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REVIEW

Therapeutic advances in immunosuppression

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SUMMARY

Immunosuppressive therapy is appropriate for the prevention or reversal of allograft rejection, and for the treatment of autoimmune disorders and allergic disease. Recent advances in our understanding of the cellular and molecular mechanisms that regulate immune responses have paralleled elucidation of the modes of action of a variety of therapeutic immunosuppressive agents, both 'old' and new. These developments have identified potential targets for more refined and specific intervention strategies that are now being tested in the clinic.

Keywords immune suppression autoimmunity transplantation allergy

INTRODUCTION

Many human diseases are characterized by excessive or inappropriate immune responses. In transplantation, the immune system attacks MHC-disparate donor tissue leading to graft rejection, in autoimmune disease it attacks normal tissues, and in allergy the immune system is hyper-responsive to otherwise harmless environmental antigens. It is now recognized that immunosuppressive therapy is appropriate for treating each of these disorders. A number of advances have recently been made in our understanding of immune regulation, and in the development and use of immunosuppressive therapies, which were reviewed at a scientific meeting at the Charing Cross Medical School, London, UK, 25–26 April, 1994. Neither the meeting nor this short review was intended to be all-embracing; the aim was to highlight some of the significant recent developments.

Traditional drug screening programmes have identified new immunosuppressants, such as tacrolimus (formerly known as FK506) and rapamycin, which are being evaluated in transplantation centres. Studies on the mechanism of action of these and other drugs, such as the corticosteroids, have had the important benefit of identifying molecular targets for further refinement of immunosuppressive compounds and for the development of new agents which affect cellular signal transduction pathways. Basic immunological research has also identified the antigens, cellular interactions, and the cytokine mediators which are necessary for the induction and maintenance of immunological disease. The predictions for therapeutic strategies derived from this preclinical work are now being successfully tested in the clinic.

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ANTIGEN-INDUCED IMMUNOSUPPRESSION

T cell-mediated immune suppression

The ability to down-regulate immune responses and to control potentially autoreactive immunocompetent cells is vital for normal immune function and survival. Regulatory mechanisms include the induction of clonal anergy (via inappropriate antigen-presenting cells), peripheral clonal deletion/apoptosis, cytokine (e.g. transforming growth factor-beta (TGF-β) or IL-10)-induced non-responsiveness, 'veto' cells, autoreactive cytolytic T cells, and both non-specific and antigen-specific T suppressor cells. At least in theory, each of these regulatory systems provides a mechanistic basis for 'therapeutic intervention'. Webb (Palo Alto) focused specifically on the recently re-vitalized controversy of 'professional' T suppressor cells [1] and reviewed recent evidence in support of 'T suppressor factors' or antigen-specific, immunosuppressive molecules. In particular, it was argued that proteins bearing T cell receptor (TCR) α and/or β chain antigens can induce antigen-specific suppression [2–4]. Furthermore, TCR-α-specific cDNAs transfected into suppressor cell lines can be used to produce antigen-specific, MHC-restricted T suppressor factors [3]. Recently, TCR-α or β peptides from complementarity determining region-2 (CDR-2) or CDR-3 have been shown to induce potent, antigen-specific suppression following administration to mice already immunized to the antigen [5,6]. The molecular basis of these findings is not yet clear, but $CD8^+$ T cells appear to be required.

Oral tolerance

Several laboratories have established that orally administered

autoantigens suppress experimental autoimmune disorders [7–11], and that oral administration of alloantigen (MHC class II allopeptides) can suppress alloreactivity and prolong allograft survival [12,13]. Two mechanisms based on antigen dosage have been delineated. Low doses of autoantigen result in the generation of antigen-specific regulatory cells that inhibit effector cell generation. Upon subsequent recognition of antigen at the target organ, they secrete the suppressive cytokine TGF-β [14]. In addition, T helper 2 (Th2) cell responses are generated in the gut, resulting in secretion of IL-4 and IL-10. Large doses of oral antigen favour Th1 clonal anergy, with no evidence of active suppression [15,16]. This is due, perhaps, to anergizing of cells involved in its generation. An advantage of the active suppression induced by low-dose antigen is that it is antigen non-specific (so-called bystander suppression) and thus it may not be essential to identify the target autoantigen(s) to suppress organ-specific autoimmune disease by oral tolerance. Sayegh (Boston) reported on initial pilot clinical trials of oral tolerance in multiple sclerosis [17], rheumatoid arthritis [18] and uveitis that have demonstrated positive clinical effects with no evidence of sensitization to autoantigens, no apparent toxicity and decreases in T cell autoreactivity. Based on these findings, oral administration of autoantigen may find a place in the therapy of human organ-specific autoimmune diseases.

TRANSPLANTATION

New immunosuppressive drugs

The potential of new experimental immunosuppressive drugs whose modes of action have recently been reviewed in detail [19–22] and are not discussed here, was evaluated by Thomson (Pittsburgh). Given similar efficacy to currently approved therapies, several questions emerge. Are these newer agents less toxic, what is the risk of infectious complications, and can they halt the progression of chronic rejection? Moreover, is the risk of lymphomas reduced, and can they be used effectively in drug combination therapy? Recently, a new form of cyclosporin A (CsA) (Neoral) has been tested in phase III trials in renal transplant patients. It has the advantage of more reproducible bioavailability than Sandimmun. It is required in lower doses and fewer dose adjustments are required. Neoral may thus prove more effective/less toxic than the 'standard' oral CsA formulation.

