IMMUNOMODULATION FOR TRANSPLANTATION TOLERANCE

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SUMMARY

Here I will discuss some new immunosuppressive reagents in the context of inducing tolerance to grafts, with particular attention to T cells which mediate cellular attack on foreign cells.

THE IDEAL

For the transplant recipient, the ideal would be to induce specific tolerance to a graft, akin to creating a 'hole' in the immune repertoire to accommodate the graft antigens. This allows the recipient to retain full immune competence – with the exception of the 'hole'. Since no immunosuppressive therapy is required the risk of adverse side-effects (ranging from nephrotoxicity to neoplasia) is avoided. If the immune repertoire is portrayed as a library, with each book being reactive against a single type of antigen, then removal of graft-specific 'books' would create the desired hole in the library (Fig. 1). In practice physical removal of graft-reactive T cells is difficult because *in vivo* these are scattered throughout the body. However, it has been shown that cannulation of the draining lymph nodes to capture cells as they leave an antigenic site can achieve deletion of a specific population of antigen-reactive cells. The method critically depends on *total* capture of antigen-reactive cells, and is not practical for clinical application. Other general approaches to creating a hole in the immune repertoire include (1) central deletion of antigen-reactive cells in the thymus, and (2) turning off the aggressive response to the graft in the peripheral circulation.

CENTRAL TOLERANCE APPROACH

A central approach to tolerance induction capitalises on one of several of the intrinsic self-control systems built into the immune system to prevent self-reactive T cells maturing: such control avoids autoimmune catastrophy. In the thymus early exposure of immature, self-reactive T cells to self-antigen results in a suicide response via apoptosis, or programmed cell death, wherein the cell collapses in a controlled manner so as to preserve the macromolecules for recycling, and to avoid any inflammatory responses (these features are in contrast with necrotic cell death). The cartoon in Fig. 2 depicts how a self-reactive T cell might be deleted in the thymus during normal maturation. Experimental introduction of graft-specific antigen into the thymus would similarly delete those immature T cells which recognise the graft antigens. Thus the effect has been to create the desired hole in the immune repertoire.

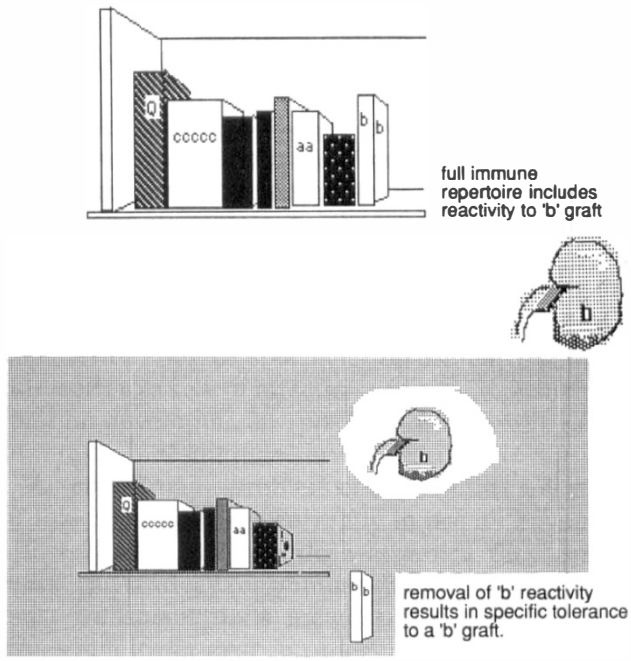

Fig. 1. *Immune repertoire portrayed as a library wherein each book is specific for a single antigen. Removal of graft-specific books (e.g. 'b' type antigens of kidney allograft 'b') creates a hole in the repertoire to accommodate the graft.*

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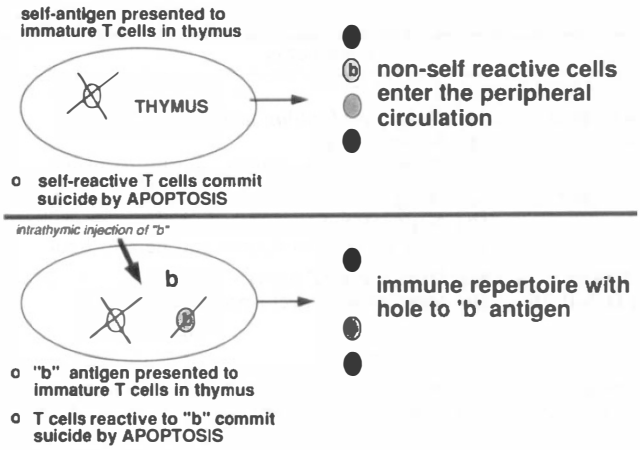

