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EDITORIAL REVIEW

The spectrum of action of new immunosuppressive drugs

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INTRODUCTION

Research on new, experimental immunosuppressive drugs (such as FK 506, rapamycin, RS61443, brequinar sodium and deoxyspergualin) with distinct and diverse modes of action has gathered considerable momentum. This surge of interest dates from reports, in 1987, of the capacity of the novel macrolide antibiotic FK 506 to inhibit selectively, $CD4^+$ T cell activation and the production of IL-2 and other cytokines [1]—a property shared with cyclosporin A (CsA). Despite its enormous contribution to the greatly improved success of organ transplantation over the past decade, CsA is potentially nephrotoxic and exhibits other side effects. Agents with a higher therapeutic index are needed. Another factor, which has promoted development of new immunosuppressive drugs within a traditionally risk-averse pharmaceutical industry, is the proven efficacy of CsA in certain autoimmune diseases with presumed T cell pathogeneses. These disorders include psoriasis, uveitis, insulin-dependent diabetes mellitus, primary biliary cirrhosis [2,3] and recently, severe, steroid-dependent asthma [4]. In these conditions, patients previously unresponsive to or intolerant of maximal existing immunosuppressive therapy may respond to CsA. Nevertheless, available therapies for autoimmune disease remain unsatisfactory. In the search for effective, safe, 'new' drugs, which can be used alone or in combination, experimental agents such as FK 506 and RS61443 have already entered the clinic, whilst 'older' drugs, such as rapamycin (a close structural analogue of FK 506) and mycophenolic acid (the active moiety of RS61443) have regained attention in a new light. The inhibitory effects of two antilymphocytic drugs, rapamycin [5] and CP 17193 [6], on experimental autoimmune disease (type 1 diabetes and lupus, respectively) are featured in this issue of *Clinical and Experimental Immunology*.

The most important of the new drugs have been classified in Table 1, according to their mode of action. Of major importance to improved understanding of signal transduction in lymphocytes and to future immunosuppressive drug design have been revelations concerning the molecular actions of FK 506, CsA and rapamycin. Both FK 506 and CsA strongly and specifically inhibit expression of early T cell activation genes, encoding IL-2, IL-3, IL-4, interferon-gamma (IFN-γ), GM-CSF and *c-myc* [7,8]. There is evidence, however, that, at least *in vitro*, FK 506 may spare IL-10 (cytokine synthesis inhibitory factor) gene transcription by cloned murine T_{H2} cells, whilst suppressing concomitant IL-4 mRNA production [9]. Thus, differential interference with T_H cell cytokine gene expression and cross-regulation of T_{H1} cell (IL-2 and IFN-γ production) may be important mechanisms whereby FK 506 inhibits immune cell activation and maintains immunosuppression. Significantly, the capacity of CsA and FK 506 to inhibit mediator release from basophils and mast cells may also contribute to their range of therapeutic effects [10].

Insight into the molecular action of FK 506 and CsA has come from studies of their specific, intracellular cytosolic receptors. These belong to the FK 506 binding protein (FKBP) and cyclophilin families of immunophilins, respectively [11,12]. The predominant binding proteins, FKBP-12 and cyclophilin A, are peptidyl-prolyl cis-trans isomerases. Although binding of drug by its respective immunophilin inhibits isomerase activity, this event does not appear relevant to immunosuppression. FK 506 and CsA are now regarded as prodrugs and their immunosuppressive effects are ascribed to formation of active complexes between the drug and its respective isomerase. These complexes interfere with signal transduction. Complexes of FK 506-FKBP and of CsA-cyclophilin bind specifically to three polypeptides, calmodulin, and the two subunits of calcineurin (a Ca^{++}-activated, serine-threonine protein phosphatase) [13]. In each case, FK 506 or CsA promotes the interaction of the normally non-interacting immunophilin and calcineurin. The drug-immunophilin complexes but neither FK 506, CsA, FKBP, nor cyclophilin alone block the Ca^{++}-activated phosphatase activity of calcineurin.

The drug-immunophilin complexes block Ca^{++}-dependent assembly of the 2-component gene transcription activator NF-AT. This is achieved by inhibiting translocation of the pre-existing cytoplasmic component of NF-AT to the nucleus [14]. The other, nuclear component of NF-AT is transcriptionally inactive in all cells other than activated T lymphocytes and is induced by signals from the T cell receptor. Its appearance is not blocked by FK 506 or CsA. FK 506 or CsA may block the dephosphorylation of the cytoplasmic component of NF-AT by calcineurin which is required for its translocation to the nucleus. In the absence of both nuclear and cytoplasmic components, binding of NF-AT to DNA and transcriptional activation of the IL-2 gene and other genes are suppressed. Whilst transcription directed by NF-AT is blocked in T cells treated with FK 506 or CsA, little or no effect is observed on other transcription factors, such as NF-KB or AP-1.

