Observations in clinical and experimental ocular autoimmunity

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Chapter 16

Intraocular Inflammatory Disease (Uveitis) and the Use of Oral Tolerance: A Status Report

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Summary

Intraocular inflammatory disease, or uveitis, is a disorder that affects mostly children and young adults. It is the cause of about 10% of the severe visual handicap in the United States. Many of the severe, sight threatening uveitic conditions are thought to be driven by putative autoimmune mechanisms, often with high dose oral prednisone used as treatment, along with cytotoxic agents, antimetabolites, and cyclosporine adjunctively. The feeding of the uveitogenic retinal S-antigen to rats immunized with the same antigen resulted in clinical protection. A pilot study in which two patients, one with pars planitis and the other with Behçet’s disease, were fed with the retinal S-antigen resulted in these patients’ immunosuppressive medication being decreased and/or stopped. The trial also provided us with information concerning dosage and expected immune responses. A randomized, masked study looking at the effect of feeding retinal antigens to uveitis patients is ongoing.

Introduction

Intraocular inflammatory disease or uveitis is a common disorder that ophthalmologists must deal with in their everyday practice. The term uveitis is an old one, harking back to the last century. Though at one time indicating where the nidus of the inflammatory response was thought to reside, i.e. the uvea, the term today has taken on a generic characteristic, and indicates only that there is an inflammatory response inside the eye. Therefore disorders such as sympathetic ophthalmia, which primarily affects the choroid of the eye, and Behçet’s disease, which primarily affects the retina, are both termed an uveitis. Further, an infectious process, such as toxoplasmosis and cytomegalovirus, both of which have a propensity for the retina, and candidiasis, which begins in the choroid, are also considered one of the many uveitides. It is the cause of about 10% of the severe visual handicap in the United States.

The mechanism of action of intraocular inflammatory disease has been debated over the years. Initially, most physicians caring for patients with this problem considered that the uveitis was a reflection of systemic infections. In one study performed at the Wilmer Eye Institute in the 1940’s, essentially all the patients examined with granulomatous uveitis were diagnosed as having either tuberculosis or syphilis (1). Over the years, these diagnoses simply could not explain what was observed in the eye. Additionally, immunologic theories and laboratory techniques permitted a more in depth evaluation of pathologic processes. The presence of circulating immune complexes, as well as in the eye were described in patients with uveitis (2,3). Because of these observations, it was suggested that the mechanism of uveitis was a type III hypersensitivity reaction. Therapeutic strategies were initially based on these concepts. Subsequent studies have suggested that uveitis patients with immune complexes have better visual outcomes than those without immune complexes (4). An explanation offered has been that immune complexes are present as a way to more rapidly clear debris and potentially toxic materials from the eye or circulation, and therefore helping as well to limit the inflammatory process.

The development of an animal model for intraocular inflammatory disease altered immeasurably our ability to study the underlying mechanisms of this disorder. Several uveitogenic antigens have been isolated and purified (5). The first and certainly one of the best characterized is the retinal S-antigen (S-Ag), which is found in the photoreceptor region of the retina. Isolated and purified by Wacker et al (6) and Faure et al (7), immunization of genetically susceptible lower
mammals with this 55kD protein will result in a bilateral uveitis several weeks later. S-Ag is also found in the pineal and a pinealitis accompanies the ocular inflammatory response. A second uveitogenic antigen that has been extensively used as a model for human uveitis is IRBP, the interphoreceptor retinoid-binding protein. This antigen, about thrice as large as the S-Ag (140kb), produces an inflammatory response that is similar to that of the S-antigen. While both the S-antigen and IRBP are capable of inducing an uveitis in genetically prone rats, mice appear to be much more susceptible to IRBP induced EAU (8).

