Experimental approaches to specific immunotherapies in autoimmune disease: future treatment of endogenous posterior uveitis?

Andrew D Dick

(Auto)immune mediated diseases are diverse and may affect almost any organ. In the past their treatment has been largely with non-specific immunosuppression and supported, in controlling pain, with analgesics and non-steroidal anti-inflammatory agents in, for example, rheumatoid arthritis. The side effects of immunosuppression with steroids and cytotoxic agents, which for any control of disease have to be taken on a long term basis, often outweigh the benefits of any treatment. However, over the past two decades the discovery of more specific immunosuppressive agents – for example, cyclosporin A (CsA), and the further understanding of underlying immune processes (for example, T cell activation, T cell receptors, cytokines) has led to a wave of interest in developing specific treatments for immune mediated diseases. Newer immunosuppressive therapies are aiming to give long term suppression of (auto)immune responses with only short term treatment and specificity for the desired antigen.

Endogenous posterior uveoretinitis (EPU) is one of the best ocular examples of putative (auto)immune disease. However, there is still conflict as to what constitutes EPU. EPU is characterised by a combination of discrete clinical signs which include vitreal infiltration with inflammatory cells (viritis), focal or diffuse chorioretinal infiltration with leucocytes (choroiditis), inner and outer retinal vessel inflammation (retinal vasculitis), and macular and optic nerve head oedema.1 2 From these building blocks of signs arises a large spectrum of clinical conditions including sympathetic ophthalmia, intermediate uveitis, birdshot chorioretinitis, and pigment epithelitis. Although these conditions vary in their presentation, degree of HLA association, and response to relatively non-specific treatment, there is growing evidence both clinically and in experimental models that these conditions are characterised by an exaggerated immune response which causes tissue destruction and, without an obvious infective aetiology, is likely to be autoimmune in nature (Table 1).1 4 The immune mechanisms generated are either directed towards autoantigens or as a response triggered, for example, by an infectious agent (that is, foreign antigen), which may show some homology with host antigens and generate cross reactivity (molecular mimicry). The points at which the inflammatory response may be inhibited successfully would depend on whether the immune response was directed against host or foreign antigen which enters the tissue. Either way, when no overt infectious or neoplastic aetiology is found treatment may be directed towards dampening the resulting inflammatory cascade and hopefully reduce tissue damage. With the former I will discuss the possibility of inhibiting specific autoreactive T cells as opposed to inhibiting inflammatory mediators (cytokines) which result as a consequence of a pathogenic immune response.

Although present treatment is successful in some cases of EPU, as well as other autoimmune conditions, it is restricted by the required long term use, resistance in some patients, and significant side effects. Therefore there is a strong need for more selective and specific immunotherapy which is rapidly being developed and is now entering the era of clinical trials. This review concentrates on both current advances and future aspects of suppressing the (auto)immune response in EPU with examples of immunosuppressive treatments of other autoimmune diseases and experimental models of autoimmune disease including experimental autoimmune uveoretinitis (EAU), the animal model of EPU.

Where to target the immune response

Discussion of the evidence for (auto)immune mechanisms in EPU is not given in detail here (for review see Forrester3). In summary, in EPU, both granulomatous and delayed type hypersensitivity responses predominate which are likely to be mediated (in association with evidence from animal model, EAU) by CD4+ (Th1) T lymphocytes5 in genetically susceptible individuals. In addition, patients with EPU and EPU in association with systemic diseases such as Behçet’s disease and sarcoidosis show evidence of immune activation with activated peripheral circulating lymphocytes,5-8 presence of autoantibodies,9 10 T cells which proliferate in culture to autoantigen,11 raised soluble circulating levels of immune activation markers,12 13 vitreous T cells which are immunoreactive against autoantigens,14 and immune-histochemical evidence of immune mediated tissue damage.15 16 Successful suppression of the immune response in (auto)immune disease depends upon whether the disease is predominantly T or B cell mediated or not. This is important because as we now know they respond