The macrolide antibiotic tacrolimus [23] that has a similar molecular action to CsA [24–26] in inhibiting transcription of IL-2 and other cytokine genes, has recently been approved by the US Food and Drug Administration for immunosuppression in human liver transplantation. Results of European and American multi-centre phase III trials of tacrolimus in primary liver transplantation demonstrated 88% 1-year patient survival, with significant reductions both in the incidence and in the severity of rejection in the tacrolimus compared with the CsA treatment arm of each trial. A second benefit of tacrolimus was the significant decrease, compared with CsA, in the cumulative steroid dose required. The principal potential side effects of tacrolimus and CsA—nephrotoxicity, neurotoxicity and diabetogenicity—appeared (on the basis of these trials) to be identical, although tacrolimus does not cause hirsutism or gum hypertrophy which can occur with CsA. Notably, tacrolimus has improved significantly the results of human small bowel transplantation [27]. The doses of tacrolimus used presently, however, are probably excessive, as was the case with CsA when it was first used clinically. Post-transplant lymphoproliferative disease remains a risk associated with the use of either CsA or tacrolimus [23], both of which can block T cell surveillance of Epstein–Barr virus (EBV)-transformed B cell hyperproliferation.

The macrolide rapamycin is a close structural analogue of tacrolimus. It mediates its antilymphocytic activity by interfering with distinct molecular mechanisms late in the G_1 phase of the cell cycle distal both to IL-2 gene expression and ligation of the IL-2 receptor (IL-2R) [28–30]. A phase I trial of oral rapamycin in combination with CsA in renal allograft recipients has shown that the drug is absorbed better than previously found in animals, and is well tolerated. It remains to be seen whether in humans, as in rats, CsA and rapamycin act synergistically to prolong organ allograft survival. Unlike CsA and tacrolimus, rapamycin does not target the enzyme calcineurin, inactivation of which has been implicated both in the immunosuppressive action of CsA and in its nephrotoxicity [24]. An important prospective benefit of combined CsA and rapamycin therapy may therefore be that the CsA dose can be markedly reduced with decreased calcineurin inhibition. Of further interest is evidence that, in small animals, rapamycin inhibits arterial intimal proliferation (presumably cytokine-mediated) following femoral artery or aortic allografting or balloon injury of the vessels [31]. Strikingly, late intervention with rapamycin (up to 21 days after allografting) was also effective in inhibiting graft vessel disease. If extrapolated to humans, this could have important therapeutic implications for the influence of rapamycin on long-term survival of vascularized (especially cardiac) organ allografts. Somewhat on the down-side, however, are reports of the apparent promiscuity of rapamycin for cell growth inhibition—it exhibits inhibitory effects on bone marrow cells *in vitro* which could indicate possible risks of leukopenia *in vivo*.

The antimetabolite mycophenolate mofetil [32] (MM; formerly RS61443), a pro-drug of mycophenolic acid, has advanced to phase III clinical trials (kidney transplantation). The drug appears to be well tolerated. Its capacity to inhibit more potently the activity of an inducible form of the enzyme inosine monophosphate dehydrogenase than the 'basal' form may explain why this DNA biosynthesis inhibitor is relatively non-myelotoxic. In rats, it prevents post-transplant proliferative arteriopathy, and when combined with CsA in primates given cardiac xenografts, it permits much longer survival than CsA plus azathioprine, with no associated vessel disease. Although clinically attainable concentrations of MM inhibit smooth muscle proliferation *in vitro*, there is, as yet, no evidence that this highly desirable effect of MM can be achieved in humans. An exciting possibility might be combination of rapamycin and MM for the prevention of the important problem of graft vessel disease. Significantly, *in vitro*, MM in clinically obtainable concentrations inhibits newly or late transformed B cells and (unlike azathioprine) does not induce chromosome breaks. This suggests that MM, compared with other antiproliferative drugs, may be associated with reduced risk of lymphomas.

Other 'new' immunosuppressive drugs under clinical study include deoxyspergualin (that inhibits induction of cytotoxic T cells), brequinar sodium (a DNA biosynthesis inhibitor

currently in phase II study which appears to have a comparatively narrow therapeutic index), and leflunomide (which inhibits transduction of growth factor receptor signals and is already in phase III trials in rheumatoid arthritis). Pharmacologic and immunologic aspects of these drugs have recently been reviewed [33–35].

Can augmentation of donor cell chimaerism promote induction of transplantation tolerance?

Stemming from the recent observations of Starzl *et al.* [36] on long-lasting, donor cell chimaerism in successful organ transplant recipients, there is much interest in the possibility that drug-free tolerance might be accomplished by the augmentation (by cell infusion) of the natural, donor-derived leucocyte chimaerism before or after transplantation [37,38]. The establishment of cell chimaerism, however, requires at least some level of protracted immunosuppressive therapy in order to obtain the (hoped for) induction of donor-specific tolerance. A clinical trial to test this approach in tacrolimus + prednisone-treated liver, kidney or heart transplant patients is presently well underway at the University of Pittsburgh Medical Centre, and a preliminary report has appeared in the *Lancet* [39]. What is the likelihood of drug-free tolerance being accomplished in humans? Although this goal can readily be achieved in laboratory rats by short courses of CsA, tacrolimus or rapamycin, establishment of drug-free unresponsiveness to organ allografts in humans is rare, although there are instances of drug weaning associated with donor cell chimaerism, 0·5–20 years post liver transplantation, with no consequent rejection up to 11·5 years later [40].