Experimental autoimmune uveitis (EAU) can be induced not only in lower mammals but in non-human primates as well (5). The disorder induced has many characteristics of severe human uveitis. Studies investigating the underlying mechanism of this model have repeatedly shown the importance of T cells. The disease cannot be induced in athymic rats, nor will a transfer of antiS-antigen antibody to naive immunocompetent hosts result in disease. However, the transfer of T cells to naive hosts will result in disease. T cell lines are efficient in transferring disease (9). These lines bear large numbers of IL-2 receptors on their cell surface and have a cytokine profile that would define them as TH-1 cells. Recent work would suggest that as the disorder progresses, TH-2 cells appear in the eye, apparently in an attempt to down regulate the inflammatory response.

Because the animal model for uveitis effectively mimics the human situation, we used this model to evaluate various immuno-modulatory approaches. This practice began with the use of cyclosporine (CsA) (10). The results demonstrated that CsA very effectively prevented the expression of EAU in Lewis rats. The response was dose dependent. Therapy could be initiated even 7 days after immunization, a point at which uveitogenic cells can be found in the draining lymph nodes of S-Ag immunized rats. The inhibition of disease was obtained also when lines of uveitogenic T-cells were transferred to naive hosts. CsA treatment did not, in our hands, induce a long-standing tolerance, since the disease would appear if therapy was stopped while S-Ag was still present at the immunization site. On the basis of these observations, CsA has been used in the treatment of uveitis in patients (see below).

EAU has become a template for the evaluation of various immunomodulatory approaches. To date, almost all new immunomodulatory agents destined for the treatment of severe intraocular inflammatory disease have been tested in this model. FK-506, with a mechanism of action thought to be virtually identical to CsA, was shown to be effective in inhibiting EAU both in rodents as well as non-human primates (11,12). Other agents, such as rapamycin and mycophenolate mofetil, have been used and found to inhibit disease (13,14). Other immunomodulatory approaches, such as T-cell vaccination (15) and the recombinantly produced material IL-2PE40, have been tried, and found useful in treating the disorder (16).

With new approaches to therapy constantly being evaluated in the animal model for human uveitis, it would be helpful to put into perspective what is presently used to treat patients. The initial drug of choice is corticosteroid. This drug can be used systemically, in periocular injections or topically. For most serious sight-threatening uveitides, most patients have posterior pole involvement, invariably necessitating the use of systemic corticosteroid. Long term use is problematic, particularly so because the initial dosages needed to effect a positive therapeutic response are relatively high, 1 to 1.5 mg/kg, as are the doses needed to maintain this effect, usually 20-40 mg of prednisone/day. Alternative approaches would include cyclosporine, as well as cytotoxic and anti-metabolic agents. Cyclosporine has begun to be used widely as a second line agent or as
a steroid sparing agent. In cases of Behçet’s disease, it may be used as an initial treatment. The use of cytotoxic agents has become less popular because of their long-term potential side effects. They, at least anecdotally, appear to be helpful in the treatment of uveitis, and if used for fairly short periods of time at low doses, in certain cases may have an acceptable risk of secondary effects. Anti-metabolites, particularly imuran and methotrexate, have undergone a resurgence of use, at lower dosages than previously used, and often as a steroid or CsA sparing agent.

Clearly there is a practical need for the development of less toxic and hopefully more specific therapies for uveitis. Oral tolerance, with its effect shown in experimental autoimmune encephalomyelitis and reports of positive therapeutic effects in 2 clinical studies would be an obvious choice. We report here in summary the results of some of the animal work using EAU as a model to evaluate various aspects of oral tolerance and the status of studies in humans.

Materials and Methods

Studies with Rats
Six to 10 week old Lewis rats were gavage fed with S-Ag or a non-specific protein [Keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA)] at a dose of 1 mg/feeding on specific intermittent days relative to immunization. In other experiments, rats were fed bovine IRBP peptide 1179-1191, LPS, GMDP (an analog of muramyl dipeptide) or combinations of the peptide and either LPS or GMDP. Rats were immunized with either S-Ag (20 µg) or IRBP peptide 1179-1191 (1 nmol), depending on the experiment. Animals were examined regularly for evidence of ocular inflammatory disease, then sacrificed at various points after immunization and the histological changes in their eyes were examined and their immune responses evaluated.