Table 1 Support for (auto)immune mediated damage in endogenous posterior uveitis (EPU)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Evidence</th>
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<tbody>
<tr>
<td>EPU</td>
<td>Supports</td>
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<tr>
<td>EAU</td>
<td>Can be targeted</td>
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<tr>
<td>T cell</td>
<td>Detected</td>
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EAU=experimental autoimmune uveoretinitis. CsA=Cyclosporin A. IRBP=interphotoreceptor binding protein. TCR=T cell receptor.
differently to various forms of treatment, be it cyclophosphamide and steroids or CsA in more T cell mediated disorders (see below). In EPU, although the putative autoantigens (S-antigen and interphotoreceptor binding protein, IRBP) are potent stimulators of EAU, the presence of autoantibodies to these antigens in humans does not correlate with disease activity and there is little evidence to suggest that they play a major pathogenic role. This is in contrast with diseases such as systemic lupus erythematosus and Wegener's granulomatosis which can also cause a posterior uveitis. That is not to say that in later stages of the disease B cells and antibody responses are not important in the regulation of the immune response. Studies in patients with uveitis have shown conflicting results with high cellular reactivity to retinal antigens in these patients and in healthy controls. The finding of potential autoreactive T cells in healthy subjects (particularly as a primary response) does not diminish the importance of cellular reactivity which can mediate tissue damage in patients with uveitis. Tests preclude accurate assessment of precursor frequency of these cells and thus it is not known at what threshold tissue damage is likely to occur. On balance it is probable that the more successful forms of immunotherapy for EPU will be via targeting the T cell response, although inhibiting B cell response or redressing idiotype response may also have a role to play in treatment of these patients.

**INHIBITING T CELL ACTIVATION** (Fig 1)

In order for T cells to recognise foreign antigen the antigen must first be processed and presented as peptide fragments in the clefts of major histocompatibility complex (MHC) antigens; in the case of EPU, MHC class II antigens on 'professional' antigen presenting cells (APCs) presenting to CD4+ (T helper) lymphocytes. The T cells recognise this peptide/MHC class II association via their own unique T cell receptor (TCR), which itself is clonally restricted. In the adult the TCR consists of both α and β subunits coded for by Vα and Vβ genes. β gene rearrangement occurs in the thymus during development, which becomes deleted or develops into clones of T cells with specific TCR Vβ regions. We will see later that this restriction of Vβ gene usage in autoreactive T cells is of fundamental importance for successful targeted therapy against, for example, the TCR itself. Activation, however, does not occur unless other costimulatory events occur. Costimulation is mediated by other T cell surface antigens and the corresponding ligands on the APC – for example, CD28/B7 interaction, but many others are now thought to be of importance, where absence of any costimulation leads to a breakdown of T cell activation. Normally T cells do not respond to 'self' antigen because during development tolerance evolves both in thymus (T cells) and extrathymically (peripheral tolerance). Breakdown of tolerance is thought to be one of the triggers leading to autoimmunity (what causes a breakdown in tolerance is not discussed in this review) and therefore the T cells become activated against self (autoantigens), in the case of EPU, retinal antigens such as S-antigen, and IRBP. Although antigen processing and presentation leading to activation of T cells are complex events, experimental models of autoimmune disease have demonstrated that disease may be prevented by selective inhibition at any of these stages (and clinical trials of these new therapies are now beginning) although some approaches are more problematical than others in the treatment of autoimmune conditions.