Fig. 2. *Cartoon to illustrate one of the mechanisms by which self-reactive T cells might be deleted during thymic development. Experimental introduction of graft antigen may similarly result in deletion of graft-reactive cells prior to their release into the periphery.*

This approach has been successful in inducing tolerance in rodents when combined with some means to remove pre-existing, mature peripheral T cells. Application in the clinical situation is limited since in adult humans the thymus is normally involuted and relatively inactive, with peripheral cells being mainly responsible for maintaining the existing immune repertoire. Thus some means of selective inactivation of peripheral graft-reactive cells is required, and new drugs and monoclonal antibodies may directly achieve this.

PERIPHERAL TOLERANCE INDUCTION

Working Hypothesis

As a simple working hypothesis we can argue that: (1) an *aggressive* immune response requires antigen *plus* interleukin-2 (IL2); (2) alone, antigen renders the antigen-reactive cells *un*responsive (anergic or suppressive).

An extrapolation of this working hypothesis says that removal of IL2 at the time of exposure to the graft would render the graft-reactive cells unresponsive. There are various observations which support this general hypothesis, for both the generation and maintenance of specific unresponsiveness.

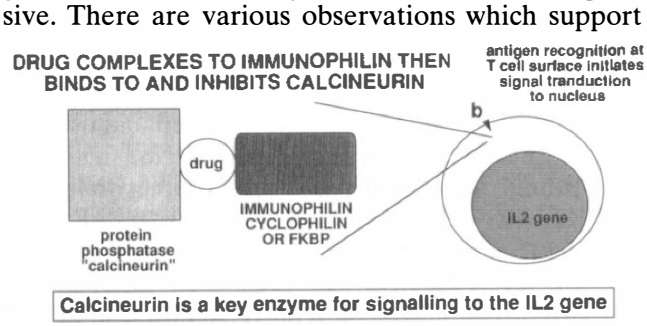

Fig. 3. *Scheme depicting the action of cyclosporin A and FK506 to inhibit interleukin-2 (IL2) induction in T lymphocytes.*

Cyclosporin A and FK506

Interestingly the specific immune-suppressive drugs cyclosporin A and FK506 each act to prevent induction of the gene encoding IL2 in T cells during antigenic stimulation (Fig. 3). These drugs caused excitement amongst molecular biologists when it was discovered that alone each drug molecule is inactive and requires complex formation with a cytoplasmic receptor to become immunosuppressive. The drug-receptor complex binds to and inhibits a calcium-calmodulin-dependent protein phosphatase called 'calcineurin' or PP2B (protein phosphatase 2B): this enzyme plays a central role in signal transduction from the cell surface to the nucleus during the activation of quiescent T cells.

Rapamycin

Rapamycin is an immunosuppressive macrolide drug chemically related to FK506 which also requires receptor binding to form an active complex (Fig. 4). The mode of action of rapamycin differs from that of FK506 since calcineurin is not inhibited and IL2 secretion is normal: however, in the presence of rapamycin the *receptor* for IL2 is unable to complete signal transduction to the nucleus upon its activation by the secreted IL2 ligand. Thus the overall effect is to blind the cell to the IL2 signal, rather than to prevent IL2 secretion *per se*. An interesting aside is that both rapamycin and FK506 share the same intracellular receptor (FKBP, or FK506 Binding Protein) and thus one may compete against the effects of the other. However, cyclosporin A is a cyclic peptide and its receptor is cyclophilin. Thus rapamycin may be used to synergise with cyclosporin A without fear of antagonism due to competition for FKBP.