In stark contrast to the above mentioned therapeutic strategy of augmenting donor leucocyte chimaerism in an effort to induce transplantation tolerance, Shockley (McGaw Park) reviewed early clinical results on pretreatment (perfusion) of kidneys with a pair of lytic MoAbs [41] recognizing CD45 (expressed on all leucocytes, but neither vascular endothelium nor renal structural components) before transplantation to deplete 'passenger' leucocytes (interstitial dendritic cells). Early results [42] suggested that this approach may reduce the incidence of early graft rejection, and that its efficacy is related to percentage of cells binding anti-CD45 MoAb.

AUTOIMMUNITY

Cytokine genes and susceptibility to autoimmune disease

Autoimmune diseases are familial and multigenic in their frequency. While strong associations with MHC genes for certain diseases are well recognized, polymorphism within cytokine genes might also influence disease susceptibility. For instance, the tumour necrosis factor-alpha (TNF-α) gene located within the MHC region has two alleles, one of which is linked to certain diseases [43]. Patients with identical MHC haplotype (A1, B8, DR3) but with the TNF2$^+$ haplotype, have a higher incidence of coeliac disease, rheumatoid arthritis, myasthenia gravis and type-1 diabetes. Sequence studies have shown that the polymorphism is located not in the transcribed portion of the molecule but in its promoter region, where there is a single base transition.

Similar associations outside the transcription region in leader and promoter sequences have been found in other cytokine genes. The IL-1 gene cluster is located on the long arm of chromosome 2, and several different polymorphisms have been identified. Of particular interest is the association between discoid lupus and the IL-1 receptor antagonist (IL-1Ra) gene, in which there is a variable number of tandem repeats (VNTR) polymorphism in intron 2 (a sequence of four tandem repeats in this microsatellite segment is the most frequent of the five alleles) [44]. The same polymorphism is strongly positive for other skin inflammatory diseases, such as psoriasis [45]. It may also be associated with ulcerative colitis (but not with Crohn's disease).

IL-1 polymorphisms appear to be linked to disease severity rather than incidence. This may be related partly to the presence of polymorphic microsatellite sequences in the IL-1Ra gene which have glucocorticoid response elements, as well as a potential binding site for the transcription factor SP-1. This might also hold promise for therapeutic intervention.

Cytokines, autoimmune disease and therapeutic intervention

Duff (Sheffield) reviewed the role of cytokines in autoimmunity, with particular reference to their potential for immunomodulation. Cytokines were categorized according to their involvement in acute (IL-1, IL-6, IL-8 and TNF-α) or chronic inflammation (interferon-gamma (IFN-γ), IL-2, IL-4, IL-5, IL-6, IL-9 and IL-10), tissue damage (IL-1, TNF-α) and fibrosis (epidermal growth factor (EGF), TGF-β, and platelet-derived growth factor (PDGF)). In addition, molecules that bind cytokines (cytokine receptors and antagonists) were reviewed from the perspective of candidate immunomodulating drugs. For instance, it was suggested that soluble IL-2R might retain binding activity after shedding and prevent T cell activation by 'mopping up' available IL-2. A further sophistication of this mechanism was described for interleukin receptor antagonists. The wide distribution of IL-1Ra reflects its role in inhibiting indiscriminate activity of IL-1. However, unlike the IL-2R, soluble IL-1R loses its ability to bind IL-1Ra and therefore does not interfere with the regulatory activity of IL-1Ra [46]. This observation has probable evolutionary significance. It also opens up the possibility of synthesizing mutant cytokine binding molecules which would act via a similar mechanism.

Several groups have attempted to modulate autoimmune disease using neutralizing MoAbs (mouse, human or humanized) to cytokines. The most marked response has been observed with antibodies to TNF-α [47] in rheumatoid arthritis. An alternative and earlier approach has been to use inhibitory cytokines (such as IFN-γ, TGF-β, IL-4, IL-6 and IL-10) in the treatment of various experimental and clinical autoimmune diseases, but no clear evidence of efficacy has yet emerged. Other inhibitory mechanisms suggested included the use of agents which disrupt cytokine generation, such as IL-1 converting enzyme inhibitor (ICE) (natural or synthetic) and inhibitors of cytokine transcription and/or release. These potential strategies have recently been reviewed [48].

Animal models for testing therapeutic modalities

The use of animal models for studies of therapeutic intervention in autoimmune disease was discussed with reference to experimental autoimmune uveoretinitis (EAU) by Forrester (Aberdeen) [49]. EAU has many resemblances to other models of autoimmune disease, especially experimental allergic encephalomyelitis (EAE) and collagen-induced arthritis [49]. However, it

has a number of advantages: the antigens (four have been identified) and their uveitogenic epitopes are fully described; the disease is highly reproducible and its severity is dose-dependent; and, because it has sharp end-points, it is ideal for studying the effects of immunosuppressive modalities [50,51].

