Observations in clinical and experimental ocular autoimmunity

de Smet, M.D.

Citation for published version (APA):
de Smet, M. D. (2000). Observations in clinical and experimental ocular autoimmunity

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Summary and Conclusions

In the foregoing chapters, a series of observations are presented on both experimental and human uveitis. The observations in the experimental model are used as a basis to better understand the human situation. However, human studies have also generated new insights on the pathophysiologic process, and these will lead to further studies in the animal model. Findings regarding fluctuations in circulating lymphocytes responsive to ocular autoantigens, their enumeration, or the presence of determinant spread as seen in humans have not yet been documented in experimental uveitis models. Indeed, it has rarely been studied in other autoimmune diseases. By contrast, while we have considerable knowledge regarding the pivotal uveitogenic determinants of S-Ag and interphotoreceptor retinoid binding protein (IRBP) in the Lewis rat (section I), we know very little of the role of peptide determinants in humans. The first plausible determinants to fulfill this role have been identified and presented in section II.

In addition to observations relating to the mechanism of immune stimulation, this thesis has also addressed the use of experimental models in testing new therapeutic modalities. For clinicians, experimental models are particularly useful in testing these novel approaches prior to initiating clinical studies. In section III, attempts at modulating experimental uveitis using several different approaches are presented. When this thesis was submitted, two of the three tested approaches have made it to human clinical trials or to clinical practice. Whether rapamycin will make it to the clinic will depend on the company’s determination to fund the appropriate trials. Rapamycin has several characteristics that make it particularly appealing, including its ability to inhibit both T and B cell proliferation, and to reduce neovascularization. Section IV presents results in human trials based on experimental data using antigen feeding, an approach which is felt to hold great promise, but which is still poorly understood. Other articles refer to cyclosporine and its clinical use. Also introduced to the treatment of uveitis on the basis of experimental results, cyclosporine has required the development of specific treatment protocols to avoid systemic toxicity. Since uveitis patients are generally healthy except for their eye disease, avoidance of systemic side effects is of crucial importance. Strategies in this direction based on the use of cyclosporine on its own, or in combination with an enzyme inhibitor are presented.

Before discussing each section in more detail, it is appropriate to reflect on the differences between the experimental model as it is currently used, and human disease. Experimental Autoimmune Uveitis (EAU) is a T cell mediated process characterized by an acute onset, a self limiting course and recurrence only under special circumstances (and in a limited number of species). In contrast, human disease in most instances is rather indolent, characterized by exacerbations and remissions over several months to years. Furthermore, S-Ag and IRBP, which are used extensively in this thesis as model ocular autoantigens, generate an inflammation centered in the retina (particularly in the rat and mice). Many human uveitic conditions affect the retina and choroid equally, or appear to cause more damage in the RPE and choriocapillaris. Other antigens such as melanocyte derived antigen may more appropriately portray human disease, and its recurrence pattern. This model deserves further investigation. At present the antigen causing EMIU is poorly characterized, and the immunopathogenic epitopes are not known. Despite its limitations, EAU has given us a template for the study of autoimmunity in general, and uveitis in particular.
This thesis has drawn on the elements of this template and hopefully may serve as a model for future studies.

Section 1: Immunologic Determinants of Ocular Autoantigens in Experimental Uveitis

Antigenic proteins are processed prior to inducing an immune response. In general, the initial immune response is directed to a limited number of determinants [see chapter 1]. In IRBP, this immunodominant determinant is located at 1182-1190 of bovine IRBP. By using amino acid substitutions along the length of the decapeptide, it was possible to study the role of individual residues with regards to various immunologic activities that characterize a given determinant, namely immunogenicity, immunodominance, and uveitogenicity (pathogenicity). Each of these terms was specifically defined in the context of these studies. Immunogenicity referred to the ability to generate an immune response. Uveitogenicity referred to the ability to cause uveitis. Immunodominance referred to the ability of a determinant to cause disease at the lowest immunizing dose (causing disease comparable to the whole molecule). Since the peptide determinant serves as a bridge between the antigen presenting cell (APC) and the T cell receptor (TCR), it was also possible to study the likely role in the TCR-MHC interaction of each residue. Analysis of activities of pivotal residues made it possible to learn about their role in the interaction between the T cell receptor and the MHC molecule on the surface of the antigen presenting cell (APC). Thus, valine at position 1188 and proline at position 1189 are critical for interaction with the TCR, while tryptophan at 1182, and aspartic acid at 1190 are essential for peptide binding to the MHC. These findings raise another issue: other experiments have shown that certain determinants were recognized by several different MHCs, and across species. It would be interesting to know if the same binding elements are crucial for TCR and MHC interactions in each of these species.

