patients with uveitis tested with S antigen and IRBP concomitantly ($n=22$) and comparing patients with active uveitis and those with inactive uveitis in respect of their responses to S antigen and/or IRBP, in the group with active uveitis ($n=10$), nine responded to S antigen, of whom five also responded to IRBP, and one did not respond to either antigen. In contrast, in the group with inactive uveitis ($n=12$), four responded to S antigen, these same four patients plus one

responded to IRBP and seven were non-responders to either antigen. Thus, S antigen was more frequently correlated to active uveitis ($p<0.05$). In patients with Behçet's disease with uveitis, responders to S antigen were more frequent in the younger age group mean age 39 (SD 10), compared with the non-responders, 47 (12), and in patients having a shorter disease period, mean 63 (46) months compared with the non-responders, 185 (157) months.

LYMPHOCYTE PROLIFERATIVE RESPONSES TO THE UVEITOPATHOGENIC PEPTIDES

In addition to testing in vitro responses to S antigen and IRBP, the responses to the peptide fragments of each of these antigens were determined. The results obtained from culture cells after 5 days of incubation with peptides M and N at 100 $\mu\text{g/ml}$ and peptides R-4 and R-14 at 20 $\mu\text{g/ml}$ were used for the following analysis. Although statistical significance could not be demonstrated, the mean proliferative responses to peptide M were high in patients with Behçet's disease with uveitis, mean 7.9 (SE 11.0), as well as in those without uveitis, 6.2 (4.4), compared with the controls, 2.2 (1.7) (Fig 1B). Patients with Behçet's disease without uveitis showed high proliferative responses to peptide N, 7.9 (7.5), compared with the controls, 3.1 (3.6) ($p<0.05$) (Fig 1C). Concerning IRBP derived peptides, the responses to R-4 and R-14 were not significantly different from the responses in the controls (Figs 2B and C).

On the basis of incidence of responders to the peptides, among patients with Behçet's disease with uveitis, 24 of 36 (67%), 13 of 36 (36%), nine of 20 (45%), and seven of 37 (19%) responded to peptide M, peptide N, R-4, and R-14, respectively (Table 2). Similarly, among patients without uveitis, 16 of 17 (94%), 13 of 17 (76%), 10 of 17 (59%), and seven of 17 (41%) responded to the respective peptides (Table 2). The incidences of responders to the respective peptides in the control group were seven of 21 (33%), eight of 20 (40%), three of 14 (21%), and three of 21 (21%) (Table 2). A statistical significance was observed between the incidence of responders to peptide M in patients without uveitis compared with the controls ($p<0.01$).

Considering the patients tested concomitantly with the native protein and its respective uveitogenic peptides, among the 11 patients with Behçet's disease with uveitis who responded to S antigen, nine also responded to peptide M (82%), and five responded to peptide N (45%). All five patients without uveitis who responded to S antigen also responded to peptide M and peptide N. In respect of IRBP, among six patients with Behçet's disease with uveitis who responded to IRBP, three responded to R-4 (50%) and two to R-14 (33%). Of six patients with Behçet's disease without uveitis who responded to IRBP, five also responded to R-4 (83%) and six to R-14 (100%). Several patients responded to a peptide fragment, but did not recognise the native protein. Among patients with Behçet's disease with uveitis, five out of 15 responders to peptide M and/or N (33%) did not recognise S antigen; while among those without uveitis, 11 of 16

Table 2 Incidence of responders to retinal antigens and their uveitopathogenic peptides among patients with Behçet's disease with and without uveitis, and in controls

Antigen tested ($\mu\text{g/ml}$)	Behçet's disease		Control
	with uveitis	without uveitis	
S antigen (4)*	14/23† (61%)	5/17 (29%)	7/23 (30%)
Peptide M (100)	24/36 (67%)	16/17‡ (94%)	7/21 (33%)
Peptide N (100)	13/36 (36%)	13/17 (76%)	8/20 (40%)
IRBP (20)	16/31‡ (52%)	6/17 (35%)	4/28 (14%)
R-4 (20)	9/20 (45%)	10/17 (59%)	3/14 (21%)
R-14 (20)	7/37 (19%)	7/17 (41%)	3/21 (21%)

*Concentration.

†The numerator refers to the number of positive responders. The denominator refers to the total number of patients tested.

‡Significance level by Kruskal-Wallis H test, $p<0.01$, between the control group and the indicated groups.