The eye has an innate immunosuppressive micro-environment (so-called immune privilege) in spite of having a rich network of professional antigen-presenting cells (dendritic cells and macrophages) within the intra-ocular compartment (the uveal tract). Resident tissue cells such as the ciliary body epithelium, the retinal pigment epithelium and retinal Muller cells are probably important in maintaining this immuno-inhibitory state by release of immunosuppressive factors, such as prostaglandin (PG)E_2 [52] (and possibly nitric oxide (NO) [53]) when activated. However, when appropriately stimulated, these cells also secrete proinflammatory cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6 and IL-8 [54], which may alter the micro-environment and break natural tolerance. Interestingly, stimulation of RPE cells with cytokines in the presence of immunosuppressive drugs, such as tacrolimus and rapamycin, enhances rather than decreases the level of cytokine release by these cells [54].

Probably all major immunosuppressive strategies have been assessed in EAU. These include drug treatments (CsA, tacrolimus, rapamycin), antibody therapy (anti-MHC class II, anti-CD4, anti-IL-2R, anti-intercellular adhesion molecule-1 (ICAM-1)), including antibody against specific antigen (retinal S antigen), and mucosal tolerance induction [51]. CsA and tacrolimus are highly effective in inhibiting EAU, but the effect persists only for tacrolimus after drug therapy has stopped. Rapamycin is unusual in that it inhibits inflammation in the retina (the site of auto-antigen) but not in the anterior segment of the eye.

Most recently oral (see above) and nasal tolerization with retinal antigens and their peptides has proved very effective in the prophylaxis of EAU [10,55,56], very similar to its effect in EAE and in allergic disease. In this regard, nasal tolerization appears to work via a 'low zone' (? specific suppressor cell) mechanism, while oral tolerance may have its predominant effect by a 'high zone' immunosuppression (? anergy). Direct blockade of effector cells (macrophages) can also be effective in blocking EAU, as shown using dichloromethylene diphosphonate-filled liposomes or macrophage-specific MoAbs (Forrester, Huitinga & Dijkstra; work in progress). The similarities and differences between EAU and other models of autoimmunity indicate that much can be learned from the study of more than one system.

Anti-CD3 MoAb in diabetic mice

Chatenoud (Paris) reported the first evidence that a short course of anti-CD3 MoAb can restore self-tolerance in adult mice with established, advanced autoimmune diabetes (>70% β cells destroyed) [57]. If extrapolated to humans, the avoidance of continuous immunosuppressive therapy would eliminate the risks of toxicity and long-term over-immunosuppression associated with previous regimens for insulin-dependent diabetes.

ALLERGY (OR ALLERGIC DISEASE)

T cell/cytokine involvement in allergy

New immunosuppressive regimens are continually being assessed for the increasingly frequent and severe forms of allergic disease. Barnes (London) commenced an overview of allergic lung disease by outlining the scale of the problem (currently a 10% incidence of asthma in children and 5–10% in adults). The traditional view that the mast cell causes the damage is not borne out by the low efficacy of mast cell stabilizers. The eosinophil appears to be the major tissue-destroying cell, with support from mast cells and neutrophils in the acute stage [58].

Asthma is well established as a T cell-mediated disease, and the sequence of events leading to tissue damage involves both inducers and triggers, particularly airway hyper-responsiveness [59]. Infiltration of cells into the lung tissue may be preceded by up-regulation of endothelial cell ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) expression in response to various stimuli, such as cytokines or mediators, e.g. platelet activating factor (PAF) or leukotrienes [60]. Priming and activation of the cells in the tissue also require GM-CSF derived from macrophages, and possibly epithelial cells.

The source of the allergen-specific Th2 cells is a subject of intense investigation. A working hypothesis suggests that allergen-induced (via release of macrophage cytokines) epithelial production of IL-8, IL-6, macrophage inflammatory protein (MIP)1α, GM-CSF and RANTES provides a selective stimulation for memory T cells and eosinophils, and that this perpetuates the disease [61].

Current therapies for asthma include a range of bronchodilators, including β2 agonists, theophylline and anticholinergics. Anti-inflammatory drugs include steroids, the cromones (e.g. sodium cromoglycate), low-dose methotrexate, gold and CsA. Steroids can block the inflammatory process at each step and have an effect on all infiltrating cells. Their critical effect, however, is on the T lymphocyte, in which they block cytokine production by binding the glucocorticoid receptor. The steroid receptor complex is internalized and transported to the nucleus, where it directly binds response elements on genes regulating protein synthesis. This leads to a generalized reduction in cytokine production including IL-5, while at the same time inducing production of lipocortin [62]. Steroids may also have a direct effect on certain lymphocyte transcription factors, such as AP-1 and NF-kB and on other genes implicated in the overall inflammatory response, such as inducible cyclo-oxygenase and nitric oxide (NO) synthase [63]. Interestingly, the IL-2 gene does not have a glucocorticoid response element which provides a rationale for the use of CsA in asthma [64]. The combined use of steroids and CsA is doubly effective by targeting two sets of genes (AP-1 and NF-AT) (see above).

