Q J Med 2003; **96**:91–102
doi:10.1093/qjmed/hcg014

Review

QJM

Clinical advances in therapies targeting the interleukin-2 receptor

A.C. CHURCH

From the Department of Respiratory Medicine, Glasgow Royal Infirmary, Glasgow, UK

Introduction

A dominant theme in modern immunological research is the elucidation of how the different branches of the immune system communicate. Identification of receptors for antigens, co-receptors such as CD4 and CD8, major histocompatibility complex (MHC) molecules and cytokine receptors, has gone a long way to giving a clearer view of the complexities of this interaction. More recently, this new information has opened up avenues for therapeutic intervention by directing novel treatments towards these various receptors. This new strategy of immune-receptor-directed therapy has been applied to an array of human disorders both in the laboratory and clinical settings.

Perhaps one of the most important, and certainly most studied, cytokines is interleukin-2 (IL-2). This molecule was first identified in 1976 by Morgan as a substance present in the supernatant of activated peripheral blood lymphocytes that was capable of driving the growth and proliferation of lymphocytes *in vivo*.[1] Recent molecular biological advances have allowed development of recombinant IL-2 and inhibitors of its cellular receptor. This review focuses on the basic biology of IL-2 and its receptor, and examines the clinical applications of IL-2 receptor-targeted therapies. Recombinant IL-2 has also been extensively used in treatment of various malignancies, but for reasons of space, I will not further examine this issue.

Methods

I searched through the databases Medline, Embase and Pre-Medline using the key words interleukin-2 and interleukin-2 receptor. I used recent reviews from these searches along with original articles on the subject.

Molecular biology of IL-2 and its receptor

IL-2 is a single polypeptide of molecular weight 15.5 kDa, 133 amino acid residues long. There is only a single IL-2 gene locus in humans, on chromosome 4. IL-2 is a globular protein containing two sets of α-helical domains, lying at right angles to each other.[2] These α-helical regions are involved in the binding to the receptor, and indeed this helical motif is found in many other cytokines, involved in binding to their respective receptors.

Il-2 is only produced by activated T-cells, especially the $CD4^+$ T-helper cell population, although $CD8^+$ T-cytotoxic cells can be stimulated *in vitro* to produce Il-2.[3] It is a potent immunomodulator, and has an important role in both the activation and maintenance of an immune response and in lymphocyte development. Interleukin-2 serves to activate numerous key cells in the immune system, including helper T cells, cytotoxic T cells, B lymphocytes, natural killer cells, tumour infiltrating lymphocytes and macrophage-monocyte cells. Perhaps of greatest interest is their interaction with the T-cells.

The first evidence that IL-2 worked through a cell-bound receptor came from the observations that there was a gradual removal of IL-2 from incubating media by activated T-cells.[4] This IL-2

Address correspondence to Dr A.C. Church, Department of Respiratory Medicine, Glasgow Royal Infirmary, Glasgow, G4 0SE. e-mail: colinchurch@zoom.co.uk

depletion correlated inversely with the number of cells present in the culture, and once IL-2 was purified, radio-labelled IL-2 was shown to bind with high affinity to stimulated T-cells.[5] In 1981, Waldmann and colleagues developed a murine monoclonal antibody against the receptor and called the antibody anti-Tac, as it bound against active T-cells only.[6] This is now known to recognize the α subunit of the receptor, a 55 kDa glycosylated type 1 membrane protein also known as CD25.[7]

Two other components of the receptor have been identified: the β and γ chains.[8,9] IL-2 in fact binds to a receptor complex made up of three chains. The individual chains have low affinities for IL-2, but when combined they act as a high-affinity complex. The α subunit on its own represents the low affinity state, the $\beta\gamma$ complex has an intermediate affinity, and the high affinity complex contains the $\alpha\beta\gamma$ chains and is the active receptor.[10] Cells expressing only $\beta\gamma$ chains can be stimulated, but only at very high concentrations of IL-2. and the biological significance of this is unclear. The ligand is thought to bind to the α and β subunits first, followed by heterodimerization of the $\alpha\beta\gamma$ chains to activate the intracellular downstream signalling mechanisms.

The α subunit gene has been identified and is located on chromosome 10 in the human.[11] The protein product of this gene is a 251 amino acid chain, with a long extracellular domain responsible for binding IL-2, a 19 amino acid hydrophobic region postulated to be the transmembrane domain, and a C-terminal intracellular domain of only 13 amino acids.[12] This intracellular domain is too short to act as an important site for signal transduction, and lacks any known consensus sequence for intracellular signalling. However, there is conserved sequence homology between humans and murine α subunits, indicating a possible important role.[13]

Cellular expression of the α subunit is tightly regulated. The gene has three important regions located either upstream or downstream of the transcribed region, which act as enhancers of transcription. One of these sites is called PRRI (positive regulatory region I): it binds NF-κB and so in activated T-cells, the NF-κB produced acts to stimulate α-subunit production.[14] Another site, PRRIII, can bind a transcription factor called Stat 5, which is itself upregulated during IL-2 stimulation, providing a positive feedback mechanism.[10] This tight genetic control explains why only certain cells express the α subunit and even then only once activated.

The β chain is a 70–75 kDa protein located on chromosome 22.[15] This type 1 membrane protein has an intracellular domain of 286 amino acid residues that contains two key signalling elements: Box 1 and Box 2. It is constitutively expressed on resting lymphocytes, monocytes/macrophages and neutrophils, and is upregulated upon T-cell activation.

The most recent chain to be identified is the γ chain.[9] This is again a type 1 membrane protein and is 347 amino acids in size giving a molecular weight of 64 kDa. The genetic locus is on the X chromosome.[16] It has a long intracellular domain that is vital for IL-2 signalling, and is constitutively expressed on lymphocytes, monocytes and neutrophils.

Both the β and γ chains are related to the cytokine receptor superfamily type 1, unlike the α subunit. These receptors are characterized by the possession of similar structural motifs: for example, four conserved cysteine residues at the N-terminus. Of significance is the fact that the β and γ subunits are constituents of other interleukin receptors. The γ chain acts in at least six other cytokine receptors and has been called the common receptor γc.[17] The β subunit is a part of the IL-15 receptor, the IL-15 molecule being very similar to the IL-2 system.[18] Thus it seems that the α subunit is the key to receptor specificity. This sharing of receptors gives rise to the phenomenon seen in the cytokine network of redundancy in functions, meaning that the same functions can be carried out by different interleukins, and helps to explain the finding that in IL-2 gene knockout mice the immune system can still function with only slight disruption.

Signal transduction from the IL-2 receptor is complex, and involves a number of different mechanisms, as the actual $\alpha\beta\gamma$ complex itself has no intrinsic enzyme activity. Perhaps one of the most important is through the Janus tyrosine kinase family and the STAT (signal transducer and activator of transcription) molecules. The Janus kinases (named after the Greek God Janus who had two faces) were first identified in the interferon system, and are important cellular kinases phosphorylating key tyrosine residues.[19] Jak1 and Jak3 are involved in the IL-2 system—Jak1 is constitutively bound to the β subunit whereas Jak3 is bound initially with low affinity, but after cellular activation with high affinity, to the γ chain. After heterodimerization of the $\alpha\beta\gamma$ units, Jak3 also interacts with the β unit and this heterodimerization activates the kinases. This leads to phosphorylation of key proteins including the β and γ subunits of the receptor,[10] which allows the STAT molecules Stat 3, Stat5a and 5b to dock on the receptor and themselves become phosphorylated.[20] Once this is complete, the Stats undergo tetramerization and translocate to the nucleus, where they can bind to promoter sequences on key genes and up-regulate the production of their protein (Figure 1).