Figure 3 Inhibition of lymphocyte proliferative responses to peptide M with monoclonal antibodies in patients with Behçet's disease. The histograms refer to four characteristic cases, two with uveitis (A and C) and two without uveitis (B and D). Background proliferation with no antigen was less than 3000 disintegrations per minute (dpm) with proliferative responses to peptide M ranging from 4000 to 48 000 (dpm), and SI ranging from 5.0 to 17.0.

responders to peptide M and/or N (69%) did not recognise the native protein. Concerning the peptides of IRBP, five out of nine (55%) patients with Behçet's disease with uveitis and five out of 11 (45%) patients without uveitis, who responded to R-4 and/or R-14, did not recognise IRBP.

INHIBITION OF THE LYMPHOCYTE PROLIFERATIVE RESPONSE WITH mAbs

The study of the inhibition of the lymphocyte proliferative responses to the antigens with mAbs was performed in responders to peptide M with Behçet's disease with (n=3) and without (n=3) uveitis. Figure 3 shows four representative cases of patients with Behçet's disease, two with uveitis (Fig 3A and 3C), and two without uveitis (Fig 3B and 3D). MAb anti-CD4 and anti-DR inhibited all responses. MAb anti-CD3 and anti-T cell accessory molecules CD2 and LFA-1 also inhibited the responses. Responses of some patients were further inhibited by mAb anti-CD8. The responses of both groups of patients with Behçet's disease were inhibited in a similar way.

Discussion

The presence of cellular proliferative response to S antigen has been described in various uveitic conditions.⁸⁻¹² Faure *et al*²⁴ reported a high frequency of positive responses to S antigen among patients with Behçet's disease with uveitis using a leucocyte migration inhibition test. In the present study, patients with Behçet's disease with uveitis showed significant high responses and a high number of positive responders to both the retinal specific antigens, S antigen and IRBP. The responsiveness to S antigen was mostly observed in patients with Behçet's disease with active uveitis, in young patients, and in patients with uveitis of short duration. Several patients responded to both S antigen and IRBP. This high reactivity to retinal antigens may reflect the severe retinal damage that takes place in Behçet's disease.²⁵ The severe retinal involvement may elicit cellular responses to various retinal anti-

gens. On the other hand, the presence of such responsiveness to these antigens may correspond to the recurrent and chronic course of Behçet's disease.

Our study showed that patients with Behçet's disease without uveitis did not differ in their responses to S antigen from the responses in the control group. Yet, more responders to IRBP were observed in the patient group without uveitis (35%) than in the control group (14%), although their responses were lower than the responses observed in patients with uveitis. Since IRBP is located in the extracellular matrix between the retinal pigment epithelium and the photoreceptor cell layer, it is possible that, in face of a subclinical inflammation, IRBP escapes the eye more easily than S antigen, which is located in the photoreceptor cell layer, and sensitises the immune system.

It was found that patients with uveitis who responded to S antigen also responded well to peptide M (82%), but less so to peptide N (45%). This indicates that peptide M is an important lymphocyte proliferative site of S antigen in patients with Behçet's disease with uveitis. Responders to either IRBP derived peptide, R-4 or R-14, were demonstrated among patients with Behçet's disease with uveitis, indicating that both sites are immunogenic.

Curiously, patients with Behçet's disease without uveitis responded fairly well to all peptides tested. The mechanisms by which the patients were sensitised to the peptides are unknown. However, it is possible that a foreign antigen having sequence homology to these self-antigens sensitises the peripheral lymphocytes of those patients, causing a cross reactivity to peptides. Indeed, various homologues to peptide M, such as yeast histone H3, that can induce EAU have already been described by Shinohara *et al*.²⁶ Patients with Behçet's disease without uveitis who responded to the peptides may have the genetic background that predisposes them to respond to and/or may have been exposed to the homologous sequence of the peptides. Therefore, patients with Behçet's disease without uveitis may lack the presence of an adjuvant,

such as microbial infection, so that clinical uveitis has not developed yet.^{27,28} In other words, the presence of lymphocyte responses to peptides in patients with Behçet's disease without uveitis might indicate a preclinical stage of ocular involvement and these patients may develop uveitis in the future, having the aforementioned predisposing factors.