New therapeutic strategies

Therapies for the future include the newer immunosuppressive drugs, and cytokine and/or Th2 cell inhibitors. Experimentally, MoAbs to IL-5 have been shown to inhibit disease in guinea pigs [65,66], as has a naturally occurring antagonist of IL-1R, if less so [67]. Direct blockade of adhesion by targeting ICAM-1 has also been attempted with some effect [68]. Less specific methods, including inhibition of inducible NO synthase or phosphodiesterase Type IV inhibition, have also been proposed, but are at an early stage [69].

Two papers presented details of strategies to be adopted if immunomodulation of allergic disease is to be attempted with

the specific aim of avoiding side effects induced by whole allergen. Briner (Waltham) described his approach to the characterization of peptides from the Feld1 allergen responsible for allergy to cat dander. Intravenous or subcutaneous inoculation of two peptides, IPC-1 and IPC-2, induces tolerance in the susceptible mouse strain B6CBAF$_1$, tested by subsequent re-challenge with peptide in Freund's complete adjuvant (FCA). This effect is long lasting (up to 110 days) and is effective for both high and low dose (? zone) tolerance. In addition, the peptides are effective in inducing tolerance to the whole protein [70]. Some tolerogenic effects can be modified, depending on the method of immunization. For instance, immunization of the animals with the peptide in Freund's incomplete adjuvant (FIA) followed by i.v. or s.c. tolerization can induce tolerance to the peptide but not to the whole protein.

Investigations of the mechanism of tolerance in immunized mice by testing for IL-2 production, for instance, were inconclusive due to the low number of T cells secreting IL-2. Therefore, transgenic mice, most of whose T cells expressed the TCR responsive to cytochrome C [71], were used to permit a clearer evaluation of the effects of peptides. In this model, responses to the cytochrome C peptide 88–103 showed that there was a marked reduction in IL-2, IL-3, IL-4 and IFN-γ production, i.e. that there was a marked decrease in T cell functions generally, suggesting a state of anergy.

Phase III clinical trials of Feld1 peptides in a 'cat room study' are currently in progress; a significant tolerogenic effect has been observed as early as 1 week after therapy and is even more marked after 6 weeks.

Lamb (London) has used mucosal immunization to induce tolerance to the house dust mite (HDM) allergens Der p1 and Der p2 of *Dermatophagoides pteronyssinus*. The sequences and functions (Der p1 is a cysteine protease) of both these proteins are known, and several overlapping peptides have been used in a series of analytical and experimental studies [72–74]. For instance, in a detailed comparative study of the proliferative responses of atopic *versus* non-atopic individuals, no quantitative differences were observed. However, the responding T cell repertoire in atopic individuals appeared to be of the Th2 type, while in non-atopic individuals, Th1 predominated. Each individual also appeared to have a single or at least a restricted array of dominant epitopes when first tested which was generally reproduced on retesting months or even years later. Responding T cells were therefore long-lived and relatively restricted, but not along MHC lines. Indeed, the considerable diversity in restriction elements within the MHC which can present allergen suggests that this is unlikely to be a useful approach to therapy.

An *in vitro* model was therefore developed to investigate the cellular and molecular basis of HDM desensitization. Experiments using the Der p1 peptide 101–119 showed that T cells when exposed to supra-optimal doses of peptide become refractory to re-challenge, even although they can respond well to IL-2. Refractoriness was associated with a decrease in TCR and CD28 expression, even though CD25 and CD2 expression was up-regulated [75]. Hyporesponsive cells were also still capable of synthesizing IL-2, IL-4, IL-10 and IFN-γ. However, on rechallenge, they failed to produce IL-4 but synthesized IFN-γ. Thus there was a shift in T cell phenotype associated with the desensitization [76].

Parallel *in vivo* studies using a murine model of Der p1 immunization showed that inhalation of Der p1 peptide (111–139) could abrogate responsiveness to the specific peptide and to the whole allergen [77]. Non-responsiveness was obtained using prior tolerization and in the presence of on-going immune responses, even if this was long-standing. Although not strictly a true type 1 hypersensitivity model (immune responsiveness was tested by allergen in FCA), similar results were obtained when ovalbumin-specific IgE responses were tested. The low dose requirements and the specificity of this response are remarkable. Even cryptic peptides can induce tolerance to the whole protein by inhalation. The mechanism of tolerance induction awaits elucidation, but it appears to be different from that of T cell activation, since it does not depend on Raf-1, an essential component of the MAP kinase cascade, whose function is necessary for TCR induction of the IL-2 gene.

CONCLUSION

There is a remarkable convergence of strategies for treatment of both autoimmune and allergic disease using both drug-mediated and antigen–allergen specific therapies. At first sight, this is a little surprising given the different pathways to disease via Th1 and Th2 cell activation. Indeed, it would appear that for each process, switching to the alternative phenotype is sufficient to halt pathogenic effects. Much remains to be done in this exciting field.