**Blocking MHC peptide presentation**

Blocking the MHC class II molecule can prevent disease in experimental models of autoimmune disease, including EAU, by the administration of monoclonal antibodies directed against the MHC molecule. Moreover, peptides may compete for binding to some MHC class II molecule, preventing its capacity to bind (pathogenic) antigenic peptide. The concept of peptide blocking therapy has been employed successfully in other models of autoimmune disease – for example, experimental autoimmune encephalomyelitis (EAE). Recently in EAU it has been shown that in vivo competition between pathogenic and non-pathogenic peptides can prevent autoimmune disease. Peptide blocking therapy is not without problems because in the clinical setting of such autoimmune diseases as EPU and multiple sclerosis (MS) the antigens involved may be different in each patient and also may bind to more than one allotype of MHC class II receptor. Selective immunosuppression may be more probable in autoimmune diseases with a high HLA association (for example, birdshot chorioretinopathy and HLA A29) where peptides recognising specific alleles of particular regions of the MHC can be targeted, increasing specificity. Overall, MHC antagonists are likely only to prevent autoimmune disease and are unlikely to suppress effectively ongoing disease; they would therefore have to be combined with other forms of treatment to be successful clinically.
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specific regions of the TCR,25 26 but antibodies against more than one region of the TCR are often required to prevent disease successfully. In human as well as animal models of autoimmune disease there is some evidence of TCR restricted expression,27 28 but overall autoreactive T cells appear to be heterogeneous, which may make selective suppression difficult to achieve – that is, clinical success will only be possible if common TCR V genes are used by pathogenic T cells in different patients. This may also be the main problem preventing other approaches to inhibitions of TCR activation – for example, enhancing the anti-idiotypic T cell response which will inhibit the pathogenic activity of T cells. This approach is possible by administering peptides corresponding to T cell receptor sequences used by autoreactive T cells, the aim being to enhance regulatory T cells which are suppressive by their anti-idiotypic activity. The TCR is associated intimately with subunits of CD3, which is integral for activation of T cells. Monoclonal anti-CD3 antibodies will inhibit T cell activation, but this is relatively non-specific.

Targeting costimulatory accessory molecules
As I have mentioned, activation of T cells also requires the presence of costimulatory signals which are mediated through other cell surface molecules and their corresponding ligands on the APC. If this costimulation is not present (for example, by presentation of antigen via non-professional APC which lack accessory molecules B7 or ICAM-1) then the T cell will be ‘tolerised’ – that is, become immunologically unreactive. Certainly the finding that the co-administration, in experimental animals, of syngeneic splenocytes coupled with antigen prevents autoimmune disease39 is likely to be a result of this mechanism. Antibodies directed towards adhesion receptors on T cells are immunosuppressive and can establish long term tolerance, but this has only been to weak antigens.30 31 Other models of autoimmune disease have been used to study the role of other accessory molecules – for example, CD40 and gp39 (the ligand for CD40, which is expressed on activated CD4+ cells) and it has been found that autoimmune disease can be inhibited by anti-gp39 antibody.32 With the increasing understanding of the immunological importance of these accessory molecule interactions, inhibition of these interactions suppressing T cell activation opens another avenue which may have therapeutic potential in the treatment of autoimmune disease, but again it is unlikely to be specific to autoreactive T cells.

Targeting the CD4 molecule
There has recently been much progress in re-establishing tolerance to autoantigens (self antigens) in a sensitised immune system by directing antibodies against CD4 or CD8. In EAU administration of anti-CD4 antibody prevents the autoimmune disease.33 In transplantation models T cell tolerance can be maintained by combining antibodies which deplete CD4 and CD8 as well as block CD4 and CD8.34 In this model it was noted that tolerance was ‘infectious’ – that is, tolerant T cells were able to induce tolerance in potentially autoreactive naive T cells, the mechanism of which is unknown but may be the unmasking of regulatory T cells which protect against further autoimmune attack (for review see Waldmann and Cobold35). In the clinical setting, Campath-1H (humanised anti-CDw52 monoclonal antibody), which is directed against an antigen on all lymphocytes and some natural killer (NK) cells, has been used to treat a number of autoimmune conditions including rheumatoid arthritis,36 although it must be noted that not all patients respond. In some patients short term treatment does provide long term remission although in other patients this requires the additional treatment with anti-CD4 monoclonal antibody. In cases of systemic vasculitis a combination of anti-CD4 and Campath-1H has resulted in long term remissions.37 Campath-1H has also been successful in the suppression of EPU although we are still awaiting long term results (manuscript in preparation). Anti-CD4+ antibody treatment with humanised monoclonal antibodies has also been used in clinical trials of rheumatoid arthritis.38 There appears to be about a 50–60% response rate and treatment caused only a small drop in CD4+ levels; therefore its effect is unlikely to be depletion, as with anti-CDw52 treatment, but to some tolerance inducing mechanism such as anergising (inactivating) the CD4+ T cell.