The cyclophilin and FKBP families of proteins are peptidyl proline *cis-trans* isomerases involved in protein folding. They are ubiquitous to cells from bacteria to man. Since cyclosporin A and the

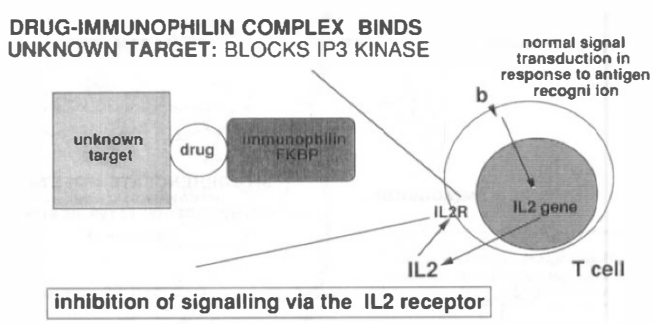

Fig. 4. *Scheme depicting the action of rapamycin to inhibit signal transduction following ligation of IL2 to the IL2 receptor at the T cell surface.*

Table I. Immunomodulatory drugs

Drug	Site of inhibition (*enzyme*1)	Comments
Inhibitors of signal transduction		
Cyclosporin A	Inhibition blocks signal transduction from the TCR to the IL2 gene promoter (*calcineurin phosphatase*)	(a) *Drug – cyclophilin active* (b) Nephrotoxic (c) High doses also neuro- and hepato-toxic
FK506	Inhibition blocks signal transduction from the TCR to the IL2 gene promoter (*calcineurin phosphatase*)	(a) *Drug – FKBP active* (b) Nephrotoxic (c) High doses also neuro- and hepato-toxic
Rapamycin	Inhibition of IP3-kinase dependent signals induced by growth factors including IL2–IL2R interaction	(a) *Drug – FKBP active* (b) Synergy with cyclosporin A
Inhibitors of cytokine gene expression		
Corticosteroids	Inhibit synthesis of many cytokines (bind glucocorticoid response element at 5′ regulator sequence of cytokine genes)	(a) *Active complex with cytoplasmic receptor* (b) Cushingoid side-effects
Inhibitors of DNA synthesis and other antimetabolites		
Azathioprine	Purine biosynthesis	(a) Myelotoxic (b) Hepatotoxic
Mycophenolate mofetil (RS-61443)	Purine biosynthetic pathway in T and B cells (other cell types can escape inhibition via an alternative pathway) (*inosine mono-P dehydrogenase*)	(a) Gastrointestinal toxicity at high doses
Mizoribine	Purine biosynthetic pathway (*inosine mono-P dehydrogenase*)	
Brequinar sodium	Pyrimidine biosynthesis in T and B cells (*dihydro-orotate dehydrogenase*)	
Deoxyspergualin (DSG) and analogue LF299	Cell maturation (detail unknown)	(a) Cytostatic (b) Reversible myelotoxicity

macrolides FK506 and rapamycin are each produced by soil-dwelling micro-organisms, these drugs may have arisen to confer an evolutionary advantage because they also inhibit the cell division cycle of common soil-dwelling organisms including yeast which might be in competition for this environmental niche. Cyclosporin A and FK506 inhibit re-entry of yeast into the cell division cycle in a similar manner to the inhibition of quiescent T cell activation into cell division. Rapamycin, by blocking signal transduction mediated by growth factors, blocks cell cycle progression at some point in G_1. In addition to blocking T cell responses to IL2, rapamycin may also inhibit autocrine-driven proliferation of certain tumours and may be especially valuable in transplant surgery to replace tumours such as primary hepatoma where immunosuppression by other drugs often results in rapid growth of metastatic disease.

The structure and properties of cyclosporin A, FK506 and rapamycin together with other new immunosuppressive drugs are given in Fig. 5 and Table I.