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REFERENCES

1 Green DR, Webb DR. Saying the 'S' word in public. Immunol Today 1993; **14**:523–5.
2 Fairchild RL, Kubo RT, Moorhead JW. DNP-specific/class I MHC-restricted suppressor molecules bear determinants of the T cell receptor α- and β-chains. J Immunol 1990; **145**:2001–9.
3 Collins M, Kuchroo VK, Whitter MJ *et al.* Expression of functional $\alpha\beta$ T cell receptor gene rearrangements in suppressor T cell hybridoma correlates with antigen binding, but not with suppressor cell function. J Immunol 1990; **145**:2809–19.
4 Green DR, Bissonnette R, Zheng H *et al.* Immunoregulatory activity of the T-cell receptor α chain demonstrated by retroviral gene transfer. Proc Nat Acad Sci USA 1991; **88**:8475–9.
5 Mohapatra S, Chen Y, Takata M, Mohapatra SS, Sehon AH. Analysis of T-cell receptor $\alpha\beta$ chains of CD8$^+$ suppressor T cells induced by tolerogenic conjugates of antigen and monomethoxypolyethylene glycol. Involvement of TCR α-complementarity determining region 3 (α-CDR3) domain in immunosuppression. J Immunol 1993; **151**:688–98.
6 Vandenbark AA, Hashim G, Ofner H. Immunization with a synthetic T-cell receptor V-region: peptide protects against experimental autoimmune encephalomyelitis. Nature 1989; **341**:541–4.
7 Thompson HSG, Staines NA. Gastric administration of type II collagen delays the onset of severity of collagen-induced arthritis in rats. Clin Exp Immunol 1986; **64**:581–6.
8 Nagler-Anderson C, Bober LA, Robinson ME, Siskind GW, Thorbecke FJ. Suppression of type II collagen-induced arthritis