Cyclosporin A, FK506, and rapamycin
Cyclosporin A (CsA) was first introduced in 1983 for the prevention of renal allograft rejection. All three agents are powerful suppressants of the immune response. Both CsA and FK506 inhibit calcium dependent TCR mediated signal transduction and thus activation of T cells.39 This also leads to a down regulation of IL-2 gene transcription40 and IL-2 receptor expression. CsA has been shown to be successful in controlling many autoimmune diseases as well as dramatically increasing the survival of renal allografts. Since the reports that CsA successfully suppressed EAU41 it has been used for a decade in humans with mixed success. Low dose treatment (5 mg/kg/day) with steroids and, in some cases, with additional cytotoxics, such as azathioprine, is able to control disease.42-45 However, as with all long term treatment side effects accrue and drug resistance is not uncommon. In the treatment of EPU, CsA induced nephrotoxicity is rare and reversible in the early stages with dose modulation or cessation of treatment.46 However, there is evidence that the renal wasting effect of CsA leads to a depletion of intracellular magnesium levels and considerable unpleasant side effects (unpublished data). Although FK506 is a more potent immunosuppressive, and is successful in suppressing EPU,47 it does not appear to be any less nephrotoxic. The main concern with long term treatment with CsA, or FK506 and cytotoxic agents in autoimmune diseases even at low effective doses, is the possible development of malignancy, and this should always be kept in mind and looked for during long term follow up. Another macrolide antibiotic, rapamycin, which has not been used clinically, is effective in suppressing EAU48 and appears to act at the effector phase of the immune response inhibiting T cell amplification and macrophage activation (J V Forrester, personal communication).

INHIBITING EFFECTOR RESPONSES (Fig 2)
When T cells have recognised antigen with the appropriate costimulation they become activated and differentiate into cells whose function is either directly cytotoxic or that of releasing cytokines which directly affect other cell differentiation and function. In organ specific autoimmune disease such as EPU, antigen is presented in combination to MHC class II antigens to CD4+ T cells. These cells can then develop into two classes of CD4+ cells, Th1 and Th2, which secrete different patterns of cytokines. EPU is an example of a Th1 mediated disease49 and cytokines such as interferon γ (IFN-γ) and interleukin 2 (IL-2) play a major role (Table 2). Although CD8+ cells (functionally cytotoxic or suppressive) are found in models of
autoimmune disease, particularly in the later stages.\textsuperscript{50} CD8+ depletion before immunisation has no effect on the course or severity of S-Ag induced EAU.\textsuperscript{51} This effect, however, may be because the monoclonal antibody used to abrogate the CD8+ response is depleting and perhaps non-depleting antibodies would have a greater effect as is the case with CD4+ monoclonal antibodies.\textsuperscript{52} However, similar studies in EAE have shown that long term depletion of CD8+ cells also has no effect on the immunoregulation of the autoimmune response,\textsuperscript{53} so the role of CD8+ cells in immune mediated disease, like EAU and EAE, remains undefined. However, there may be an immunoregulatory role, re-establishing self-tolerance by CD8+ T cells during mucosal tolerance induction which is discussed later.