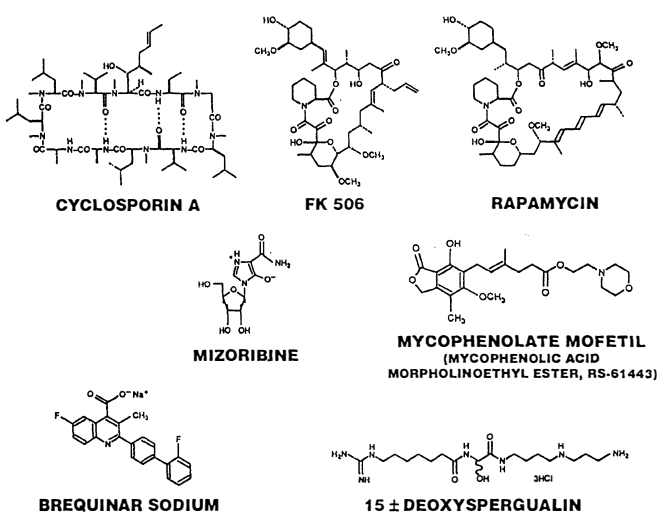

Fig. 5. *Chemical structures of some new immunomodulatory drugs.*

Monoclonal Antibody Therapy to Modulate the Immune Response

Monoclonal antibodies (mabs) have exquisite specificity for target antigens and provide powerful reagents. In the context of our hypothesis, mabs against IL2, or the IL2 receptor, are directly relevant and have been used both experimentally and clinically to reduce the IL2 signal at the time of transplantation. OKT3 is a mab directed against the T cell receptor complex required for antigen recognition: our hypothesis would predict that use of OKT3 would be immunosuppressive but not tolerogenic since it would prevent antigen recognition by graft-reactive T cells. An alternative approach which leaves antigen recognition intact is to target the CD4 co-receptor with mab. CD4 is expressed on 'helper' T cells and binds to major histocompatibility complex (MHC) class II to

Table II. Immunomodulatory monoclonal antibodies

Aim	Example mab or epitope	Comments
Block antigen recognition	T cell receptor (TCR complex) Class II	(a) Immunosuppressive
T cell depletion	OKT3 (CD3 of TCR complex)	(a) Immunosuppressive (b) First dose effect (c) Antiglobulin response
T and B cell depletion	Campath-1-H (CD52)	(a) Immunosuppressive (b) First dose effect (c) Humanised mab
Inhibit IL2 synthesis	CD4 co-receptor for TCR CD8 co-receptor for TCR CD28 (B7 or CTLA4)	(a) Potentially tolerogenic (b) B7 or CTLA4 bind CD28 to induce co-stimulatory pathway for IL2 synthesis
Inhibit IL2 effect	Anti-IL2 Anti-IL2 receptor α chain	(a) Potentially tolerogenic (b) α chain expressed on activated T cells only
Inhibit lymphocyte trafficking and adhesion to target cell	LFA-1 – ICAM-1 VLA-4 – VCAM-1 CD2 – LFA-3	(a) T cell – APC epitopes (b) Immunosuppressive (c) Tolerogenic in rodents

General comments

(1) Mouse or rat immunoglobulin (mab) for human therapy usually induces '*anti-globulin responses*' (i.e. patient-derived antibody against the foreign protein) within 10 days of daily intravenous therapy. This effectively neutralises the therapeutic mab and prevents further beneficial effect. Repeated treatment with mab in the face of an antiglobulin response may cause anaphylactoid reactions.

(2) Mabs derived from rodents may be *humanised* by genetically engineering the mab idiotype (site responsible for antigen recognition) onto a human immunoglobulin. Humanised Campath-1-H (derived from rat Campath-1-G directed against the CD52 lymphocyte antigen) has been used clinically to reverse rejection episodes: importantly no antiglobulin responses to Campath-1-H were observed.

(3) Mabs which cause lysis of lymphocytes (e.g. depleting mabs such as OKT3 and Campath-1-H) are often associated with a '*first dose effect*' due to release of cytokines from the lysed lymphocytes, the first dose causing the most lysis. The symptoms are flu-like and may be severe (OKT3) or relatively mild (Campath-1-H).

(4) Blocking mabs do not lyse the target cell but mask the target antigen, or cause it to be internalised or shed (antigenic modulation). This allows the target cell to become altered in function, such as becoming suppressive instead of aggressive.

(5) The *T cell receptor complex (TCRC)* is made up of the TCR αβ heterodimer *plus* the CD3 antigen *plus* ζ and n chains; to form a complex for specific antigen recognition (TCR αβ) and signal transduction (CD3 plus ζn).

cooperate in recognition of antigenic peptide presented to the T cell receptor by MHC class II (see Fig. 6): in the absence of CD4 the T cell is unable to secrete IL2 in response to presented antigen. (A similar scenario applies to CD8, which is a co-receptor (reacting with MHC class I) on cytotoxic T cells that recognise antigen in the context of MHC class I.) CD4 and CD8 cooperate directly with the TCR for IL2 induction: T cells also express CD28 which provides a separate, co-stimulatory pathway for IL2 induction and also may be targeted in therapy (see Table II).