In the course of certain studies regarding factors affecting the intracellular trafficking of IRBP, we found that it was strongly bound, through an ATP dependent process, to a cytosolic protein of molecular weight 70 kD. Immunoprecipitation and partial sequencing identified this protein as being part of the chaperone family of proteins, a highly conserved protein family involved in preventing protein denaturation, and degradation. Its role in the immune process is not clear. There is considerable evidence in the literature to suggest that chaperones influence antigen presentation. Their presence may also promote an immune response as has been clearly demonstrated in certain forms of cancer and suggested in Behçet’s disease. Certainly modifications in the peptide sequence will modify the affinity for the IRBP chaperone (personal communication – K. Rengarajan). Expression of hsp in the inflamed retina follows a defined pattern, starting in the ganglion cell layer and progressing to the RPE which expresses high level of hsp only in late stages of the disease. However, the exact role of this particular chaperone in the immunologic process remains to be defined.

Next human S-Ag (hS-Ag) was studied in the Lewis rat to identify its immunologic and immunopathologic determinants. Using overlapping peptides of the whole sequence, several sites were identified capable of generating an immunopathogenic response. The most active site was identified at position 340-360, similar to findings by other authors who had used the bovine sequence. Of interest, this most pathogenic sequence was not the most proliferative suggesting a
separation between pathogenicity and immunogenicity. This observation, also made by other investigators is of critical importance, since the identification of a highly proliferative site in humans does not necessarily imply a role in pathogenesis.

Finally, the more pathogenic determinants of S-Ag in the Lewis rat were tested in other rat strains, some of which shared the same MHC background while others did not. This study attempted to identify determinants which had the ability to cross histocompatibility boundaries. In the case of determinant 340-360, this was certainly the case. However, even in strains that shared the same MHC background, there was considerable variation in the degree of immunogenicity and pathogenicity of each tested determinant. While the causes of this variation were not investigated in this study, factors such neuro-endocrine modulation, the T cell priming to a Th1 or Th2 profile play a critical role. Thus, factors extrinsic to the ability to appropriately process and recognize an antigen play a role in the phenotypic expression of disease. In outbred species, these external influences are even more likely to play a critical role. In the light of these results and others, interpretation of proliferation results in humans must be done with care.

Section II: Immunologic Responses to Ocular Autoantigens in Humans

To obtain a profile of the immune response to retinal autoantigens in humans, two patient populations were tested in the US and in Japan. The profile in both countries was similar. Patients with retinal pathology were more likely to have a positive immune response to S-Ag or IRBP. Furthermore, several patients responded to both antigens or fragments thereof. This pattern was seen in 26 of 82 patients tested. The result is even more significant if one considers that about half of the patients did not show any significant proliferative response to any antigen (defined in this case as a proliferation above the mean of controls plus 2 standard deviations). Since most of these patients had a well established disease pattern for several years, it is assumed that sensitization to the second retinal autoantigen (and likely more) took place some time after the initial inflammatory episode began. A proliferative response in control subjects was noted in up to one third of individuals. Current thoughts regarding autoimmunity support the concept of self recognition in both B and T cell networks both in normal individuals and in pathological states.

In lymphocyte cultures from patients, fluctuations in triplicate wells were frequently observed. Often one of the three triplicate wells would deviate in its response level. The reason for this deviation was generally felt to be due to an inhibitory microenvironment preventing growth of responsive cells. However, the cause could equally have been a low circulating number of responsive T cells. To test this hypothesis, cultures were set up in a way to minimize both effects. A statistically significant number of wells were placed in culture (490 wells), and T cell cytokine stimulants were given in each well. This assay was shown by others to act as a modified limiting dilution assay (LDA). Thus, it was possible to estimate the number of circulating responsive T cells in the peripheral blood of patients and controls. Patients have a 100 to 1000 fold higher number of responsive circulating cells to S-Ag as compared to controls. Results were in keeping with those previously published using an IL-2 release assay.

Having shown that this method allowed enumeration of responsive T cells in the peripheral blood of patients and controls, we wanted next to determine the profile of these cells over time. We de-
cided to limit our study to a well defined population of patients, namely those with Behçet’s disease. A group of five patients were followed prospectively for several months using the standard proliferation assay (as was used in the first study presented in this section), as well as by the modified LDA assay described above. Despite the limited nature of the study, several observations were possible. (1) The standard assay is poor at predicting the timing of an inflammatory recurrence, but a stimulation index higher than the mean + 2x standard deviation correlated with clinical exacerbation some time in the future. (2) Following an ocular attack, the standard assay gave stimulation indices often below their baseline, making it an unsuitable test to follow patients prospectively. (3) The LDA appeared to correlate fairly well with ocular activity—demonstrating an increase in peripheral responsive cells shortly after an episode of ocular activity. (4) The increase was maintained for a few months before decreasing back to baseline. The curve was similar to the one observed in experimental models of inflammation or infection. Thus, the standard assay is able to provide information on the relevance of a given antigen to a disease process (without saying anything on its role in the disease), while the LDA assay might be useful in prognostication. Future studies may help to delineate the predictive value of LDA assays, but a simpler methodology than the one used in the current study would be needed.