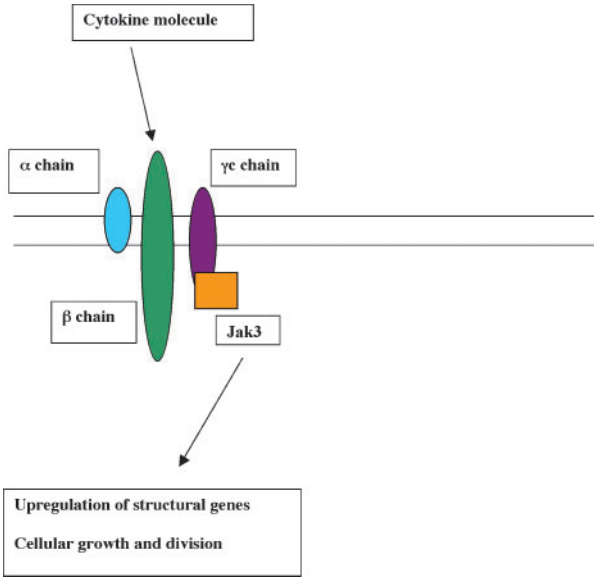

Figure 1. Outline of cytokine signalling. Defects in the γc chain leads to failure to bind either with Jak3 or with the ligand. This leads to XSCID.

Other important tyrosine kinase pathways are through the Src family[21,22] and phosphatidyl inositol-3-kinase (PI3 kinase).[23] p56lck was the first kinase identified to be associated with the IL-2 receptor complex. The exact role which these molecules have is still unclear, as knockout mice for p56lck still have IL-2 signalling and proliferation. The PI3 kinase system has important downstream signals, including that of p70S6 kinase, which has been identified as one of the targets of rapamycin, a new immunosuppressive agent used in transplantation.[24]

The ras-raf-MAP kinase pathway is also activated through a complex recruitment of molecules to the receptor, including Grb2, which then activates p21ras.[25] This allows eventual activation of MAP kinase, which in turn activates various transcription factors such as c-fos and c-jun. Other transcription factors activated by the IL-2 receptor include c-myc and NF-κB.[10] This allows the activation of the IL-2 target genes including key mitogenic genes such as the cyclins. The cyclins act to control cellular division and Il-2 stimulation leads to the movement of the cell from G1 to S stage.

IL-2 acts as an autocrine and a paracrine signal. In response to interaction with an antigen-presenting cell and IL-1 secreted by that cell, there is secretion of IL-2 and up-regulation of IL-2Rα on the T-helper cells called Th0. There is further differentiation of these cells into two different pathways—either Th1 or Th2 response. These T cells are defined by their characteristic cytokine secretion in that Th1 cells secrete IL-2 (among others) whereas Th2 cells do not. IL-2 is the stimulus for a number of cellular changes. It enables cell division by allowing cells to progress into the S phase of the cell cycle; in B-cells it stimulates antibody production; it stimulates the differentiation of NK cells into lymphokine-activated killer cells, which have enhanced non-MHC restricted cytolytic activity; and finally it can also act on monocyte-macrophage cells, possibly to enhance their cytotoxicity.[26]

More recently it has been shown that IL-2 also serves as an important cell viability signal, and experimentally, if it is removed from proliferating T-cells, it can induce apoptosis. Perhaps more confusingly, in T- and B-cell development, IL-2 may also be a necessary requirement for the process of negative clonal selection—the process by which autoreactive lymphocytes are removed by apoptosis.[27]

The development of the IL-2 receptor as a target

As a key immune signalling molecule, the interleukin-2 receptor presented itself as a possible useful clinical target. The scientific rationale for targeting more specifically the α subunit of the complex is the fact that in general resting T and B lymphocytes do not express this subunit. Waldmann has shown that < 5% of the circulating lymphocyte population express the α subunit, and those that do are immature developing thymocytes, which are entering a stage of irreversible commitment to T-cell lineage (called triple negative cells, as they are $CD4^-$ $CD8^-$ $CD3^-$).[28,29] As mentioned already, only after stimulation of their receptors with adjuvant cytokines (IL-1, IL-6 and TNF), do mature T and B cells start to express the α subunit.

However, in conditions such as organ-allograft rejection, T-cell-mediated autoimmune diseases and certain haematological malignancies, the α subunit is continuously expressed on the cell surface.[30] In particular, HTLV-1 induced adult T-cell leukaemia/lymphoma, hairy B-cell leukaemia, Hodgkin's disease and cutaneous T-cell lymphoma have been shown to be constitutively expressing the α subunit on their cellular surface. In contrast, in certain T-cell-mediated diseases, such as transplant rejection or autoimmune diseases, the α subunit is expressed as a result of T-cell activation, and these T-cells are only located in the area of the pathology, for example the synovial fluid in rheumatoid arthritis. By selectively targeting these diseases using specific anti-IL-2α receptor antibodies, maximum damage to those pathogenic cells could be inflicted

with minimal side effects to other non-α subunit expressing cells.

There are various theories as to how the monoclonal antibodies work. The most obvious one is by direct binding to the α subunit with high affinity and effectively preventing IL-2 from binding. However, the development of an IL-2 knockout mouse showed that this may not be the only mechanism, as in this model, where the IL-2 is non-functional, the mouse could still reject an allograft.[31] Therefore it was proposed that in fact the monoclonal antibodies act through the ADCC (antibody-directed cellular cytolysis) and complement fixation processes. However, this would mean that the cells with the α subunit on them should disappear from the circulation. Recent evidence has shown that there is only a very small decrease in α-subunit-positive lymphocytes after treatment with humanized monoclonal antibodies. Thus it may be through a combination of both direct blocking and cytolytic mechanisms that the antibodies achieve their effect.[32]

The development of IL-2 receptor antibodies

As mentioned above, the initial discovery of the α subunit of the receptor complex was by the development of a murine monoclonal antibody against activated T cells and this was named anti-Tac. Since this, many adaptations have been made to the basic structure of anti-Tac in an attempt to improve its efficacy.