In one set of experiments, some Lewis rats were splenectomized prior to feeding while control rats received sham operations.

In another set of experiments, female Lewis rats were treated intraperitoneally with the anti-CD8 antibody, 0X8 (9 mg total), while control animals received PBS or an isotype-matched irrelevant antibody. The rats were fed either S-Ag or BSA, as above, at specific points during the injection schedule, then subsequently immunized with S-Ag (20 _g).
Evidence of disease was evaluated for three weeks then the rats were sacrificed and their immune responses measured.

Studies with Mice:
Six - 12 week old mice deficient in CD8 lymphocytes [82m (-/-)] and immunologically intact 129/J control mice were fed intermittently with either ovalbumin (OVA) or myosin prior to immunization with OVA (20 _g). Cellular immune responses were measured by the lymphocyte proliferation assay two weeks after immunization.

In vitro Human Studies
2 x 105 cells isolated from the blood of the patient were placed into microtiter wells. At the initiation of the cultures, each well was pulsed with 20 _g/well of purified bovine retinal S-antigen. Cultures were performed in sextuplicate and harvested on day 5. Sixteen hours before the termination of the culture, thymidine was added to the wells. The results are expressed as stimulation
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index, which is obtained by dividing the counts/min obtained in the wells pulsed with retinal S-antigen by those counts obtained in the controls wells which just received culture medium. A stimulation index of 2 or more is considered as evidence of an anamnestic response to the retinal S-antigen (indicated by the dashed line).

Results

Feeding S-Ag Before the Immunization with S-Ag (17)
Feeding S-Antigen 3 times (-7, -5, -3) before immunization with S-Ag resulted in abrogation of the disease. The observation appeared to be dose dependent, with animals receiving 1 mg at each feeding being essentially totally protected. Lower doses resulted in an attenuated form of the disease, when measured by a masked observer approximately 2 weeks after immunization. Proliferative responses of T-cells taken from the draining lymph nodes to the immunizing antigen were decreased as compared to those animals fed as a control antigen.

Feeding antigen after immunization
The effect of oral tolerance as a means of inhibiting disease after immunization has been evaluated as well. Animals fed S-Ag 3, 5, and 7 days after immunization were noted to have either no disease or a disease process which was far less severe than control animals.

Role of the spleen in oral tolerance
The effect of splenectomy on oral tolerance was also evaluated (18). It was noted that animals that were splenectomized soon after birth developed severe uveitis even if fed with S-Ag at a dose which was capable of protecting animals who had received sham operations. This would suggest that an intact gut-spleen-ocular axis is important for the development of oral tolerance.

Effects of bacterial products on oral tolerance
Interest has been raised as to whether oral tolerance could be enhanced if bacterial products are fed at the same time as the uveitogenic antigens. Lewis rats immunized with an immuno-dominant fragment of IRBP, peptide 1179-1191, developed disease in all cases. The development of EAU could be exacerbated by feeding the rats with LP or GMDP prior to immunization. In contrast, the disease was markedly diminished when these bacterial products were fed along with the IRBP peptide used later for immunization (19).

Role of CD8 cells in oral tolerance
Rats which received injections of the anti-CD8 antibody OX8 and were fed S-Ag prior to immunization with S-Ag responded similarly to their controls. As can be seen in Figures 1 A and 1 B, the use of the antibody did not affect the induction of oral tolerance, as compared to treatment with PBS. As well, Figure 2 shows that the proliferative responses from the draining lymph nodes of S-antigen immunization gave identical proliferative responses.

Mice:
Cellular immune responses from CD8 deficient mice [B2m (-/-)] and their 129/J controls were very similar; feeding with OVA markedly reduced the cellular response against this antigen (Figure 3).
Figures 1A and 1B. Effect of anti-CD8 monoclonal antibody therapy on the induction of oral tolerance in experimental autoimmune uveitis (EAU). In panel A, animals received OX8 (anti-CD8) therapy and then were fed either S-Ag or BSA. In panel B, the results after PBS therapy are shown. The monoclonal antibody therapy did not abrogate the effect of oral S-Ag therapy.