The findings that several patients from both groups with Behçet's disease responded to IRBP derived peptides, R-4 and/or R-14, but did not recognise IRBP itself may indicate that these peptides are 'cryptic determinants'.^{29,30} Further, the non-responsiveness to S antigen but responsiveness to peptide M of patients with Behçet's disease without uveitis could be due to the differences in the fragments expressed when bovine S antigen is cleaved and when peptide M is cleaved.³¹ However, the non-responsiveness to S antigen cannot be ignored but responsiveness to peptide M could be due to the differences in molar concentration between them. Peptide N evoked responses in lymphocytes from only patients with Behçet's disease without uveitis, therefore, it could be speculated that it is involved in some suppressive mechanism that impedes the manifestation of the eye inflammation.

In spite of the extensive cross-reactivity and similarities of bovine S antigen and human S antigen³² and of bovine IRBP and human IRBP,³³ and the similarities between the responses to human and bovine S antigen tested simultaneously by Nussenblatt *et al*,⁸ several of the differences observed between the patients with uveitis and those without uveitis could in part be related to the bovine source of the antigens.³¹ Intraocular inflammation may increase the number of circulating clones to less frequent peptide determinants and these may manifest themselves as a response to bovine S antigen or IRBP.

The study on the inhibition of the lymphocyte proliferative responses to peptide M showed that the surface molecules involved in the T cell activation in vitro were similar in both groups of patients with Behçet's disease, with uveitis and without uveitis. CD4⁺ T cells, as well as antigen presenting cells (APCs) with HLA-DR molecules, were revealed to be important in the lymphocyte proliferative responses in all patients tested. These findings are consistent with the reports about the importance of CD4⁺ T cells,^{6,7} and the presence of Ia⁺ cells³⁴ in the induction of EAU. Expression of MHC class II antigens in both the infiltrating cells and ocular resident cells has also been described in enucleated eyes from uveitis patients.³⁵ Hirose *et al* reported the predominant role of enriched CD4⁺ T cell fraction in the lymphocyte proliferative response to retinal antigens in uveitis patients.³⁶ The addition of APC was essential for the lymphocyte response.³⁶ Further, accessory molecules, such as LFA-1 and CD2, which are important for cell-cell adhesion during activation of the T cell inhibited the lymphocyte proliferative responses to peptide M. CD3 associated with T cell receptor (TCR) molecules form the CD3-TCR complex. The CD3 molecule was also involved in the T cell response to peptide M. Curiously, the present

study demonstrated that CD8⁺ T cells are also involved in the proliferative responses to peptide M. The primary proliferative responses of purified CD8⁺ cells are reported to occur and not to be blocked by anti-CD4 mAb in a mixed lymphocyte reaction against allo-MHC class I.³⁷ One of the responses inhibited by mAb anti-CD8 was also inhibited by mAb anti-HLA class I (data not shown).

The present study has shown that patients with Behçet's disease without uveitis did not have their lymphocytes proliferating to bovine S antigen as much as those patients with uveitis. However, a certain percentage of patients responded to bovine IRBP and also to the uveitogenic peptides, peptides M and N, from the sequence of bovine S antigen, and to R-4 and R-14, from the sequence of bovine IRBP. These findings suggest that cellular autoimmunity to the retinal antigens may play a pivotal role in the induction of uveitis in Behçet's disease. Further longitudinal studies in patients without uveitis may clarify whether cellular autoimmunity plays a primary role in the pathophysiology of the uveitis in Behçet's disease.

The authors would like to express their gratitude to: Dr K Hayashi, Dr Y Izawa, for the patients with Behçet's disease with uveitis; Dr S Hirohata, Dr T Hashimoto, Dr A Takeuchi, Dr M Mizoguchi, for the patients with Behçet's disease without uveitis; Dr T Shinohara, for the generous gift of peptides M and N; Dr B Wiggert, Dr G J Chader, for the generous gift of IRBP, R-4 and R-14; Dr Y Fujino, for important advice; Dr I Gery, for relevant discussion and advice; Mrs B Parker, for reviewing the English language of the manuscript. J H Yamamoto is supported by a scholarship from the Japanese Ministry of Education, Science and Culture.