Initial clinical uses of the unmodified murine anti-Tac were hampered by various problems. Murine antibodies have short half-lives, and are rapidly cleared from the circulation. This dictates more frequent administration of the antibody, which induces a host immune response. As the antibody is murine in origin, the host recognizes it as foreign and frequently develops human anti-murine antibodies (HAMA) that act to neutralize it. Furthermore, it fails to activate the human immune effector systems such as complement and macrophage dependent phagocytosis (antibody-directed cellular cytolysis ADCC), as it lacks critical signalling elements of the human antibody structure.[33,34]

To overcome these problems, genetic engineering has created both chimaeric and humanized forms of the antibody. In the chimaeric constructs, the murine variable regions are fused to the human immunoglobulin constant domains, and so binding to the α subunit is maintained, as the variable regions contain the complementarity determining regions (CDR) which determine antibody specificity. In the humanized forms, more extensive remodelling is performed such that all that remains of the murine antibody are the CDRs, with the rest of the framework being provided by the $IgG_{1\kappa}$ antibody, yielding a monoclonal antibody which is more than 90% human origin.[35] Queen *et al*. were the first to develop these adapted antibodies, and furthermore went on to show that they activated complement and ADCC, were less immunogenic, and had longer half-lives.[36]

Currently, two marketed specific anti-IL2 receptor antibodies exist, called basiliximab and daclizumab. These have been used extensively in clinical practice, and have already achieved an important role in organ transplantation. Basiliximab (Simulect) is a chimaeric monoclonal antibody which is produced by recombinant DNA techniques from a mouse-myeloma cell line. Daclizumab (Zenapex) is a humanized monoclonal antibody, similarly produced by recombinant technology.

Further adaptations have been made to the anti-IL2 receptor antibody to create novel mechanisms for treatment of certain conditions. These include the creation of both radio labelled anti-Tac constructs and immunotoxin-linked conjugates. Both α- and β-emitting radioisotopes have been attached to the antibody, including ^{212}bismuth and ^{90}yttrium.[37] One major advantage of these radiolabelled antibodies is that they can penetrate a tissue area, and despite binding to only one cell, the emitting radiation can work over a distance of several cell diameters, eliminating surrounding cells at the same time. They also do not need to be internalized into the cell, unlike the immunotoxin-antibody conjugates. These structures have in essence part of a toxic protein, such as diphtheria or Pseudomonas toxin, attached to the antibody, which then delivers this cytotoxic agent to the target cell. LMB2 (anti-Tac Fv-PE38) is one important example of this type of agent.[38] This is built from the fusion of a single chain of the variable region of the anti-Tac to a truncated Pseudomonas toxin molecule. Pseudomonas exotoxin A is a 66 kDa protein with three identifiable domains—the critical one is domain III, which harbours the ADP-ribosyltransferase that inactivates elongation factor 2 (EF-2), a key cell signalling molecule, and by doing so leads to cell death.[39] By removing domain 1, which was the Pseudomonas receptor signal, allowing entry of the toxin to non-Tac expressing cells, the molecule could be targeted to Tac-expressing cells only.

A similar strategy has been to attach a cytocidal moiety to the interleukin-2 molecule itself. The principal example of this is with the FDA-approved drug called denileukin difitox (DAB(389)-IL-2 or

Ontak). This has been created by recombinant technology using an *E. coli* expression system to yield a protein molecule which has IL-2 linked to diphtheria toxin domains A and B. This toxin is very similar to the Pseudomonas toxin described above and once inside the target cell it inactivates EF-2 and leads to apoptosis. The prototype molecule was DAB(486)-IL-2,[33] but because of a short half-life *in vivo*, further molecular engineering led to the shortened form of DAB(389)-IL-2 being developed.[40] This has now been adopted into clinical practice.

Clinical application of anti-IL2 receptor antibody

We will discuss the application of antibody therapy to the conditions of neoplasia, autoimmune diseases and organ allograft rejection.

Neoplasia

The first malignancy to be targeted using receptor directed therapy was HTLV-1 induced adult T-cell lymphoma/leukaemia. This is a malignant proliferation of mature T cells which have a predisposition to invade sites such as lungs, skin, and the central nervous system and can cause profound immunosuppression leading to opportunistic infections. It is common in Japan, the Caribbean and central Africa, reflecting the distribution of the causal agent—a human type C retrovirus called human T-lymphotropic virus type 1.[41] This has a typical retroviral structure with long terminal repeats, *gag*, *env* and *pol* genes, and integrates into the T-cell genome. There is then transcription from the viral genome of a protein called Tax. This is a 42 kDa protein which acts to up-regulate the transcription of certain genes, including IL-2, IL-2 receptor α subunit and NFκB genes, by binding to sequences in the promotor region of these genes. This leads to the constitutive expression of IL-2Rα at up to 5–10 times greater than the maximally stimulated normal T cell (correlated with a high level of soluble IL-2Rα found in the serum of these patients).[42]

There is also secretion of IL-2, and it is postulated that at least initially there is an autocrine feedback onto these cells from the secreted IL-2, causing further IL-2 production and cellular division. As the disease state progresses, chromosomal abnormalities and genetic mutations can occur, rendering the cells eventually IL-2-independent and free to divide unregulated.[12] Prognosis is dismal—average life expectancy is 9 months in acute ATL and 24 months in the chronic form. Treatment therapies are poor. By interrupting the cytokine-mediated growth of the malignant cells using anti-Tac and thereby leading to apoptotic cell death by deprivation of IL-2, it was envisaged that a useful clinical treatment could be developed.

This led to Waldmann and his group undertaking a clinical trial using murine unmodified anti-Tac against this disease.[43] In this trial, 19 patients were enrolled, 10 of whom had failed chemotherapy. Six patients showed signs of remission—two had complete and four had partial remissions—lasting between 1 month and 9 years, as shown by genetic analysis. Only mild side-effects were seen: fever in two patients and a transient pancytopenia in one. However, the development of human anti-murine antibodies limited further uses of this treatment. Furthermore, the murine antibodies were not cytocidal and as the cancer cells became IL-2-independent, the treatment became ineffective.

This contributed to the application of radiolabelled murine antibodies to the disease. In this study, 18 patients were enrolled with advanced disease and given an infusion of the anti-Tac antibody with attached ^{90}yttrium to a dose of 5–10 mCi. Sixteen patients survived long enough to be evaluated (at least 3 weeks). Of those 16, seven developed a partial remission and two developed a complete remission. A partial remission was defined as at least a 50% reduction in leukaemic cell count, a 50% reduction in the size of all measurable lymph nodes, and no new lesion for 1 month, while a complete remission was defined as the disappearance of all measurable disease for more than 1 month. Toxicity was mainly restricted to granulocytopenia and/or thrombocytopenia in 12/18 patients, occurring at 4–5 weeks after starting treatment, although significant hepatic upset occurred in three patients. Six patients, out of the 15 patients evaluated, were found to have developed anti-murine antibodies.[44] This hampered repeated infusions, and as a follow-up to this further clinical trials are being initiated using the radionuclide attached to the newer more humanized antibodies.[45]

Other strategies to combat neoplasia include the use of the immunotoxin-linked antibody to the IL-2 receptor. A phase I trial was recently reported in which LMB-2 (anti-Tac(Fv)-PE38), as explained above, was used in the treatment of chemoresistant haematological tumours.[46] This group took 35 patients in total with various haematological cancers and treated them with LMB-2, which was administered intravenously on alternate days for three doses—this constituted one cycle. Patients could go on to receive further cycles at higher dosages if they still met eligibility criteria, and did

not get significant toxicity or develop neutralizing antibodies to the immunotoxin after the initial treatment. Sixteen of the trial patients had been previously treated with high-dose chemotherapy and bone-marrow transplantation and had relapsed.