Animal Studies: Discussion
Oral tolerance appears to be a mode of immunosuppression which protects against EAU. The effect is dose dependent and can be induced when feeding begins before or even after immunization. While fragments have been reported to be effective in preventing the expression of EAU, we have not seen a particularly remarkable effect with fragments of the retinal S-Ag. The immunodominant fragment of the retinal S-antigen in human disease is not clear since patients' cells will respond to several fragments when tested in vitro. Additionally, usually these fragments are not those to which rat lymphocytes respond (21). Experiments not shown here have not been overwhelmingly convincing in demonstrating a bystander suppression effect such as has been reported in other animal models. It may be that doses of the antigen used in our experiments induced anergy and therefore no bystander suppression was induced. As well, experiments would suggest that CD8+ cells are not necessary for the induction of oral tolerance. This finding needs to be discussed in the context of an earlier finding that the addition of anti-CD8 antibody to cultures of splenically derived lymphocytes from S-antigen fed rats reversed the downregulation noted in proliferation assays (17). We have seen as well the importance of the ocular splenic axis in our system. This mimics other immunosuppressive mechanisms involving the eye, most notably ACAID (22). Of interest was the finding that CD8 positive cells were not needed for the induction of oral tolerance.

Based on the observations that the EAU model would be a good “predictor” of an effect in human disease, a pilot study involving two patients was initiated.

Human Studies
Two patients were included in a pilot study initiated about 2 and one half years ago. The first patient is a 28 year old man with pars planitis (a common form of intermediate uveitis), who had been taking prednisone orally. This medication controlled his ocular inflammatory activity with maintenance of good visual acuity. However, he would suffer a recurrence of his uveitis
with any significant decrease in his prednisone dosage. The second patient is a 42 year old white woman with over a 12 year history of Behçet's disease (23). She was begun on cyclosporine in 1983 and has required this medication as well as a low dosage of prednisone in order to maintain remission of her ocular disease. In 1986 she received a trial of leukeran but this resulted in multiple ocular attacks necessitating a return to cyclosporine therapy. These patients were chosen because of the documented recurrences of their ocular disease that occurred with dosage reduction, and because they manifested in vitro cell mediated proliferative responses to the retinal S-antigen.

Both patients in this pilot phase of the study initially received 30 mg of bovine derived retinal S-antigen, given orally, three times a week with a subsequent decrease in S-antigen dosing to once a week and their subsequent course can be seen on figures 4 and 5. The figures also show the results of in vitro proliferative assays to the retinal S-antigen performed over time.

Figure 2. Panel showing the proliferative responses of cells from draining lymph nodes of S-Ag immunization site. Some animals were treated with the anti-CD8 antibody systemically and others received only PBS. Those fed S-Ag had significantly lower stimulation indices than those fed BSA, whether they were treated beforehand with antibody or not.

Figure 3. Lymphocyte proliferation response of β2m(-/-) mice and their 129/J controls after three feedings with either 0.3mg OVA or a control antigen (myosin).
Figure 4. Flow sheet showing clinical course and in vitro proliferative responses to the retinal S-antigen in Behçet’s disease patient participating in the pilot study evaluating the induction of oral tolerance with S-antigen.

Figure 5. Flow sheet showing clinical course and in vitro proliferative responses to the retinal S-antigen in a pars planitis patient participating in the pilot study evaluating the induction of oral tolerance with S-antigen.
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Modulation of Human Uveitis with Ocular Antigens

| UVEITIS CANDIDATE FOR FEEDING | S-AG + GOOD CONTROL OF OCULAR DISEASE ON CSA, STEROID, ETC GETS RECURRENCES WITH TAPER |