- Mochizuki M, Iwase K, Numaga J, Hayashi K, Ota Y. Behçet's disease in Japan. In: Belfort RJ, Petrilli AM, Nussenblatt RB, eds. *Proceedings of the First World Uveitis Symposium*. Sao Paulo:Roca, 1988: 401.
- Gery I, Mochizuki M, Nussenblatt RB. Retinal specific antigens and immunopathogenic processes they provoke. In: Osborne N, Chader GJ, eds. *Progress in Retinal Research*. New York: Pergamon, 1986: 75-109.
- Chader GJ. Interphotoreceptor retinoid-binding protein (IRBP): a model protein for molecular biological and clinically relevant studies. *Invest Ophthalmol Vis Sci* 1989; 30: 7-22.
- Nussenblatt RB, Kuwabara T, Monasterio FM, Wacker WB. S-antigen uveitis in primates. A new model for human disease. *Arch Ophthalmol* 1981; 99: 1090-2.
- Hirose S, Kuwabara T, Nussenblatt RB, Wiggert B, Redmond TM, Gery I. Uveitis induced in primates by interphotoreceptor retinoid-binding protein. *Arch Ophthalmol* 1986; 104: 1698-702.
- Mochizuki M, Kuwabara T, McAllister C, Nussenblatt RB, Gery I. Adoptive transfer of experimental autoimmune uveoretinitis in rats: immunopathogenic mechanisms and histological features. *Invest Ophthalmol Vis Sci* 1985; 26: 1-9.
- Caspi RR, Roberge FG, McAllister CG, El-Saied M, Kuwabara T, Gery I, *et al*. T cell line mediating experimental autoimmune uveoretinitis (EAU) in the rat. *J Immunol* 1986; 136: 928-33.
- Nussenblatt RB, Gery I, Ballantine EJ, Wacker WB. Cellular immune responsiveness of uveitis patients to retinal S-antigen. *Am J Ophthalmol* 1980; 89: 173-9.
- de Smet MD, Yamamoto JH, Mochizuki M, Gery I, Singh VK, Shinohara T, *et al*. Cellular immune responses to patients with uveitis to retinal antigens and their fragments. *Am J Ophthalmol* 1990; 110: 135-42.
- Nussenblatt RB, Mittal KK, Ryan S, Green WE, Maumenee AE. Birdshot retinochoroidopathy associated with HLA-A29 antigen and immune responsiveness to retinal S-antigen. *Am J Ophthalmol* 1982; 94: 147-58.
- Doekes G, van der Gaag R, Rothova A, van Kooyk Y, Broersma L, Zaai MJM, *et al*. Humoral and cellular immune responsiveness to human S-antigen in uveitis. *Curr Eye Res* 1987; 6: 909-19.
- Nussenblatt RB, Mittal KK, Fuhrman S, Sharma SD, Palestine AG. Lymphocyte proliferative responses of patients with ocular toxoplasmosis to parasite and retinal antigens. *Am J Ophthalmol* 1989; 107: 632-41.
- Yamamoto JH, Wiggert B, Chader GJ, Shinohara T, Gery I, Nussenblatt RB, *et al*. Cellular immune responsiveness of Japanese uveitis patients to retinal antigens and their uveitogenic peptides. In: Usui M, Ohno S, Aoki K, eds. *Ocular immunology today*. Amsterdam: Elsevier, 1990: 357-60.