Inhibition of the cytokine network has been the focus of recent developments of immunotherapy in animal models, the success of which has resulted in the onset of clinical trials in autoimmune diseases. This is mainly because the cytokines which activated CD4+ cells produce play a central role in the induction and perpetuation (and control) of an autoimmune response. Based on murine experimental models it is now thought that in humans CD4+ cells can also be characterised by their cytokine secretion profile into Th0, Th1, and Th2.\textsuperscript{54} Th1 cells secrete IFN-γ and also tumour necrosis factor (TNF-α), which activate macrophages enhancing antigen presentation, phagocytosis, and both superoxide and nitric oxide production; they also mediate delayed type hypersensitivity reactions,\textsuperscript{55} one of the hallmarks of EPU. Activation of naive T cells also leads to synthesis of IL-2 and upregulation of IL-2 receptors on the cell surface which in turn stimulates further T cells activation and T cell proliferation. The interaction between cytokines and subsets of CD4+ cells is complex; however, it is slowly becoming unravelled. In brief, it is known that Th2 cytokines, IL-4 and IL-10, inhibit the function of Th1 cells and their cytokine secretion, particularly IL-1, TNF-α, IL-6, and IFN-γ, and therefore interaction between these two subsets of cells plays a major role in regulation of each of their effector functions.\textsuperscript{54, 55} In EAU there is evidence to suggest that these two subsets of cells also play a role, as seen when in early stages of the disease IL-2 and IFN-γ are major cytokines and later IL-4 dominates.\textsuperscript{56} The role of individual cytokines in the immune response and in models of autoimmune disease is becoming more understandable and, with the recent and ever escalating development of ‘gene knockout mice’ where expression of individual cytokines is absent, the effect of immune mediated disease in the absence of important cytokines can be observed.

### Inhibiting cytokines in experimental models of autoimmune disease: progression to clinical trials

The cytokine network within the local environment of inflammation plays an integral role in the immunoregulation of the immune response. I have already discussed the role of currently used treatments such as CsA and FK506 and their effects of inhibiting not only signal transduction via the TCR but also IL-2 production and IL-2R expression. Within the eye rapamycin and FK506 appear to modulate cytokine release by activated retinal pigment epithelial cells, particularly increasing the release of GM-CSF, IL-8, and IL-6, although the role of these cytokines in EAU still requires further clarification (J V Forrester, personal communication).

Using animal models there have been many attempts to block cytokine action with specific monoclonal antibodies in the hope of suppressing the immune response. This success has now led to clinical trials which also show promise, although these trials have been open and uncontrolled, and thus the efficacy of treatment cannot be derived from their results. The role of TNF-α has been receiving particular attention recently. TNF-α can protect higher organisms against infection but can also induce lethal shock and profound inflammation. Many of the clinical trials at present are attempting to show an effect in the treatment of Gram negative sepsis with TNF-α infusions. In models of autoimmune disease TNF has been targeted because of the regulatory effect of this cytokine on other major pro-inflammatory cytokines including IL-1 and IL-6. This is well shown by a murine transgenic model of humanised TNF which expresses high levels of TNF-α and develops arthritis by 4 weeks of age which can be blocked by anti-TNF monoclonal antibody.\textsuperscript{57} Further to this collagen induced arthritis can be inhibited with anti-TNF antibody treatment.\textsuperscript{58} Inhibiting the effector response is more effective with concomitant therapy with anti-CD4 monoclonal antibody; the combined therapy inhibits active disease.\textsuperscript{59} Progression to clinical trials has

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### Table 2: Important proinflammatory cytokines

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<thead>
<tr>
<th>Name</th>
<th>Origin</th>
<th>Major actions</th>
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<tbody>
<tr>
<td>IL-1</td>
<td>Macrophages, lymphocytes</td>
<td>Activation of T+B cells</td>
</tr>
<tr>
<td>IL-2</td>
<td>T cells</td>
<td>Proliferation of activated T cells</td>
</tr>
<tr>
<td>IL-6</td>
<td>Lymphocytes</td>
<td>Stimulation of T cells, polyclonal B cells</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Macrophages, T cells</td>
<td>Induction of secretion of other cytokines (e.g. IL-6, IL-1, IL-2, and MIP-1)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Macrophages, T cells</td>
<td>Th1 activation and proliferation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Upregulation of MHC class II expression on immune and resident non-immune cells</td>
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already occurred with the development of humanised antibodies (human immunoglobulin constant region linked to mouse anti-human variable regions against cytokine). TNF-\(\alpha\) monoclonal antibody (constant region of human IgG1 and high affinity neutralising murine anti-human TNF-\(\alpha\) antibody) has been administered by intravenous infusion to several patients with rheumatoid arthritis. Overall there was a dramatic improvement in clinical signs with a concomitant decrease in serum IL-6 and C-reactive protein levels. Additionally, with this form of treatment there was no apparent fever and malaise; these side effects have been reported with both CD4+ and CD8+ monoclonal antibody therapies in rheumatoid arthritis. However, in common with all these trials several questions arise about safety and efficacy of treatment. The first is that the effect appears to be limited (about 3 months), although some patients do continue in remission for longer periods but this is unpredictable. It seems that repeated treatment is required and so the question of safety with long term therapy has to be addressed; the possibility of recurrent infections which may be fatal and the development of malignancy are as yet unknown.