**RANDOMIZED STUDY**

TREAT FOR 21 DAYS
WITH 30 MG PO TIW S-AG, RETINAL MIXTURE, PLACEBO BUT MAINTAIN STANDARD THERAPY

BEGIN TO TAPER STANDARD THERAPY OVER THE NEXT 8 WEEKS
CONTINUE TO "FEED" ANTIGEN BUT DECREASED TO ONCE A WEEK

ATTEMPT TO TAKE PATIENT COMPLETELY OFF STANDARD THERAPY
CONTINUE MAINTAINANCE FEEDING THERAPY ONCE A WEEK FOR AT LEAST 6 MONTHS

*Figure 6. Outline of double masked study protocol evaluating the use of orally administered retinal antigens and the induction of oral tolerance.*

The patient with Behçet’s disease (Figure 4) had a minor inflammatory episode as the decrease of her cyclosporine therapy began, but this problem stabilized, and she remained free of activity for over two years. She suffered a recurrence at this point, necessitating a short term course of cyclosporine and prednisone which then was stopped. She was placed on a maintenance of 50 mg of imuran and continued to be fed with S-antigen. Of interest in both cases was the fact that the stimulation indices for the S-antigen in the main appeared to elevate with decreases in immunosuppressive or dosing before an attack.

*Figure 5 summarizes the clinical course of the pars planitis patient. As one can see, there was an initially high stimulation index of about 9. After three weeks of S-antigen therapy, the stimulation index fell to below two. With the discontinuation of prednisone therapy and as well a decrease in the frequency of S-antigen administration, an increase of the stimulation index back to the original level was noted, but no change in the clinical status of the patient was seen. With time, a decrease in the stimulation index was noted, but again this increased as the S-antigen feeding was stopped, which was done in an attempt to see if long term immunologic tolerance*
had been permanently induced. The patient remained off all medication from week 24 till week 62 when he returned because of an ocular inflammatory attack. He was treated with prednisone and feeding of the retinal S-antigen (three times a week) was reinstituted. Tested two weeks after the initiation of therapy, the stimulation index had once again fallen to well below two. He had a mild drop in vision in one eye with his local physician giving him a periorcular injection, but has not required systemic immunosuppression; he has continued to take S-antigen and has excellent vision in both eyes.

The results of these two pilot study patients were most helpful. While of course not proving the potential universal therapeutic efficacy of this approach, it did give us an indication that such an approach could be useful since neither clinical course would have been expected, based on the natural history of either patient’s disease. Both have retained good vision for the past 2 and one half years. The stimulation indices appear to be a sensitive indicator of either a change in immunosuppression or of an impending attack. Because of these positive therapeutic responses in these two patients, a randomized double masked study has begun where the efficacy not only of the retinal S-antigen but also a retinal mixture made up of soluble retinal antigens at low concentrations, is being tested (Figure 6). Patients with intermediate or posterior uveitis of a non-infectious cause necessitating systemic immunosuppressive therapy are potential candidates. Their circulating lymphocytes are tested for evidence of responsiveness to the retinal S-antigen. If they are positive, patients are randomized either to placebo, a retinal mixture, S-antigen (bovine source), or a combination of the retinal mixture and 5-antigen. Once fed one of these combinations for 3 weeks while maintaining their immunosuppressive therapy necessary to maintain good vision, they are tapered off from their therapeutic regimen over 10 weeks while continuing the feeding of the retinal materials or placebo. The intent is to induce a tolerogenic state with the feeding, therefore obviating the need for further immunosuppressive therapy (or at a lower dose). The end point of the study is time to recurrence of disease and the dosage of immunosuppressive agents used at the time of recurrence.

In summary, we have presented here our experience with oral tolerance, first in an animal model for human disease and then in a pilot study with 2 patients. The randomized masked study we hope will give more information about the potential utility of this approach.

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Conclusions

“Oui, le rêve dont se nourrit obscurement la Recherche humaine, c'est au fond, de parvenir à maîtriser, par delà toutes affinités atomiques ou moléculaires, l’Energie de fond dont toutes les autres énergies ne sont que les servantes: saisir, réunis tous ensemble, la barre du Monde, en mettant la main sur le Ressort même de L’Evolution.”

Pierre Teilhard de Chardin: Le Phénomène humain, 1940