by intragastric administration of soluble type II collagen. Proc Natl Acad Sci USA 1986; **83**:7443–6.
9 Higgins P, Weiner HL. Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein and its fragments. J Immunol 1988; **140**:440–5.
10 Nussenblatt RB, Caspi RR, Mahdi R *et al.* Inhibition of S-antigen induced experimental autoimmune uveoretinitis by oral induction of tolerance with S-antigen. J Immunol 1990; **144**:1689–95.
11 Zhang JA, Davidson L, Eisenbarth G, Weiner HL. Suppression of diabetes in NOD mice by oral administration of porcine insulin. Proc Natl Acad Sci USA 1991; **88**:10252–6.
12 Sayegh MH, Zhang ZJ, Hancock WW, Kwok CA, Carpenter CB, Weiner HL. Down-regulation of the immune response to histocompatibility antigen and prevention of sensitization by skin allografts by orally administered alloantigen. Transplantation 1992; **53**:163–6.
13 Sayegh MH, Khoury SJ, Hancock WH, Weiner HL, Carpenter CB. Induction of immunity and oral tolerance with polymorphic class II major histocompatibility complex allopeptides in the rat. Proc Natl Acad Sci USA 1992; **89**:7762–6.
14 Miller A, Lider O, Roberts AB, Sporn M, Weiner HL. Suppressor T cells generated by oral tolerization to myelin basic protein suppress both *in vitro* and *in vivo* immune responses by the release of TGF-β following antigen specific triggering. Proc Natl Acad Sci USA 1992; **89**:421–5.
15 Whitacre CC, Gienapp IE, Orosz CG, Bitar D. Oral tolerance in experimental autoimmune encephalomyelitis. III. Evidence for clonal anergy. J Immunol 1991; **147**:2155–63.
16 Melamed D, Friedman A. Direct evidence for anergy in T lymphocytes tolerized by oral administration of ovalbumin. Eur J Immunol 1993; **23**:935–42.
17 Weiner HL, Mackin GA, Matsui M *et al.* Double-blind pilot trial of oral tolerization with myelin antigens in multiple sclerosis. Science 1993; **259**:1321–4.
18 Trentham DE, Dynesius-Trentham RA, Orav EJ *et al.* Effects of oral administration of collagen on rheumatoid arthritis. Science 1993; **261**:1727–30.
19 Thomson AW, Starzl TE. New immunosuppressive drugs: mechanistic insights and therapeutic advances. Immunol Rev 1993; **136**:71–98.
20 Allison AC, Lafferty KJ, Fliri H, eds. Immunosuppressive and anti-inflammatory drugs. Ann NY Acad Sci 1993;**696**:1–419.
21 Kahan BD. New immunosuppressive drugs — pharmacologic approaches to alter immunoregulation. Therapeutic Immunol 1994; **1**:33–44.
22 Thomson AW, Starzl TE, eds. Immunosuppressive drugs: developments in anti-rejection therapy. London: Edward Arnold, 1994.
23 Peters DH, Fitton A, Plusker GL, Faulds D. Tacrolimus. A review of its pharmacology, and therapeutic potential in hepatic and renal transplantation. Drugs 1993; **46**:746–94.
24 Sigal NH, Dumont FJ. Cyclosporin A, FK-506, and rapamycin: pharmacologic probes of lymphocyte signal transduction. Annu Rev Immunol 1992; **10**:519–60.
25 Schreiber SL, Crabtree GR. The mechanism of action of cyclosporin A and FK 506. Immunol Today 1992; **13**:136–42.
26 Liu J. FK 506 and cyclosporin, molecular probes for studying intracellular signal transduction. Immunol Today 1993; **14**:290–5.
27 Todo S, Tzakis AG, Abu-Elmagd K *et al.* Cadaveric small bowel and small bowel-liver transplantation in humans. Transplantation 1991; **52**:369–73.
28 Sehgal SN, Molnar-Kimber K, Ocain TD, Weichman BM. Rapamycin: a novel immunosuppressive macrolide. Med Res Rev 1994; **14**:1–22.
29 Kuo CJ, Chung J, Fiorentino DF, Flanagan WM, Blenis J, Crabtree GR. Rapamycin selectively inhibits interleukin-2 activation of p70 S6 kinase. Nature 1992; **358**:70–73.
30 Flanagan WM, Crabtree GR. Rapamycin inhibits $p34^{cdc2}$ expression and arrests T lymphocyte proliferation at the G1/S transition. Ann NY Acad Sci 1993; **696**:31–37.
31 Gregory CR, Huie PH, Billingham ME, Morris RE. Rapamycin inhibits arterial intimal thickening caused by both alloimmune and mechanical injury. Transplantation 1993; **55**:1409–18.
32 Allison AC, Eugui EM. Mycophenolate mofetil (RS 61443): mode of action and effects on graft rejection. In: Thomson AW, Starzl TE, eds. Immunosuppressive drugs: developments in anti-rejection therapy. London: Edward Arnold, 1994:141–60.
33 Suzuki S. Deoxyspergualin: mode of action and effects on graft rejection. In: Thomson AW, Starzl TE, eds. Immunosuppressive drugs: developments in anti-rejection therapy. London: Edward Arnold, 1994:187–202.
34 Makowka L, Sher LS, Cramer DV. The development of brequinar as an immunosuppressive drug for transplantation. Immunol Rev 1993; **136**:51–70.
35 Bartlett RR, Anagnostopulos H, Zielinski T, Mattar T, Schleyerbach R. Effects of leflunomide on immune responses and models of inflammation. Springer Semin Immunopathol 1993; **14**:323–44.
36 Starzl TE, Demetris AJ, Murase N, Ildstad S, Ricordi C, Trucco M. Cell migration, chimerism, and graft acceptance. Lancet 1992; **339**:1579–82.
37 Starzl TE, Demetris AJ, Murase N, Thomson AW, Trucco M, Ricordi C. Donor cell chimerism permitted by immunosuppressive drugs: a new view of organ transplantation. Immunol Today 1993; **14**:326–32.
38 Delaney CP, Thomson AW, Demetris AJ, Starzl TE. Xenobiotics, chimerism and the induction of tolerance following organ transplantation. Therapeutic Immunol 1994; in press.
39 Fontes P, Rao AS, Demetris AJ *et al.* Bone marrow augmentation of donor-cell chimerism in kidney, liver, heart, and pancreas islet transplantation. Lancet 1994; **344**:151–5.
40 Reyes J, Zeevi A, Ramos H *et al.* Frequent achievement of a drug-free state after orthotopic liver transplantation. Transplant Proc 1993; **25**:3319–23.
41 Bindon CI, Hale G, Clark M *et al.* Therapeutic potential of monoclonal antibodies to the leucocyte common antigen. Transplantation 1985; **40**:538–44.
42 Brewer Y, Taube D, Bewick M *et al.* Effect of graft perfusion with two CD45 monoclonal antibodies on incidence of kidney allograft rejection. Lancet 1989; **2**:935–7.
43 Wilson AG, de Vries N, Pociot F, di Giovine FS, van der Putte LBA, Duff GW. An allelic polymorphism within the human tumour necrosis factor alpha promoter region is strongly associated with HLA-Al, B8 and DR3 alleles. J Exp Med 1993; **177**:557–60.
44 Tarlow JK, Blakemore AIF, Lennard A *et al.* Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86 bp tandem repeat. Human Genetics 1993; **91**: 403–4.
45 Cork MJ, Mee JB, Tarlow JK *et al.* Interleukin one receptor antagonist polymorphism: allelic association with inflammatory dermatoses. J Invest Dermatol 1993; **100**:448.
46 Symons JA, Young PR, Duff GW. The soluble type II interleukin-1 (IL-1) receptor binds and blocks processing of IL-1β precursor and loses affinity for IL-1 receptor antagonist. Proc Natl Acad Sci USA. 1994; in press.
47 Elliott MJ, Maini RN, Feldmann M *et al.* Treatment of rheumatoid arthritis with chimeric monoclonal antibody to tumour necrosis factor alpha. Arthritis Rheum 1993; **36**:1681–90.
48 Pugh-Humphreys RGP, Thomson AW. Cytokines and their receptors as potential therapeutic targets. In: Thomson AW, ed. The cytokine handbook, 2nd edn. London: Academic Press, 1994:525–66.
49 Forrester JV. Uveitis: pathogenesis. Lancet 1991; **338**:1498–501.
50 Gery I. Retina specific antigens and the immunopathologic process they provoke. Prog Ret Res 1986; **5**:75–109.
51 Forrester JV, Liversidge JM, Dua HS, Dick A, Harper F, McMenamin PG. Experimental autoimmune uveoretinitis: a

model system for immunointervention. Curr Eye Res 1992; **11**(Suppl.):33–40.

52 Liversidge JL, McKay D, Mullen G, Forrester JV. Retinal pigment epithelial cells modulate lymphocyte function at the blood–retina barrier by autocrine PGE_2 and membrane bound mechanisms. Cell Immunol 1993; **149**:315–30.