In man, FK 506 is effective in the prevention and reversal of liver, heart and kidney allograft rejection [15], in permitting the long-term survival of small bowel allografts [16] and in sup-

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Table 1. Modes of action of 'new' immunosuppressive drugs

Effect: drug (structure)	Pharmaceutical Co.	Use as immunosuppressant in man to date	References
Inhibitors of cytokine production			
CsA (cyclic peptide)	Sandoz Ltd., Basel, Switzerland	Yes	[7,12]
FK 506 (macrolide)	Fujisawa Pharmaceutical Co., Osaka, Japan	Yes	[1,8,12]
Inhibitor of cytokine action			
Rapamycin* (macrolide)	Wyeth-Ayerst Research, Princeton, NJ	No†	[20,21]
Inhibitors of DNA synthesis			
Mizoribine = Bredinin	Sumitomo Chemical Co., Takarazuka, Japan	Yes	[31]
RS61443 (morpholinoethylester of mycophenolic acid)	Syntex, Palo Alto, CA	Yes	[25,26]
Brequinar sodium (quinoline carboxylic acid derivative)	Dupont-Merck Pharmaceutical Co., Wilmington, DE	No	[34]
Inhibitors of cell activation/maturation			
Deoxyspergualin (polyamine)	Nippon Kayaku, Tokyo, Japan	Yes	[36]
CP 17193* (pyrazaloquinoline)	Pfizer Inc., Groton, CT	No	[39]

* Studies on these agents are featured in this issue.

† Phase I clinical studies in progress.

This is not a complete list of experimental immunosuppressive drugs. Other agents of interest include cyclosporin G (Sandoz Ltd., which may be less nephrotoxic than CsA in man), SK&F 105685 (SmithKline Beecham Pharmaceuticals, King of Prussia, PA), an azaspirane which may induce non-specific suppressor cell activity or modulate cell surface adhesion molecules, and prostaglandin E analogues.

pressing rejection of transplanted pancreatic islets [17]. Early experience also indicates that it is effective in treatment of psoriasis, nephrotic syndrome, uveitis and pyoderma gangrenosum [15,18]. Although, like CsA, FK 506 is potentially nephrotoxic, its range of side effects is more restricted than that of CsA. Since immunosuppressive activity and not immunophilin binding or inactivation of PPIase activity determines the nephrotoxicity associated with CsA analogues [19], it may prove difficult to design non-nephrotoxic drugs that retain the potency of CsA or FK 506.

The macrolide rapamycin (Rapa) is a more powerful inhibitor of T lymphocyte proliferation than CsA. It inhibits murine and human T and B cell activation by a variety of pathways, including those insensitive to FK 506 or CsA, e.g. IL-2-mediated proliferation of IL-2-dependent T cell lines and activation of human B cells by pokeweed mitogen (PWM). It binds to the same cytosolic receptor (FKBP) as FK 506, but not to cyclophilin [11]. In contrast to FK 506, Rapa does not block mRNA expression for early T cell activation genes [20] or inhibit IL-2R expression. Scatchard analysis of the interaction between radiolabelled, recombinant IL-2 (r^{125} I-IL-2) and IL-2R shows that Rapa affects neither the avidity nor the number of IL-2R binding sites [21]. It may however, retard the intracellular incorporation of r^{125} I-IL-2/IL-2R complexes. Rapa inhibits not only IL-2- but also IL-4-induced proliferation of CTLL and D10.G4 cell lines [22] and growth of an IL-6-dependent line (MH60.BSF-2) [23]. The mechanisms whereby Rapa causes these inhibitory effects have yet to be elucidated. Complexes of FKBP-Rapa fail to bind and inhibit calcineurin phosphatase activity [13] and the target of these complexes remains to be defined. Rapa does not block translocation of the cytoplasmic component of NF-AT to the nucleus after T cell activation. This is consistent with its failure to block NF-AT binding activity and NF-AT-directed transcription [12,14].