The use of monoclonal antibodies against TNF-\(\alpha\) in other models of autoimmune disease is also likely to be of benefit. Anti-TNF-\(\alpha\) antibody has already been shown to inhibit the development of EAE, and TNF receptor fusion protein (linked to human immunoglobulin), TNF-p55R-huIgG, which neutralises TNF action, suppresses active EAE (J Sedgwick, personal communication). TNF-p55R-huIgG treatment does not prevent the infiltration of T cells into the central nervous system, but the animals do not develop signs of the disease, which means that the effector function appears to be blocked. Similar studies in retinal antigen induced EAU are presently under investigation and treatment with the fusion protein reduces severity and delays onset of EAU (manuscript in preparation). Studies using TNF-\(\alpha\) monoclonal antibody in endotoxin induced uveitis (EIU), a model of anterior uveitis, have shown that TNF-\(\alpha\) is not directly involved in the pathogenesis of the disease, and indeed in these circumstances it may even be protective. However, this model has distinct pathogenetic differences from retinal antigen-induced EAU, being mainly mediated by neutrophils and thus has a different immune activation and immunoregulatory cytokine profile. However, the point of this paradoxical effect emphasises the need for adequate knowledge of the effects and underlying immunobiology of immunoregulation by cytokines in animal models of (auto)immune disease before entering into clinical trials. IFN-\(\gamma\) was used to treat MS and, rather than reducing inflammation, it induced MHC class II expression and made the disease worse, although interestingly and without explanation IFN-\(\beta\) reduces the incidence of recurrences of MS, as documented by serial magnetic resonance imaging (for review see Ebers).

Similarly, in EAU, IFN-\(\gamma\) upregulates MHC class II expression on resident cells and it is the blocking of such functions that will probably have a profound effect on the immunoregulation of autoimmune disease, such as EPU. There are also paradoxical effects of other cytokines. I have already mentioned TNF and a similar inflammatory mediator, IL-1, which is involved in the pathogenesis of many diseases. For example it is uveitogenic but is also able to inhibit other models of autoimmune disease. There are now trials treating rheumatoid arthritis with recombinant IL-1 receptor (IL-1Ra) and a fusion protein of the IL-1R. In both studies treatment was shown to be beneficial but only in some clinical variables. The treatment was noted to have only minor adverse effects, which may be expected as both IL-1Ra and soluble IL-1R are naturally occurring molecules.

ACAI (anterior chamber associated immune deviation) is a phenomenon where an altered immune response is generated by placing antigen directly into the anterior chamber. The anterior chamber is thought to be immunosuppressive mediated via certain cytokines such as transforming growth factor \(\beta\) (TGF-\(\beta\)), a small secreted polypeptide which has suppressive actions on T cells. In EAE, there also appears to be a suppressive effect of TGF-\(\beta\) even in the presence of IFN-\(\gamma\). TGF-\(\beta\) mediated suppression has the advantage of not being restricted by antigen specificity. TGF-\(\beta\) is both suppressive and proinflammatory. As is seen in the ACAID model TGF-\(\beta\) in the aqueous seems to be important for the maintenance of an immunosuppressive environment. Although also associated with fibrosis in wound healing, it may be of benefit in promoting healing – for example, in full thickness macular holes. TGF-\(\beta\), therefore, does have differential effects which appear to depend on cell type, state of immune activation, and site which may limit its clinical efficacy.