53 Liversidge J, Grabowski P, Ralston S, Benjamin N, Forrester JV. RPE cells express an inducible form of nitric oxide synthase and produce nitric oxide in response to inflammatory cytokines and activated T cells. Immunology 1994; in press.

54 Kuppner MS, McKillop S, Forrester JV. Cytokine release by activated retinal pigment epithelial cells. 1994; submitted for publication.

55 Dick AD, Cheng YF, McKinnon A, Liversidge J, Forrester JV. Nasal administration of retinal antigens suppresses the inflammatory response in experimental allergic uveoretinitis. Brit J Ophthalmol 1993; **77**:171–5.

56 Dick AD, Cheng YF, Liversidge JL, Forrester JV. Intranasal administration of retinal antigen suppresses retinal antigen induced experimental autoimmune uveoretinitis. Immunology 1994; **82**:625–31.

57 Chatenoud L, Thervet E, Primo J, Bach J-F. Anti-CD3 antibody induces long-term remission of overt autoimmunity in nonobese diabetic mice. Proc Natl Acad Sci USA 1994; **91**:123–7.

58 Djukanovic R, Wilson JW, Britten KM *et al.* Quantitation of mast cells and eosinophils in the bronchial mucosa of symptomatic and healthy control subjects using immunohistochemistry. Am Rev Respir Dis 1990; **142**:863–71.

59 Corrigan CJ, Kay AB. CD4 T lymphocyte activation in acute severe asthma: relationship to disease severity and atopic status. Am Rev Respir Dis 1990; **141**:970–7.

60 Montefort S, Gratziou C, Goulding D *et al.* Bronchial biopsy evidence for leukocyte infiltration and upregulation of leukocyte-endothelial cell adhesion molecules six hours after local allergen challenge of sensitised asthmatic airways. J Clin Invest 1994; **93**:1411–21.

61 Barnes PJ. Cytokines as mediators of chronic asthma. Am J Resp Crit Care Med 1994; in press.

62 Beato M. Gene regulation by steroid hormones. Cell 1989; **56**:335–44.

63 Barnes PJ, Belvisi MG. Nitric oxide and lung disease. Thorax 1993; **48**:1034–43.

64 Paliogianni F, Raptis A, Ahuja SS, Najjar SM, Boumpas DT. Negative transcriptional regulation of human interleukin 2 (IL-2) gene by glucocorticoids through interference with nuclear transcription factors AP-1 and NF-AT. J Clin Invest 1993; **91**:1481–9.

65 Gulbenkian AR, Egan RW, Fernandez X *et al.* Interleukin 5 modulates eosinophil accumulation in allergic guinea pig lung. Amer Rev Resp Dis 1992; **146**:263–6.

66 Chand N, Harrison JE, Rooney S *et al.* Anti-IL-5 monoclonal antibody inhibits allergic late phase bronchial eosinophilia in guinea pigs: a therapeutic approach. Europ J Pharmacol 1992; **21**:121–3.

67 Selig W, Tocker J. Effect of interleukin-1 receptor antagonist on antigen induced pulmonary responses in guinea pigs. Europ J Pharmacol 1992; **213**:331–6.

68 Wegner CD, Gundel L, Reilly P, Haynes N, Letts LG, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. Science 1990; **247**:456–9.

69 Barnes PJ, Adcock IM. Anti-inflammatory action of steroids: molecular mechanisms. Trends Pharmacol Sci 1993; **14**:436–41.

70 Briner TJ, Kuo MC, Keating KM, Rogers BL, Greenstein JL. Peripheral T cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen *Feld1*. Immunology 1993; **176**:1355–64.

71 Berg LJ, Pullen AM, Fazekas Mathis DSG, Benoist DC, Davis MM. Antigen/MHC-specific T cells are preferentially exported from the thymus in the presence of their MHC ligand. Cell 1989; **58**:1035–46.

72 O'Hehir RE, Verhoef A, Panagiotopoulou E *et al.* Analysis of human T cell responses to the group II allergens of dermatophagoides species: localisation of the major antigenic sites. J Allergy Clin Immunol 1993; **92**:105–13.

73 Wedderburn LR, O'Hehir RE, Hewitt CR, Lamb JR, Owen MJ. In vivo clonal dominance and limited T cell usage in human $CD4^+$ cell recognition of house dust mite antigens. Proc Natl Acad Sci USA 1993; **90**:8214–8.

74 Yssel H, Johnson KE, Schneider PV *et al.* T cell activation-inducing epitopes of the house dust mite allergen Der p1 and lymphokine production patterns by Der P1-specific $CD4^+$ T cell clones. J Immunol 1992; **148**:738–45.

75 O'Hehir RE, Lamb JR. Induction of specific clonal anergy in human T lymphocytes by staphylococcal enterotoxin. Proc Natl Acad Sci USA 1990; **87**:8884–8.

76 Schall TJ, O'Hehir RE, Goedell DV, Lamb JR. Uncoupling of the cytokine mRNA expression and protein secretion during the induction phase of T cell anergy. J Immunol 1992; **148**:381–7.

77 Hoyne G, O'Hehir RE, Wraith DC, Thomas WR, Lamb JR. Inhibition of T cell antibody responses to house dust mite allergen by inhalation of the dominant T cell epitope. J Exp Med 1993; **178**:1783–8.