- 14 Behçet's Disease Research Committee of Japan. Behçet's disease. Guide to diagnosis of Behçet's disease. *Jpn J Ophthalmol* 1974; **18**: 291.
- 15 Dorey C, Cozette J, Faure JP. A simple and rapid method for isolation of retinal S-antigen. *Ophthalmic Res* 1982; **14**: 249-55.
- 16 Redmond TM, Wiggert B, Robey FA, Nguyen NY, Lewis MS, Lee L, *et al.* Isolation and characterization of monkey interphotoreceptor binding protein, a unique extracellular matrix component of the retina. *Biochemistry* 1985; **24**: 787-93.
- 17 Shinohara T, Dietzschold B, Craft CM, Wistow G, Early JJ, Donoso LA, *et al.* Primary and secondary structure of bovine retinal S antigen (48-kDa protein). *Proc Natl Acad Sci USA* 1987; **84**: 6975-9.
- 18 Donoso LA, Merryman CM, Shinohara T, Dietzschold B, Wistow G, Craft C, *et al.* S-antigen. Identification of MabA9-C6 monoclonal antibody binding site and the uveitopathogenic sites. *Curr Eye Res* 1986; **5**: 995-1004.
- 19 Donoso LA, Merryman CF, Sery TW, Shinohara T, Dietzschold B, Smith A, *et al.* S-antigen: characterization of a pathogenic epitope which mediates experimental autoimmune uveitis and pinealitis in Lewis rats. *Curr Eye Res* 1987; **6**: 1151-9.
- 20 Singh VK, Nussenblatt RB, Donoso LA, Yamaki K, Chan C-C, Shinohara T. Identification of a uveitopathogenic and lymphocyte proliferative site in bovine S-antigen. *Cell Immunol* 1988; **115**: 413-9.
- 21 Borst DE, Redmond TM, Elser JE, Gonda MA, Wiggert B, Chader GJ, *et al.* Interphotoreceptor retinoid-binding protein. Gene characterization, protein repeat structure and its evolution. *J Biol Chem* 1989; **264**: 1115-23.
- 22 Sanui H, Redmond TM, Hu L-H, Kuwabara T, Margalit H, Cornette JL, *et al.* Synthetic peptides derived from IRBP induce EAU and EAP in Lewis rats. *Curr Eye Res* 1988; **7**: 727-35.
- 23 Sanui H, Redmond TM, Kotake S, Wiggert B, Hu L-H, Margalit H, *et al.* Identification of an immunodominant and highly immunopathogenic determinant in the retinal interphotoreceptor retinoid-binding protein (IRBP). *J Exp Med* 1989; **169**: 1947-60.
- 24 Faure J-P, Kozak Y. Cellular and humoral reactions to retinal antigens; specific suppression of experimental uveoretinitis. In: Helmsen RJ, Suran A, Gery I, Nussenblatt RB, eds. *Immunology of the eye: II*. Washington: IRL Press, 1981: 33-48.
- 25 Spencer WH. In: *Ophthalmic pathology. An atlas and textbook*. 3rd ed. Philadelphia: Saunders, 1985: 1160.
- 26 Shinohara T, Donoso LA, Tsuda M, Yamaki K, Singh VK. S-antigen: structure, function and experimental autoimmune uveitis (EAU). In: Osborne N, Chader GJ, eds. *Progress in retinal research*. New York: Pergamon, 1988: 51-66.
- 27 Abbas AK, Lichtman AH, Pober JS. *Cellular and molecular immunology*. Philadelphia: Saunders, 1991: 369.
- 28 Eto K, Santhanakrishnan S, Suzuki S, Shinohara T. Auto-immune inflammation induced by micro-organisms possessing cross-reactive antigens. *Invest Ophthalmol Vis Sci* 1992; **33**: 931.
- 29 Gammon G, Shastri N, Cogswell J, Wilbur S, Sadegh-Nasseri S, Krzych U, *et al.* The choice of T-cell epitopes utilized on a protein antigen depends on multiple factors distant from, as well as at the determinant site. *Immunol Rev* 1987; **98**: 53-73.
- 30 Lipham WJ, Redmond TM, Takahashi H, Berzofsky JA, Wiggert B, Chader GJ, *et al.* Recognition of peptides that are immunopathogenic but cryptic. *J Immunol* 1991; **146**: 3757-62.
- 31 Gregerson DS, Fling SP, Obritsch WF, Merryman CF, Donoso LA. A new perspective of S-antigen from immunochemical analysis. *Curr Eye Res* 1990; **9** (suppl): 145-53.
- 32 Mirshahi M, Boucheix C, Collenor G, Thillaye B, Faure J-P. Retinal S-antigen epitopes in vertebrate and invertebrate photoreceptors. *Invest Ophthalmol Vis Sci* 1985; **26**: 1016-21.
- 33 Redmond TM, Wiggert B, Robey FA, Chader GJ. Interspecies conservation on structure of interphotoreceptor retinoid-binding protein. *Biochemistry* 1986; **24**: 19-26.
- 34 Fujikawa LS, Larson CA, Holmes A. Immunomodulation of experimental autoimmune uveoretinitis: the role of class II antigen expression and interferon- γ . *Invest Ophthalmol Vis Sci* 1991; **32**: 932.
- 35 Hooks JJ, Chan C-C, Detrick B. Identification of the lymphocytes, interferon-gamma and interleukin-2, in inflammatory eye diseases. *Invest Ophthalmol Vis Sci* 1988; **29**: 1398-402.
- 36 Hirose S, Tanaka T, Nussenblatt RB, Palestine AG, Wiggert B, Redmond TM, *et al.* Lymphocyte responses to retinal-specific antigens in uveitis patients and healthy subjects. *Curr Eye Res* 1988; **7**: 393-402.
- 37 Sprent J, Schaefer M. Antigen-presenting cells for unprimed T cells. *Immunol Today* 1989; **10**: 17-23.