One patient developed complete remission (hairy cell leukaemia) which was ongoing after 20 months of follow-up. Seven patients developed partial responses—one with cutaneous T-cell lymphoma, three with hairy cell leukaemia, one with chronic lymphocytic leukaemia, one with Hodgkin's and one with adult T-cell leukaemia/lymphoma. The definitions of complete and partial remissions are as outlined above. In fact all four patients with hairy cell leukaemia, which by entry into the study was refractory to treatment, responded to the immunotoxin therapy, leading the group to advocate this as a future salvage therapy in unresponsive disease.[47] Toxicity was mild and consisted of transient transaminitis and fever. Importantly, vascular leaky syndrome, previously noticed with the administration of other immunotoxins, was not seen. Six patients were identified as having developed neutralizing antibodies after the first cycle, which is a lower rate than that observed in studies with other immunotoxins, and may reflect the smaller size of the PE38 toxin and the absence of the immunogenic murine constant domain.

Treatment of cutaneous T-cell lymphoma (CTCL), including mycosis fungoides, has also been investigated using Ontak. In a double-blind phase III trial, 71 patients with chemoresistant tumours were treated with either 9 or 18 μg/kg/day intravenously for 5 consecutive days every 3 weeks for up to 8 cycles.[48] These patients were eligible if they were found to have CD25-positive malignant cells. Up to 30% of these patients achieved a response (20% obtained a partial remission, while 10% achieved a complete remission) with a median duration of 6.8 months. Furthermore, assessments of the 71 patients showed that those who responded also had significant increases in their quality of life from baseline.[49] Consequently, in 1999 the FDA liscensed the drug for use in CTCL.

Toxicity from Ontak consists of mainly either hypersensitivity reactions or a vascular leak syndrome, occurring either immediately or after 10 days, respectively. Flu-like symptoms occurred in 60–70%. Antibodies to Ontak did develop in almost all patients and were found to result in an increased rate of clearance of the drug. The antibodies are directed towards the diptheria toxin only, and not the IL-2 element of the molecule.[49] As a result there is some cross-reactivity with those patients who have had diptheria toxoid administered previously.

Autoimmunity

In many autoimmune conditions, the Th1 response is the predominant factor responsible and the IL-2 receptor expressed on these active T-cells is being used as a target for intervention. Nussenblatt *et al*. looked at the use of daclizumab in the treatment of autoimmune non-infectious uveitis.[50] As in animal models of uveitis the T-cells in the posterior segment of the eye had increased Tac expression, various groups considered using monoclonal antibodies to the IL-2 receptor as a treatment for uveitis. Guex-Crosier *et al*. found that an infusion of unmodified Anti-Tac into a primate model of uveitis had a beneficial effect.[51]

To confirm this in humans, a non-randomized open-label study was conducted. Ten patients were recruited who had posterior uveitis and were either finding their current immunosuppressive dosage too much, or agreed to have their systemic treatment weaned. The uveitis was a result of different conditions ranging from sarcoid to Vogt-Koyanagi-Harada disease, but perhaps more importantly was the fact that Behçet's disease patients were excluded from the trial.

The patients had their immunosuppression tapered off over 8 weeks and then commenced initially fortnightly infusions of daclizumab in a dose of 1 mg/kg as their only form of immunosuppression. The infusion interval was gradually increased until patients received the drug every 4 weeks, with follow-up for 52 weeks. Eight patients developed statistically significant improvements in visual acuity, in the eye with the poorer vision at baseline. Mild transient rashes appeared in six patients, but resolved either spontaneously or after topical steroid administration.

Similar studies have been completed in graft vs. host disease. Again, in animal experiments, removal of IL-2Rα-subunit-positive cells actually reduces the ability of that animal to go on to develop GVHD, identifying an important role of IL-2 in the pathogenesis of this condition. In a phase I study, Anasetti *et al*. treated 20 patients who had steroid-resistant acute GVHD with daclizumab, in doses of up to 1.5 mg/kg. Six patients showed a response—two complete, and four with partial resolution.[52]

A larger study conducted in the USA used multiple doses of daclizumab to treat 43 patients with GVHD following bone marrow transplantation along with adjuvant immunosuppression. Twenty-four patients received 1 mg/kg on days 1, 8, 15, 22

and 29 (called regimen 1) and 19 received the same dose but on days 1, 4, 8, 15 and 22 (called regimen 2) and were followed-up for both response at day 43 and survival at day 120. A complete response was identified as complete resolution of rash, normalization of bilirubin and absence of diarrhoea, while a partial response was classed as a reduction of at least one grade. In regimen 1, the complete response rate was 29% while it was 47% in regimen 2. Survival rates were 29% and 53%, for regimens 1 and 2, respectively.[53]

No side-effects from the infusion or development of neutralizing antibodies were seen. The more intensive early dosing in regimen 2 may have led to greater receptor saturation of the IL-2 receptor on the T-cells, but it could also have saturated the soluble IL-2 receptor, which could have negated some of the overall effect of the antibody.

Experience with anti-IL-2 receptor antibodies in dermatological conditions is yielding fascinating results. Psoriasis is characterized by a Th1 response in plaques. Krueger *et al*. looked at patients with psoriasis who had moderate to severe disease and had been treated with at least one form of systemic therapy, for example PUVA. Nineteen patients were identified, and stopped all therapy for 4 weeks prior to commencing the trial.[54] A loading dose of 2 mg/kg of daclizumab was given, followed by infusions of 1 mg/kg at weeks 2, 4, 8 and 12. Patients were found to improve initially as assessed by the PASI score with the peak improvement seen at 8 weeks. This correlated with the time of maximal receptor blockade as measured by cytotoxic assay. After 8 weeks however, as the dosing interval increased, there was a corresponding fall in receptor saturation and in relapse of disease. Throughout, toxicity was not a problem. This study shows that clinical improvement was achieved by IL-2 receptor blockade, but unless saturation is complete then relapse can occur. Another clinical study is required to investigate the use of more frequent dosing regimens.

Further studies have confirmed the efficacy of the anti-IL-2 receptor antibodies in treatment of psoriasis.[55,56] Moreover, case reports are emerging of its usefulness in other dermatological conditions. Kagi treated a patient with severe atopic dermatitis, who was not responding to cyclosporin treatment, with the standard 40 mg of basiliximab given over 2 infusions. Benefits for the patient were immediate and lasted for 4 weeks.[57]

Finally, in a Rhesus monkey model of rheumatoid arthritis, daclizumab leads to a reduction in joint inflammation and erosion. This awaits investigation in formal human trials.[58]

Clinical experience with the immunotoxin Ontak and its predecessor DAB(486)-IL-2, has revealed them to be important new additions to the treatment of both severe psoriasis and rheumatoid arthritis. In one study, 19 patients with active rheumatoid arthritis refractory to conventional treatment, including methotrexate, were given infusions of varying dosages of DAB486-IL-2. Nine patients treated with either the intermediate or high dosage schedule had a substantial or meaningful response as assessed by clinical and functional examination.[59] In addition, a number of studies have established the usefulness of Ontak in the treatment of psoriasis, and this remains an ongoing area of active research in order to establish the optimal dosage regimen.[60,61] In a multicentre dose escalation study, the higher dosage regimen produced a $>50\%$ decrease in psoriasis Area and Severity index score, in 7 of the 15 patients treated with Ontak.[60]