Inhibiting effector cells

I have already discussed the possibilities of targeting cells responsible for mediating inflammatory response in autoimmune disease, including CD4+ and CD8+ cells. Macrophages are also directly involved in the pathogenesis of CD4+ (Th1) mediated diseases such as EAE and EAU. In EAE selectively depleting macrophages with a specific drug (dichloromethyl ketone) encapsulated in a mannosylated liposome (being able to cross the blood-brain barrier) can inhibit EAE.

Before the effector response can occur the cells obviously have to enter the target organ, a complex process which involves not only changes in the blood-retinal or blood-brain barrier but adhesion of activated cells to endothelium. Cell adhesion is a multistep process which involves the initial attachment of cell to vessel wall endothelium by selectins, such as E-selectin, which in turn activates integrins on leucocytes (as a result of the cellular conformational changes that have occurred), such as leucocyte functional antigen 1 (LFA-1). These bind to endothelial adhesion molecules like intercellular adhesion molecule 1 (ICAM-1). Expression of these molecules has been demonstrated in chronic intraocular inflammation.

One method, therefore, of suppressing the immune response is by targeting the adhesion process via monoclonal antibodies directed towards adhesion molecules expressed on circulating activated cells such as ICAM-1 and LFA-1. Suppression of EAU occurs with monoclonal antibodies directed against these cell surface adhesion molecules. However, these molecules are also important for cell-cell interaction during cell activation and therefore their effect in suppressing EAU may be secondary to inhibition of cell activation, rather than inhibition of homing.

There are many potential applications of targeting cytokines, especially with the advancement of recombinant technology and development of humanised antibodies for clinical use and the understanding of the immunobiology of the cytokine network in immune mediated diseases. Focusing on TNF-\(\alpha\) had already been shown to be successful and testament, as I have mentioned, may be improved when it is used in combination – for example, targeting both CD4+ T cells and cytokines. Overall we are entering a very exciting generation of new, more specific immunotherapies which will directly affect our future
treatment of EPU and other ocular immune mediated conditions.

**Mucosal administration of antigen induces tolerance**

The aim of all immunosuppression is to subdue successfully the immune response, the effect of which is long lasting and with no or at least minimal (acceptable) side effects. I have discussed already the advances made in trying to achieve specific immunotherapy which fits the above criteria. Induction of tolerance (immunological unresponsiveness) is a natural and powerful method of inducing suppression, which occurs during early thymic development of the T cell response and continues into adult life (peripheral tolerance). Monoclonal antibody treatment directed toward CD4 or CD8 T cells is meant to redress the cellular balance and achieve peripheral tolerance, even after the treatment has stopped. There are also other methods of inducing peripheral tolerance, besides neutralising or depleting peripheral CD4+ T cells, which include generating active suppression of CD4+ T cells by specific CD8+ T cells or bystander suppression by tissue resident cells (a role which resident choroidal macrophages may play). These methods are inherently attractive forms of treatment but the problem is which is the best approach and by which route inducing tolerance will suppress the inflammatory response and as a result redress the immunoregulatory balance (Fig 3)?