Kahan and his colleagues have reported mutually synergistic interactions between Rapa and CsA both *in vitro* and *in vivo* [23,24]. Rapa augments the inhibitory effects of CsA on human peripheral blood lymphocyte (PBL) activation by various stimulants and enhances the capacity of CsA to suppress cytotoxic cell generation and precursor frequency during alloactivation *in vitro*. Rapa is a potent inhibitor of cardiac allograft rejection in rats. Moreover, minimally effective doses of Rapa and CsA, when combined, allow 100% cardiac graft survival beyond 50 days in all graft recipients [23]. Although no data are yet available concerning effects of Rapa in man (phase I studies are in progress), its efficacy and safety when combined with CsA will be of considerable interest.

The antipurine RS61443 is a semi-synthetic derivative (morpholinoethylester) of mycophenolic acid (MPA) to which it is rapidly hydrolysed *in vivo*. It is a non-competitive inhibitor of inosine monophosphate dehydrogenase (IMPDH), the rate controlling enzyme in the *de novo* biosynthesis of guanine nucleotides, which are essential for nucleic acid and protein synthesis. MPA suppresses human T and B cell DNA synthesis stimulated by mitogens or alloantigens and inhibits antibody production both *in vitro* and *in vivo*. MPA also prevents generation of allospecific, cytotoxic T cells [25,26]. Since RS-61443 has been reported to be neither nephro-, hepato-, nor myelotoxic, it may represent a more effective and safer alternative to azathioprine for the control of graft (including xenograft) rejection. In rodents, short-term treatment can induce a state of donor-specific tolerance [27]. In dogs, triple

therapy, consisting of RS61443, CsA and methylprednisolone, prolonged renal allograft survival from 8 days (untreated controls) to 122 days, without major side effects (including bone marrow suppression) or infectious complications [28]. In preliminary clinical trials, RS61443 used adjunctively with CsA and prednisone has proved relatively safe and effective for prevention and rescue of kidney allograft rejection [29,30]. Like MPA, the imidazole nucleoside mizoribine MZB inhibits the activity of IMPDH in mammalian cells and prevents lymphocyte proliferation [31]. It exhibits equal potency to azathioprine, but little bone marrow suppression or hepatotoxicity and is therefore, like MPA, a candidate to replace azathioprine in clinical practice [32,33].

The anti-metabolite brequinar sodium (BQR) is a novel, quinoline carboxylic acid analogue, with broad anti-tumour activity in mice. It inhibits dihydroorotate dehydrogenase, an enzyme in *de novo* pyrimidine biosynthesis, resulting in depletion of precursors required for RNA and DNA synthesis. BQR inhibits MLR and cell-mediated immunity in mice and is very effective, either alone or in combination with CsA, at subtherapeutic doses, in prolonging experimental organ allograft survival in the rat [34]. The potency of BQR in experimental organ transplantation is similar or superior to that of CsA. Treatment of kidney or liver allograft recipients with BQR for 30 days was sufficient to induce indefinite graft survival. In long-term liver allograft survivors, the unresponsiveness was donor-specific [34]. Based on these findings, BQR (like RS61443) may prove a valuable new adjunctive immunosuppressive agent for treatment of organ graft rejection. Its immunosuppressive properties have recently been reviewed [35].

Fifteen-deoxyspergualin (15-DSG) is a semi-synthetic polyamine with anti-tumour activity which exhibits a novel spectrum of immunosuppressive activity in animals and in allogeneic and xenogeneic transplantation models [36]. DSG may have a predominant effect on monocyte/macrophage function, including inhibition of oxidative metabolism, lysosomal enzyme synthesis, IL-1 production and cell surface expression of MHC class II antigens. Apart from influencing monocytes/macrophages, DSG also inhibits *in vivo* generation of cytotoxic T cells, either directly or indirectly. It does not affect IL-2 production. On the other hand, the recovery of secondary, cytotoxic T cell activity (that is susceptible to DSG) following addition of IFN-γ suggests that suppression of IFN-γ production may be the main effect of DSG on T cell populations. DSG may also affect B cell activation, differentiation and maturation, leading to inhibition of antibody production.

The precise biochemical action of DSG is uncertain. It is well known, however, that high levels of naturally occurring polyamines inhibit ornithine decarboxylase, thus elevating intracellular ornithine concentration which, in turn, suppresses cytotoxic T cell differentiation, but not IL-2 secretion. In man, it is effective (either alone or in combination with other agents) as rescue therapy in renal transplantation [37,38].

It is clearly important to put these recent developments in perspective. Whilst progress continues in development of specific molecular intervention strategies to interfere with adverse immune reactions at the level of antigen, T cell receptor, MHC or adhesion molecule expression, the new immunosuppressive drugs described in this review are likely to play major roles in forthcoming clinical trials and to be incorporated in the range of immunosuppressants of the future.

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