In vivo, treatment of mice with a brief course of CD4 mab plus CD8 mab to block the respective co-receptors at the time of heart grafting results in stable tolerance to the graft. Importantly, this tolerance is 'infectious' in the sense that potentially aggressive cells become unreactive in the presence of tolerant cells which share the same antigenic target. Thus transfer of tolerant spleen cells to a naive recipient will transfer the tolerant state. Such adoptive transfer of tolerance is successful at least five times away from the original recipient (i.e. the only recipient treated with mabs): this implies that the capacity for graft-specific tolerance has been amplified. Thus, the concept of a simple 'hole' in the immune repertoire is not sufficient to explain this peripheral tolerance. Instead it is thought that graft-reactive cells in the original mab-treated recipient become switched from an 'aggressive' to a 'suppressive' mode, and carry the suppressive regulatory property upon adoptive transfer where it is dominant over the naive recipient's graft-reactive cells.

The use of mabs to interfere with the immune response to a foreign graft has been successful in rodents, but clinical application is restricted by the patient making an immune response against the foreign mab protein (i.e. an anti-globulin response). This often occurs by day 10 and effectively obviates further therapeutic effect of the mab in addition to risking side-effects of an anaphylactoid nature.

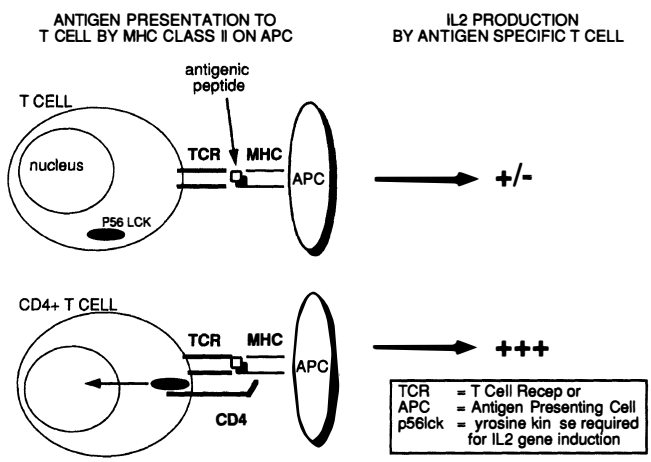

Fig. 6. *Requirement for CD4 co-receptor function for IL2 secretion induced by antigenic peptide presentation to the T cell receptor.*

Preclinical research is needed to identify therapeutic strategies using mabs in combination with conventional immunosuppressive drugs, and in Cambridge mabs against dog lymphocytes have been generated to allow transposition of methods to achieve tolerance induction from rodents to a large animal model. By using mabs in conjunction with azathioprine and cyclosporin A (given with four dose reductions for a total of 56 days) we have shown that CD4 and CD8 mabs significantly prolong renal allograft survival between strongly mismatched dogs, with operational tolerance occurring in some cases. Moreover, by combining the mabs (rat immunoglobulin) with azathioprine and cyclosporin A, the antiglobulin response was prevented, opening the way to more prolonged, effective mab treatment.

The reagents discussed above relate directly to induction of graft tolerance in the context of controlling IL2 levels. A range of other specific reagents are also available to modulate immune responses, including anti-metabolites which inhibit lymphocyte DNA replication (RS-61443, or mycophenolic acid, selectively inhibits T and B cell purine biosynthesis and Brequinar sodium inhibits pyrimidine biosynthesis). Alternatively cell maturation and antigen presentation may be inhibited by deoxyspergualin (DSG) or a closely related analogue (L299) to provide complementary reagents to other drugs. Monoclonal antibodies against adhesion molecules reduce lymphocyte trafficking to and from the graft site; whilst inflammatory cytokines such as tumour necrosis factor (TNF) may be removed by their respective mab. (TNF mab is currently undergoing clinical trials for treatment of rheumatoid arthritis, with good results compared with conventional high-dose steroid therapy.) Overall, the specificity of new immunodulatory reagents promises to provide powerful strategies for tolerance induction to foreign grafts in the near future.

SOME FURTHER READING

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