Finally, using the same overlapping peptides of human S-Ag that were used in section I, we tested a number of patients and controls. To insure relevance to the disease process, a substantial number of controls were tested. The data were analyzed using 2 approaches. The first aimed at identifying determinants for which the proliferative response in patients was statistically significantly higher than in controls—in essence these determinants would provide an immunodominant (proliferative) response in all patients with a given disease. Such determinants were identified in Behçet’s disease and in Sarcoidosis. They were located adjacent to immunopathogenic determinants in the rat with which they shared part of the sequence. Next the data was analyzed for determinants of clinical relevance, as indicated in the previous study by stimulation indices above the mean of controls plus 2 standard deviations. Several patients had responses to multiple determinants not limited to the immunodominant determinants identified above. A similar observation was made by others in patients with multiple sclerosis where it was proposed to be caused by determinant spreading. With each disease recurrence, the immune response appears to shift to a new determinant. In experimental autoimmune encephalitis, this determinant spread follows a predictable course, and modulation of the response to these determinants can limit disease recurrence. This is the first observation of such a phenomenon in uveitis. Its relevance to ocular autoimmune needs further study.

Section III: Novel Therapeutic Strategies in Uveitis

Over the years, the EAU model of uveitis has been useful to test new therapeutic modalities. Favorable observations with FK506 (tacrolimus) in the rat model were extended to the primate model, and are the subject of the first paper in this section. Side effects were limited to a dose of 0.5 mg/kg/d and were largely limited to liver toxicity. At the lower doses of 0.25 mg/kg/d or 0.125 mg/kg/d, no toxicity was observed. Protection from uveitis was induced in a majority of animals though not all were protected, particularly when the drug was given a few days prior to the onset of disease (in the afferent phase of the immune response). Immunohistochemical study of the ocular infiltrating cells revealed a shift in response to a more inhibitory profile (CD8), with a reduction in the number of infiltrating B cells.
A similar study was carried out in rats using rapamycin, an inhibitor of lymphocyte growth factor signal transduction. A dose of 0.1 mg/kg/d was found effective at inhibiting EAU induction even when treatment was initiated 7 days after immunization.

We also looked at the ability of using IL-13 to block further development of uveitis once it had appeared in one eye. IL-13 is a pleomorphic cytokine produced by Th-2 lymphocytes. As IL-10 and glucocorticoids, it has a downregulating effect on cell mediated immunity. IL-13 inhibited inflammation both in the affected eye as well as in the contralateral eye. The effect extended beyond the treatment period, and lasts at least for the 4 week follow-up period. Modulation of disease by promoting a Th2 environment as suggested here appears to be a promising line of investigation.

Section IV: Therapeutic Strategies for the Treatment of Human Uveitis

Cyclosporin e was introduced about 20 years ago as a potent mediator of T cell activity. It has been used extensively in uveitis with beneficial results, particularly in Behçet's disease. However, a few years after its introduction, it became clear that Cyclosporine was responsible for significant side effects, particularly in the kidney. Appropriate treatment schemes were required to monitor patient response to treatment, and to monitor for side effects. A summary of the current knowledge regarding cyclosporin e and its use in Ophthalmology is provided in the first chapter in this section. Since then, trials have been made to start at lower initial doses, and it has been used to treat dry eye syndrome using a topical preparation.

The next two articles present a novel treatment combination in an attempt to reduce the required oral dose of cyclosporine. Since cyclosporine is metabolized primarily by hepatic cytochrome P450, the use of an inhibitor of this enzyme (ketoconazole) leads to a reduced need for the medication, and more stable systemic drug levels. Additional benefits include an increased efficacy due to more stable blood levels throughout the day, and a reduction in renal toxicity.

The final article presents a novel therapeutic approach. The use of antigen feeding to prevent ocular inflammation was introduced a few years ago as a highly promising approach to modulate autoimmune inflammation. The mechanism is felt to involve antigen presentation in intestinal Peyer patches where a Th2 profile is induced in exposed lymphocytes. These cells are then free to circulate to other target organs such as the eye where their action may lead to a down regulation of inflammation through secretion of Th2 type cytokines. The pilot results of an S-Ag feeding study in 2 patients are presented in the last article. Two patients with posterior uveitis were able to be tapered off medication. Stopping S-Ag feeding caused an increase in lymphocyte responsiveness which was followed by disease recurrence. Based on these promising results, a trial of bovine S-Ag versus crude bovine retinal extract was initiated. Results from this study were rather surprising. S-Ag fed patients had prolonged remissions compared to controls and lower doses of anti-inflammatory medications were needed. Patients receiving retinal extract did worse than controls. While the reason for these results is not known, the literature does support paradoxical immune responses. Thus, while a promising approach, additional studies are required to identify the exact determinants responsible for the ocular inflammatory response. Given our observations with S-Ag fragments, it would seem that additional information would also be needed regarding the influence of antigen spreading on the feeding response.
Many unanswered questions remain. While our understanding of human disease still lags behind our understanding of the experimental model, approaches similar to those proposed here, supplemented by obtaining intraocular material (through ocular punctures or biopsies) should help us to fill in part of this gap in years to come.