The vast majority of exogenous antigenic insults the body receives are via the mucosal tract, either ingested, where there is interaction with the mucosal associated lymphoid tissue (MALT) of the gastrointestinal tract, or where airborne antigens interact with respiratory mucosa. Most antigens we see when presented in either of these ways result in a state of immunological tolerance. Because of this antigen induced immunosuppression there have been various attempts to induce tolerance in autoimmune disease. One of the most successful methods is that of oral tolerance, where feeding milligram doses of antigen before immunisation with the antigen has been successful in preventing the onset of EAU, EAE, and collagen induced arthritis. In these models, the suppression of the effector cell response is both antigen and disease specific. Tolerance may be induced with smaller quantities of antigen (microgram doses) via the respiratory tract, and inhalation of retinal antigens by this method prevents the onset of EAU and collagen induced arthritis, which appears to be antigen specific. Mucosal tolerance induction in models of autoimmune disease appears to be driven by the generation of suppressor CD8+ cells, although in collagen induced arthritis it has been suggested that oral tolerance generates active peripheral suppression via both CD8+ and CD4+ cells driven through the action of their regulatory cytokines, particularly IL-4, IL-10, and TGF-β. Interestingly, in the immature neonatal system oral administration of antigen in tolerogenic doses enhances immune mediated diseases such as EAE when the animal
is adult, which suggests that when antigen is presented to the immature mucosal lymphoid system (capable of generating, like the thymus, its own maturing T cells) it may contribute towards the pathogenesis of autoimmune disease later in life. We obviously have a delicate balance between possible exacerbation of (auto)immune disease and suppression when antigens are administered via mucosal lymphoid tissue, which is perhaps dependent upon previous exposure. Although in experimental models mucosal tolerance can prevent disease onset, it must be established that the inflammatory response is not exacerbated by this mode of treatment. In EAU, active disease is suppressed with nasal administration of antigen but there is still target organ infiltration (that is, rod outer segment leucocytic infiltration) which does not occur when tolerogenic doses of antigen are given before immunisation. In EAE, chronic relapsing disease may be suppressed by myelin basic protein feeding. A full appraisal of whether mucosal tolerance therapy suppresses ongoing active disease is required before clinical trials can commence in uveitis. It may be that co-therapy with specific CD4+ mononuclear antibodies which would induce a period of immunosuppression could increase the efficacy of tolerance induction, so that once the cellular balance was redressed, continued mucosal tolerance therapy would maintain the antigen specific suppression and control any further exacerbations of disease. However, another problem with mucosal tolerance therapy is the unconfirmed evidence that the antigens which produce animal models of autoimmunity are important in humans. In some models like EAU, the circumstantial evidence is strong for S-Ag and IRBP to be more than just the putative autoantigens in human disease. It is possible that mucosal tolerance does initiate an effect by stimulating bystander suppression which may, in turn, be mediated by TGF-β, so that the lack of known autoantigens or the antigen specific nature of the therapy may not be a drawback to clinical use. Despite these potential problems a clinical trial has begun by feeding bovine myelin to patients with chronic relapsing MS. Provisional results have been published after a year of oral feeding of protein to patients, and although the efficacy of this form of treatment has not been shown, tolerance therapy was without any adverse effects, and the initial results indicated less exacerbation of MS in the treated group (relapse was documented in 6/15 patients in the treated group compared with 12/15 in control group). The potential of this form of suppression by induction of mucosal tolerance has been demonstrated recently by the topical administration of retinal antigens into the conjunctival sac which suppresses EAU in an antigen specific manner, and is thought to be mediated via the conjunctival associated lymphoid tissue (CALT). By whatever mechanisms tolerance is induced this form of therapy promises exciting developments in the treatment of autoimmune disease.

The future

I have focused on many experimental methods which successfully suppress or abolish immune responses in models of autoimmune disease. Much of the work only supports suppression of the primary response and prevention of the disease, which is largely because many of these models are not chronic and relapsing in nature; only recently are data concerning suppression of chronic disease (more pertinent to situation in humans) becoming available. At present, however, even with the advent of CsA therapy in autoimmune disease, we are still restricted by unacceptable side effects, a high incidence of resistance to therapy, and relapses which occur despite continued immunosuppression. The objectives of newer specific immunotherapies are to suppress the immune response for longer and with an acceptable risk/benefit ratio. The most specific treatments, such as inhibiting TCR/MHC/peptide binding, are still in their infancy and may be hampered in the future by the broad T cell repertoire which is apparent in autoimmune disease and the variety of autoantigens to which individual patients are sensitised. More encouraging are the developments of inhibiting the effector mechanisms via targeting CD4+ T cells, cytokines, or inducing tolerance via presentation of antigen to the mucosal tissue. All these methods are now entering the era of clinical trials. And already, in the limited pilot studies which are being performed, they are showing promise in the treatment of autoimmune diseases such as uveitis, psoriasis, rheumatoid arthritis, multiple sclerosis, and chronic inflammatory bowel diseases.

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