Transplantation

Acute allograft rejection is a T-cell-mediated process in which IL-2 is a very important component, and is a risk factor for future chronic allograft dysfunction and loss.[62] Animal studies were the first to show the promise of anti-IL-2-receptor antibodies in the prevention of cellular rejection. Kirkman[63] and his group showed that the use of the murine anti-Tac AMT-13 prolonged cardiac allograft survival in mice, and this was confirmed by similar experiments in primates by Cooper *et al*.[64] Renal allografts were also shown to be aided by the administration of anti-Tac in primate experiments.[65]

It was not until the development of the chimaeric or humanized forms that antibody therapy in transplantation was used to the full. Following on from a phase I trial reported in 1997,[66] Vincenti and his group conducted an extensive randomized, double-blind, placebo-controlled phase III trial analysing daclizumab in renal transplantation.[67] Patients receiving their first cadaveric kidney transplant were eligible, and all received triple immunosuppression of cyclosporin, azathioprine and prednisolone. They recruited 260 patients (126 to the daclizumab arm) and the primary endpoint was biopsy-proven rejection. The infusion was given over 15 min, the first within 24 h prior to transplantation, followed by four more doses at 2, 4, 6 and 8 weeks after transplant. This regimen had been previously established by pharmacokinetic studies to saturate the receptors for up to 120 days, and although receptor saturation can be prolonged beyond this, it is not felt necessary, as most episodes of rejection would occur within this time period. This is now the recommended dosage schedule.

Fewer patients in the daclizumab group developed rejection during the first six months after transplantation (22% vs. 35% in the placebo group; $p = 0.03$), time to develop rejection was longer (73 vs. 30 days) and the numbers of rejection episodes were lower in the daclizumab group. Moreover, this was not associated with any adverse reactions, nor were there any differences in infection or cancer rates between the two groups. However, in the short term at least, there was no difference in graft or patient survival, either. The findings of this trial were established by a second European trial that recruited 275 patients and found that in the daclizumab treatment arm only 28% developed rejection, compared with 47% in the placebo control. Interestingly, this was despite only using dual immunosuppression of cyclosporin and prednisolone.[68]

A comparable study has also been published using basiliximab in renal transplantation.[69] Again, entry criteria were for patients who were receiving their first cadaveric transplant, and specifically excluded were patients with previous transplants, high panel reactive antibodies (>80%) or had a current infection. Of 380 patients initially recruited, 333 patients completed the study at 12 months follow up; 165 in the basiliximab treatment arm. All patients received dual immunosuppression of cyclosporin and prednisolone and were matched for variables such as sex, HLA-mismatches and cold ischaemia time, which represents the time between organ retrieval and transplantation. Patients were given basiliximab infusions 2 h prior to transplantation and then 4 days after transplant. This has been shown to produce receptor saturation.

There was a significant reduction in the incidence of rejection in the basiliximab group at both 6 and 12 months (34% vs. 52% at 6 months and 38% vs. 55% at 12 months). Graft and patient survival at 12 months were similar. As before, there were no significant adverse events and no differences between groups in terms of infection rates or malignancies confirming the safety profile of the antibody.

Three-year follow-ups for the daclizumab trials using triple or dual immunosuppression have been completed.[70] They confirmed the persistence of a significant reduction in acute rejection episodes compared to placebo at 1 year (43% vs. 28%; $p < 0.001$). As before, there was no significant difference in the 3-year graft survival between the different groups. Reassuringly there was no increased risk of malignancies or infection seen in the daclizumab group. Using the same data from these daclizumab trials, Bumgardner investigated whether daclizumab had any specific effect on patients who develop delayed graft function following transplantation.[71] This was defined as urine output <30 ml/h, decline in serum creatinine of <0.5 mg/dl or need for dialysis within the first 24 h. They found a significant reduction in the time to, and the development of, acute rejection in the patients treated with daclizumab who developed delayed graft function.

Further studies have confirmed beyond doubt both the efficacy and safety profile of the monoclonal agents in prevention of acute rejection in first transplants.[72] However, whether these agents can help to prolong graft survival in the long term remains to be seen.

Since these landmark trials, the monoclonal antibodies have been used in other organ transplants. Beniaminovitz *et al*. used daclizumab in preventing rejection in cardiac transplantation.[73] Of 55 patients, 28 received antibody treatment at transplantation and every 2 weeks thereafter to a total of five doses as per standard protocols in other organ transplantation trials. Adjuvant immunosuppression consisted of cyclosporin, mycophenolate and prednisolone. Patients were matched for patient characteristics, although the cold ischaemia time in the control group was found to be significantly shorter. Despite this, the incidence and severity of acute rejection, time to rejection and need for rescue therapy were all significantly lower in the daclizumab group. Overall survival at 1 year was similar between groups, as were rates of infection or *de novo* malignancies

Daclizumab has also been investigated in the context of liver and intestinal[74,75] transplantation, and was found to reduce the incidence of acute rejection and be of advantage as an induction agent. In liver transplantation, Calmus and his group looked at induction with 40 mg basiliximab in 188 patients, along with cyclosporin and steroids. At 6 months, there was a lower incidence of biopsy-confirmed acute rejection in the basiliximab-treated group when compared to the placebo group. Furthermore, the safety of the anti-IL-2-receptor antibodies was confirmed.[76] There have also been some concerns about using the antibodies in patients who require a liver transplant secondary to hepatitis C infection. In some reports, use of these monoclonal antibodies was associated with an increased rate of hepatitis C recurrence after transplant. In pancreas-kidney transplants, monoclonal antibodies are effective in reducing the incidence of biopsy proven rejection at 6 months and also safe when compared to induction with anti-thymocyte globulin.[77] Similar findings have been seen in kidney-only transplants.[78]

A further use of the new agents may be to allow the adoption of an immunosuppressive regimen that uses a low dose of the calcineurin inhibitors. Agents such as cyclosporin can be nephrotoxic, and minimizing their use should be beneficial. Vincenti reported on a study in which patients were given daclizumab, mycophenolate and prednisolone only in renal transplant induction.[79] There was a higher incidence of acute rejection in this group, compared to expected rates, but graft survival at 12 months was 96%. Receptor saturation studies showed that the dosing regime was adequate, and it could be that a combination of low-dose calcineurin inhibitors with the monoclonal antibodies would be more effective while less toxic.

One other important consideration to be accounted for is the overall cost of these new agents. A cost analysis has shown that by using basiliximab at induction in renal transplants, a net saving of $1554 could be made in the first year. This was a comprehensive study based on the multicentre trial conducted by Nashan *et al.*,[69] and included costing for hospitalization, immunosuppressive drug use, dialysis, patient and graft survival.[80] This study lends further weight to the argument for routine use of these agents in clinical practice.

Functional implications of blocking IL-2 mediated signalling

It is interesting to speculate what functional consequences may arise as a result of long-term blockage of key immune signalling pathways. With regards to this, both nature and science have already given us an insight in the form of naturally occurring mutations and recombinant technology allowing targeted disruption of specific genes in mice. In the case of IL-2Rα and IL-2Rγ, mutations have been identified in humans as well as being created in mice.

IL-2Rα deficiency in mice gives rise to a phenotype consisting of a haemolytic anaemia, lymphadenopathy and an inflammatory bowel condition not unlike ulcerative colitis in humans. Surprisingly T, B and natural killer (NK) cell deve lopment is intact, although there is a polyclonal expansion of peripheral lymphoid cells, suggesting a role for the α subunit in regulating the overall size and homeostasis of the lymphoid system.[10] B-cell activity is increased, and results in hypergammaglobulinaemia, although as the mice get older, B-cell numbers fall. The T cells have a characteristic defect—there is absence of CD1, which is involved in antigen presentation, and also regulation of apoptosis. Absence of the IL-2Rα and CD1 leads to high levels of the gene product bcl-2, which protects cells from entering into apoptosis.[81] It is hypothesized that the CD4 CD25+ve T cells are vital for the suppression of autoreactive clones and regulation of peripheral immune tolerance.[82] Disruption of this system leads to the observed murine phenotype mediated by a self-directed immune system. In humans, deficiency of the IL-2Rα has been seen as a result of a frameshift mutation, inherited as a homozygous mutation. This leads to a phenotype of profound immunosuppression with recurrent viral, fungal and bacterial infections presenting early in life.

Perhaps the most studied abnormality has been that of the γc chain deficiency. Mutations in this common chain, which plays an vital role in six other cytokine receptor systems (including IL-7 and IL-15) leads to the human disease of X-linked severe combined immunodeficiency (XSCID), as the γ chain gene locus is on the X chromosome. Again, the patient presents in childhood with failure to thrive, recurrent infections and lymphopenia.[83] The disease is characterized by defects principally in T and NK cellular development.[84,85] The T-cell defects arises from the interruption not of IL-2 signalling but of the IL-7 system, which seems to be a key cytokine for T-cell development. Similarly, loss of IL-15-mediated effects leads to NK cell abnormalities. In γc-knockout mice, identical effects are seen, except there is a more profound B-cell abnormality not seen in humans.

The loss of the γc chain leads to failure of downstream signalling from the cytokine receptors via the Jak3 kinase. Recently mutations in the Jak3 kinase itself have been identified, and not surprisingly lead to an identical phenotype to that of the γc mutation.[85]

It remains to be seen if any adverse long-term effects of the administration of the anti-IL-2α monoclonal antibodies will develop. Most studies to date have not involved children, and concern remains that treatment with these drugs may have negative effects on the future development of their immune systems.

Future developments

The future is exciting. Through the discovery of the intricate signalling system of the IL-2 system, new treatments have become available for use in oncology, autoimmune disorders and of course transplantation. However, as more is discovered more opportunities for designing newer agents become available. The redundancy of the cytokine

system, especially between IL-2 and IL-15 means that the IL-2Rα monoclonal antibodies can be bypassed by the action of IL-15. Furthermore the antibodies cannot target NK cells, which are IL-15-dependent (and lack the IL-2Rα subunit), and these NK cells themselves have been implicated in organ rejection. Development of antibodies to both IL-2Rα and IL-15Rα subunits may provide a more complete level of immunosuppression, although the full extent to which IL-15 is important in immune responses remains to be fully elucidated.[86] Furthermore the identification of Jak3-deficient humans and the creation of Jak3 knockout mice has shown that this can lead to an immunodeficiency syndrome almost identical to that seen in X-linked severe combined immunodeficiency disorder, which results from a mutation in the common γ chain gene.[32] Targeting of Jak3 may therefore provide another immunosuppressive avenue.

In conclusion, many new medical treatments are entering clinical practice as a result of a better understanding of cytokine signalling, and will soon play an important part in almost every field of patient care. The monoclonal anti-IL-2-receptor antibodies appear to be efficacious, safe and cost-effective.

References

1. Morgan DA, Ruscetti FW, Gallo RC. Selective in vitro growth of T-lymphocytes from human bone marrow. *Science* 1976; **193**:1007–8.
2. Brandhuber BJ, Boone T. Three-dimensional structure of IL-2. *Science* 1987; **238**:1707–9.
3. Schwartz RH. A cell culture model for T lymphocyte clonal anergy. *Science* 1990; **248**:1349–6.
4. Smith KA. T-cell growth factor. *Immunol Rev* 1980; **51**:337–57.
5. Robb RJ, Munck A, Smith KA. T cell growth factor receptors: quantitation, specific and biological relevance. *J Exp Med* 1981; **154**:1455–74.
6. Uchiyama T, Nelson DL, Fleisher TA, Waldmann TA. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T-cells. *J Immunol* 1981; **126**:1398–403.
7. Leonard WJ, Depper JM, Crabtree GR, Rudikoff J, Pumphery J, Robb J, *et al*. Molecular cloning and expression of cDNA for the human interleukin-2 receptor. *Nature* 1984; **311**:626–31.
8. Tsudo M, Kozak RW, Goldman CK, Waldmann TA. Demonstration of a new (non-Tac) peptide that binds interleukin-2: a potential participant in a multichain interleukin-2 complex. *Proc Natl Acad Sci USA* 1986; **83**:9694–8.
9. Takeshita T, Asao H, Ohtani K, Ishii N, Kumaki S, Tanaka N, *et al*. Cloning of the α-chain of the human IL-2 receptor. *Science* 1992; **257**:379–82.
10. Nelson BH, Willerford DM. Biology of the interleukin-2 receptor. *Adv Immunol* 1998; **70**:1–81.
11. Leonard WJ, Depper JM, Kronke M, Peffer NJ, Svetlik PB, *et al*. Structure of the human interleukin-2 receptor gene. *Science* 1985; **230**:633–9.
12. Waldmann TA. The interleukin-2 receptor. *Ann Rev Biochem* 1989; **58**:875–911.
13. Anderson DM, Kumaki S, Ahdieh M, Bertles J, Tometsko M, Loomis A, *et al*. Functional characterisation of the human interleukin-15 receptor alpha chain and close linkage of IL-15RA and IL-2RA genes. *J Biol Chem* 1995; **270**:29 862–9.
14. Lin BB, Cross SL, Halden NF, Roman DG, Toledano MB, *et al*. Delineation of an enhancerlike positive regulatory element in the interleukin-2 receptor alpha-chain gene. *Mol Cell Biol* 1990; **10**:850–3.
15. Shibuya H, Yoneyama M, Nakamura Y, Harada H, Hatakeyama M, *et al*. The human interleukin-2 receptor beta-chain gene: genomic organization, promoter analysis and chromosomal assignment. *Nucleic Acids Res* 1990; **18**:3697–703.
16. Noguchi M, Adelstein S, Cao X, Leonard WJ. Characterization of the human interleukin-2 receptor gamma chain gene. *J Biol Chem* 1993; **268**:13601–8.
17. Leonard WJ, Noguchi M, Russell SM, McBride OW. The molecular basis of X-linked severe combined immunodeficiency: the role of the interleukin-2 receptor gamma chain as a common gamma chain, gamma c. *Immunol Rev* 1994; **138**:61–86.
18. Bamford RN, Grant AJ, Burton JD, Peters C, Kurys G, *et al*. The interleukin (IL) 2 receptor beta chain is shared by IL-2 and a cytokine, provisionally designated IL-T, that stimulates T-cell proliferation and the induction of lymphokine-activated killer cells. *Proc Natl Acad Sci USA* 1994; **91**:4940–4.
19. Bach EA, Aguet M, Schreiber RD. The IFN-gamma receptor: a paradigm for cytokine receptor signalling. *Ann Rev Immunol* 1997; **15**:563–93.
20. Lin JX, Leonard WJ. The role of Stat5a and Stat5b in signalling by the IL-2 family cytokines. *Oncogene* 2000; **19**:2566–76.
21. Hatakeyama M, Kono T, Kobayashi N, Kawahara A, Levin SD, *et al*. Interaction of the IL-2 receptor with the src-family kinase p56lck. *Science* 1991; **252**:1523–8.
22. Kobayashi N, Kono T, Hatakeyama M. Functional coupling of the src-family protein tyrosine kinases p59fyn and p53/56lyn with the interleukin 2 receptor. *Proc Natl Acad Sci USA* 1993; **90**:4201–5.
23. Merida I, Diez E, Gaulton GN. IL-2 binding activates a tyrosine-phosphorylated phosphatidylinositol-3-kinase. *J Immunol* 1991; **147**:2202–7.
24. Kuo CJ, Chung J, Fiorentino DF, Flanagan WM, Blenis J, *et al*. Rapamycin selectively inhibits interleukin activation of p70 S6 kinase. *Nature* 1992; **358**:70–3.
25. Graves JD, Downward J, Izquierdo-Pastor M, Rayter S, Warne PH, *et al*. The growth factor IL-2 activates p21ras proteins in normal human T-lymphocytes. *J Immunol* 1992; **148**:2417–22.
26. Abbas AK, Lichtman AH, Pober JS. *Cellular and Molecular Immunology*, 2nd edn. 1994.
27. Sakaguchi S, Sakaguchi N, Asano M, *et al*. Immunologic self tolerance maintained by activated T-cells expressing IL-2 receptor alpha chains. *J Immunol* 1995; **155**:1151–64.

28. Waldmann TA. The structure, function and expression of interleukin-2 receptors on normal and malignant T-cells. *Science* 1986; **232**:727–32.
29. Nelson BH, Willerford DM. Biology of the interleukin-2 receptor. *Adv Immunol* 1998; **70**:1–81.
30. Waldmann TA. The interleukin-2 receptor. *J Biol Chem* 1991; **266**:2681–4.
31. Li XC, Roy-Chaudhury P, Hancock WW, *et al*. IL-2 and IL-4 double knockout mice reject islet allografts: a role for novel T cell growth factors in allograft rejection. *J Immunol* 1998; **161**:890–6.
32. Soulillou JP. Immunosuppression through inhibition of interleukin-2/interleukin-2 receptor interaction. *Transplant Proc* 1999; **31(Suppl.)**:49–51S.
33. Strom TB, Kelley VR, Murphy JR, *et al*. Interleukin-2 receptor directed therapies. *Ann Rev Med* 1993; **44**:343–53.
34. Lobuglio AF, Wheeler RH, Trang J, Haynes A, Rogers K, *et al*. Mouse/human chimeric monoclonal antibodies in man: kinetics and immune response. *Proc Natl Acad Sci USA* 1989; **86**:4220–4.
35. Williams KA, Thiel M, Zola H. The new generation anti-CD25 monoclonal antbodies. *Transplantation* 1999; **68**:1228–9.
36. Queen C, Schneider WP, Selick HE, Payne PW, *et al*. A humanised antibody that binds to the interleukin-2 receptor. *Proc Natl Acad Sci USA* 1989; **86**:10029–33.
37. Kozak RW, Atcher RW, Gansow OA, Friedmann AM, Hines JJ, *et al*. Bismuth-212-labeled anti-Tac monoclonal antibody: alpha-particle-emitting radionuclides as modalities for radioimmunotherapy. *Proc Natl Acad Sci* 1986; **83**:474–8.
38. Kreitman RJ, Bailon P, Chaudhary VK, Fitzgerald DJP, Pastan I. Recombinant immunotoxins containing anti-Tac (Fv) and derivatives of Pseudomonas exotoxin produce complete regression in mice of an interleukin-2 receptor expressing human carcinoma. *Blood* 1994; **83**:426.
39. Hwang J, Fitzgerald DT, Adhyas S, Pastan I. Functional domains of pseudomonas exotoxin identified by deletion analysis of the gene expressed in *E. coli*. *Cell* 1987; **48**:129–36.
40. Foss FM. DAB(389)IL-2 (ONTAK) : a new fusion protein technology. *Clin Lymphoma* 2000; **(Suppl. 1)**:S27–31.
41. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci USA* 1980; **77**:7415–19.
42. Uchiyama T, Hori T, Tsudo M, Wano Y, Umadome H, *et al*. Interleukin-2 receptor (Tac antigen) expressed on adult T cell leukemia cells. *J Clin Invest* 1985; **76**:446–53.
43. Waldmann TA, White JD, Goldmann CK. The IL-2 receptor:a target for monoclonal antibody therapy of HTLV-1 induced adult T-cell leukaemia. *Blood* 1993; **82**:1701–12.
44. Waldmann TA, White JD, Carrasquillo JA, Reynolds JC, Park CH, *et al*. Radioimmunotherapy of IL-2 receptor alpha-expressing Adult T-cell leukaemia with Yttrium-90 labelled anti-Tac. *Blood* 1995; **86**:4063–75.
45. Morris JC, Waldmann TA. Advances in Interleukin-2 receptor targeted treatment. *Ann Rheum Dis* 2000; **59(Suppl.)**:i109–14.
46. Kreitman RJ, Wilson WH, White JD, Jaffe ES, Giardina S. Phase 1 trial of recombinant immunotoxin anti-Tac Fv-PE38 in patients with haematologic malignancies. *J Clin Oncol* 2000; **18**:1622–36.
47. Kreitman RJ, Wilson WH, Robbins D, Marguiles I, Stetler-Stevenson M, *et al*. Responses in refractory hairy cell leukaemia to a recombinant immunotoxin. *Blood* 1999; **94**:3340–8.
48. Olsen E, Duvic M, Frankel A, Kim Y, Martin A, *et al*. Pivotal phase III trial of two dose levels of denileukin diftitox for the treatment of cutaneous T-cell lymphoma. *J Clin Oncol* 2001; **19**:376–88.
49. Duvic M, Kuzel TM, Olsen EA, Martin AG, Foss FM, *et al*. Quality-of-life improvements in cutaneous T-cell lymphoma patients treated with denileukin diftitox. *Clin Lymphoma* 2002; **4**:222–8.
50. Nussenblatt RB, Fortin E, Schiffman R, Rizzon L, Smith J, *et al*. Treatment of non-infectious intermediate and posterior uveitis with the humanized anti-Tac monoclonal antibody: a phase I/II clinical trial. *Proc Natl Acad Sci USA* 1999; **96**:7462–6.
51. Guex-Crosier Y, Raber J, Chan CC, Knete MS, Benichou J. Humanized antibodies against the alpha-chain of the IL-2 receptor and against the beta-chain shared by the IL-2 and IL-15 receptors in a monkey uveitis model of autoimmune diseases. *J Immunol* 1997; **158**:452–8.
52. Anasetti C, Hansen JA, Waldmann TA. Treatment of acute Graft-versus-host disease with humanized anti-Tac. *Blood* 1994; **84**:1320–7.
53. Przepiorka D, Kernan NA, Ippoliti C, Pappadopoulous EB, Giralt S, *et al*. Daclizumab, a humanized anti-IL-2 receptor alpha chain antibody for treatment of acute graft-versus-host disease. *Blood* 2000; **95**:83–9.
54. Kreuger JG, Walters IB, Myazawa M, Gilleaudeau P, Hakimi J, *et al*. Successful in vivo blockade of CD25 on T-cells by administration of humanized anti-Tac antibody to patients with psoriasis. *J Am Acad Dermatol* 2000; **43**:448–58.
55. Owen CM, Harrison PV. Successful treatment of severe psoriasis with basiliximab, an IL-2 receptor monoclonal antibody. *Clin Exp Dermatol* 2000; **25**:195–7.
56. Mrowietz U, Zhu K, Christophen E. Treatment of severe psoriasis with anti-CD25 monoclonal antibody. *Arch Dermatol* 2000; **136**:675–6.
57. Kagi MK, Heyer G. Efficacy of Basiliximab, a chimeric anti-IL-2 receptor monoclonal antibody in a patient with severe chronic atopic dermatitis. *Br J Dermatol* 2001; **145**:350–1.
58. Brok HP, Tekoppele JM, Hakimi J, Kerwin JA, Nijenhuis EM. Prophylactic and therapeutic effects of Daclizumab on collagen induced arthritis in rhesus monkeys. *Clin Exp Immunol* 2001; **124**:134–41.
59. Sewell KL, Parker KC, Woodworth TG, Reuben J, Swartz W, *et al*. DAB486IL-2 fusion toxin in refractory rheumatoid arthritis. *Arthritis Rheum* 1993; **36**:1223–33.
60. Martin A, Gutierrez E, Muglia J, McDonald CJ, Guzzo C, *et al*. A multicenter dose escalation trial with denileukin diftitox in patients with severe psoriasis. *Am Acad Dermatol* 2001; **45**:871–81.
61. Bagel J, Garland WT, Breneman D, Holick M, Littlejohn TW, *et al*. Administration of DAB389IL-2 to patients with recalcitrant psoriasis: a double blind phase II multicenter trial. *J Am Acad Dermatol* 1998; **38**:938–44.

62. Matas AJ, Gillingham KJ, Payne WD, Najanan JS. The impact of an acute rejection episode on long term renal allograft survival. *Transplantation* 1994; **57**:857–9.

63. Kirkman RL, Barrett LV, Koltun WA, Diamantstein T. Prolongation of murine cardiac allograft survival by the anti-IL-2 receptor monoclonal antibody AMT-13. *Transplant Proc* 1987; **19**:618–19.

64. Cooper MM, Robbins RC, Waldmann TA, Gansor OA, Clark RE. Use of anti-Tac antibody in primate cardiac xenograft transplantation. *Surgical Forum* 1988; **39**:353–5.

65. Reed MH, Shapiro ME, Strom TB, Milford EL, Carpenter CB, *et al*. Prolongation of primate renal allograft survival by anti-Tac, an anti-human IL-2 receptor monoclonal antibody. *Transplantation* 1989; **47**:55–9.

66. Vincenti F, Lantz M, Bimbaum J, Garovoy M, Mould D. A phase I trial of humanized anti-IL-2 receptor antibody in renal transplantation. *Transplantation* 1997; **63**:33–8.

67. Vincenti F, Kirkman R, Light S, Bumgardner G, Pescovitz M, *et al*. IL-2 receptor blockade with Daclizumab to prevent acute rejection in renal transplantation. *N Engl J Med* 1998; **338**:161–5.

68. Nashan B, Light S, Hardie LR, Lin A, Johnson JR. Reduction of acute renal allograft rejection by Daclizumab. *Transplantation* 1999; **67**:110–15.

69. Nashan B, Moore R, Amlot P, Schmidt AG, Abeywickrama K. randomized trial of basiliximab versus placebo for control of acute cellular rejection in renal allograft recipients. *Lancet* 1997; **350**:1193–8.

70. Bumgardner GL, Hardie I, Johnson RW, Lin A, Nashan B, *et al*. results of 3 year phase III clinical trials with Daclizumab prophylaxis for prevention of acute rejection after renal transplantation. *Transplantation* 2001; **72**:839–45.

71. Bumgardner GL, Ramos E, Lin A, Vincenti F. Daclizumab prophylaxis for prevention of acute rejection in renal transplant recipients with delayed graft function. *Transplantation* 2001; **72**:642–7.

72. Nair MP, Nampoory MR, Johny KV, Costandi JN, Abdulhalim M, *et al*. Induction immunosuppression with interleukin-2 receptor antibodies in renal transplant recipients. *Transplant Proc* 2001; **33**:2767–9.

73. Beniaminovitz A, Itescu S, Lietz K, Donovan M, Burke EM, *et al*. Prevention of rejection in cardiac transplantation by blockade of the IL-2 receptor with a monoclonal antibody. *NEJM* 2000; **342**:613–19.

74. Abu-Elmagd K, Fung J, McGhee W, Martin D, Mazariegos G, *et al*. The efficacy of Daclizumab for intestinal transplantation: preliminary report. *Transplant Proc* 2000; **32**:1195–6.

75. Carreno MR, Kato D, Weppler JM, Mathew JM, Fuller L, *et al*. Induction therapy with daclizumab as part of the immunosuppressive regimen in human small bowel and multiorgan transplants. *Transplant Proc* 2001; **33**:1015–17.

76. Neuhaus P, Kittur D, Salizzoni M, Nashan B, Clarien PA, *et al*. Improved treatment response with basiliximab immunoprophylaxis after liver transplantation:results from a double-blind randomised placebo-controlled trial. *Liver Transplant* 2002; **8**:132–42.

77. Hesse UJ, Troisi R, Jacobs B, Van Vlem B, de Hemptinne B, *et al*. A single centers clinical experience with quadruple immunosuppression including ATG or IL2 antibodies and mycophenolate mofetil in simultaneous pancreas-kidney transplants. *Clin Transplant* 2000; **14**:340–4.

78. Sollinger H, Kaplan B, Pescovitz, Philosophe B, Roza A, *et al*. Basiliximab versus antithymocyte globulin for prevention of acute renal allograft rejection. *Transplantation* 2001; **72**:1915–19.

79. Nashan B. The IL2 pathway in clinical immunosuppression. *Transplant Proc* 2001; **33**:3072–4.

80. Keown PA, Balshaw, Krueger H, Baladi JF. Economic analysis of basiliximab in renal transplantation. *Transplantation* 2001; **71**:1573–9.

81. Cory S. Regulation of lymphocyte survival by the bcl-2 gene family. *Annu Rev Immunol* 1995; **13**:513–43.

82. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha chains: breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995; **155**:1151–64.

83. Buckley RH. Primary immunodeficiency diseases due to defects in lymphocytes. *N Engl J Med* 2000; **343**:1313–24.

84. Leonard WJ. XSCID: from molecular cause to gene therapy within seven years. *Mol Med Today* 2000; **6**:403–7.

85. Leonard WJ. The molecular basis of XSCID: defective cytokine receptor signalling. *Annu Rev Med* 1996; **47**:229–39.

86. Macchi P, Villa A, Gillani S, Sacco MG, Frattini A, *et al*. Mutations of Jak3 gene in patients with severe autosomal severe combined immune deficiency. *Nature* 1995; **377**:65–8.