improvement of some facilities such as the psychiatry block, eye clinic, catering department, etc., numerous cosmetic changes to wards and departments were possible, but no expansion of the ward situation was permitted. Despite these efforts some very unsatisfactory facilities still remain. Nevertheless King Edward VIII Hospital remains one of the major training hospitals in the country for both undergraduates and postgraduates. Paramedical training in such fields as physiotherapy, radiotherapy, nuclear medicine, ultrasonography, pharmacy, speech and occupational therapy and medical technology is well established.

Over the years the increase in patient numbers overwhelmed available facilities to an extent that patients sleep on mattresses on the floor at night. The daily average of 150 'floor beds' greatly reduces physical ward space and increases cross-infection. It also places a considerable burden on the nursing staff. There also developed an urgent need to increase service departments such as catering, laundry, maintenance, laboratories, mortuary, central sterilizing and the internal communications system.

The atmosphere in this hospital is, as a result of the pressure of work, one of constant and purposeful activity, demanding unremitting effort and awareness on the part of every member of staff. This common purpose evokes deep loyalty and dedication among all staff at the hospital. However, with little relief of the situation over the years, there is a sense of frustration regarding the enormous pressures of having to admit ever-increasing numbers of patients, and the necessity of transferring patients to Clairwood before this is medically justified, thus destroying continuity in medical care. There is clearly a pressing need for greatly expanded facilities — beds, clinics and equipment — to relieve this hard-pressed hospital.

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**Review Article**

**The application, mechanism of action and side-effects of immunosuppressive agents in clinical transplantation**

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**Summary**

The conventional agents (azathioprine and steroids) have been the mainstay of organ allograft immunosuppression for the past 20 years. The main drawback of the immunosuppressive agents at present in use is that they act nonspecifically with sequential general depression of the immune system. The introduction of cyclosporin, an undecapeptide of fungal origin, which selectively inhibits T-cell-dependent immunoreaction has made a significant impact on organ allograft survival rates. Clinical application has been complicated because of renal or hepatotoxicity. Thoracic duct drainage is of historical interest but the use of antilymphocyte serum, despite its chequered history, has recently been shown to be safe and effective in cadaver kidney transplant recipients. There has also been a resurgence of interest in the use of total lymphoid irradiation as an immunosuppressive agent. The introduction of xenogenic monoclonal antibodies with anti-T-cell specificity opened a new era in clinical immunology and OKT3-PAN has emerged as a powerful major immunosuppressive agent with low toxicity.

The introduction of new chemical immunosuppressants together with the refinement of existing agents has resulted in improved renal allograft survival and rekindled interest in transplantation of other organs — liver, pancreas and heart-lung preparations. It is accepted that with few exceptions organ allograft recipients need lifelong immunosuppression to combat rejection of the transplanted organ. Unfortunately, the agents at present in use have the disadvantage of causing generalized immunosuppression of the recipient, which in turn significantly increases susceptibility to infection. It is clear that donor-specific immunosuppression is the ultimate goal if safety is to be improved. Current results, world-wide, indicate that to ensure initial acceptance of an organ allograft major and hazardous treatment whether by irradiation, chemotherapy or antibody manipulation is required. Although immunologists and transplantation biologists desire to manipulate the immune system to accept an organ allograft without penalty, this objective is probably still a long way off.

This review briefly discusses the application, mechanism of action and side-effects of immunosuppressive agents at present in use in clinical transplantation practice (Table 1).

**Conventional immunosuppression**

Conventional immunosuppression of organ allografts with azathioprine (AZA) and steroids was the sheet-anchor of treatment before the discovery of cyclosporin (CSA). AZA, an antimetabolite, inhibits protein synthesis by competing with or blocking specific surface receptors. It is a purine antagonist and like 6-mercaptopurine is cycle-specific in its action, thus effective against proliferating cells.
After ingestion, AZA is rapidly converted to 6-mercaptopurine. Both substances inhibit immune responses that initiate cell proliferation including antibody production and graft rejection. The optimum time for administration of these agents is after exposure to antigen but unfortunately a considerable species variation in immunosuppressive effect has been observed. Side-effects of AZA are related to its effect on bone marrow, which includes leucopenia, thrombocytopenia and anaemia.

Steroids are administered, either in large or small doses, as prophylaxis against rejection or for treatment of rejection episodes.1-7 Steroid administration results in depression of protein, RNA and DNA synthesis, death of small lymphocytes in the blood, thymus, lymph nodes, and spleen, impaired cellular immunity, inhibition of T-cell migration to the sites of antigen disposition, inhibition of lymphokine release from lymphocytes, reduction of monocytes and blocking of the interaction between lymphocytes and monocytes.1,7

Important side-effects of steroid administration have been steroid diabetes, stunted growth in children, peptic ulceration, cushingoid appearance, hypertension, cataract formation, psychiatric disturbances, osteoporosis and avascular necrosis of the femoral head.

In clinical practice AZA and steroids are given together since adequate immunosuppression is not provided by either drug alone in large doses. Generally speaking, AZA and steroids result in 50 - 60% 1-year cadaver renal allograft survival, and in view of attendant side-effects it is imperative to continue the search for more effective and safe immunosuppressive agents.3 The application of CSA as the only immunosuppressive agent has been disappointing and in patients with nephrotoxicity it has been mandatory to revert to AZA plus steroid therapy.5,7

Cyclosporin (CSA)

CSA, a fungal derivative, has been found to be markedly effective as an immunosuppressant in man and in a variety of animal species with significant suppression of organ allografts. CSA, a cyclic undecapeptide of molecular weight 1202 daltons, contains 11 amino acids, one of which is unique. It is neutral, hydrophobic and almost completely eliminated by hepatic metabolism and broken down to at least 17 metabolites.8-12

From in vitro animal experiments it is apparent that CSA exerts its maximal effect very early after exposure of the recipient to a tissue allograft. In man CSA inhibits the proliferative response of lymphocytes to concanavalin A, phytohaemagglutinin and pokeweed mitogen in vitro as well as complete inhibition of the mixed lymphocyte reaction (MLR).10

It has been suggested that CSA is directed predominantly against helper T lymphocytes, either preventing production of interleukin 2 (IL 2) (T-cell growth factor) or inhibiting the response to IL 2.1,2 Borel et al's12 original experiments showed that CSA suppresses both humoral and cell-mediated immunity, fails to suppress the antibody response to lipopolysaccharides in nude mice, inhibits lymphocyte proliferation, affects mitogenic triggering but not mitosis, has no influence on preformed cytotoxic cells and is not lymphotoxic or myelotoxic.12,14 White and Cane1 showed that CSA had no effect on macrophage function and confirmed that the drug acted on activated but not resting T cells during the phase of the priming process. It is clear that CSA exerts a highly specific effect on the T-lymphocyte population and the likely mechanism by which such specificity is achieved is through the presence of specific surface ligands or receptors.10

Considerable experience has been gained with the use of CSA in renal, hepatic, pancreatic, heart and kidney transplantation either alone or in combination with antilymphocyte globulin (ALG) or conventional immunosuppressive agents.15-19

Unfortunately, the clinical use of CSA is difficult to evaluate because of its narrow therapeutic window. CSA can be measured in plasma or whole blood samples by radio-immunoassay or by high performance liquid chromatography. Serum trough-level determination has been of some value in avoiding nephrotoxicity. Documented side-effects include nephro- and hepatotoxicity, hypertension, hyperkalaemia, hyperuricaemia, tremors, paraesthesiae, muscle weakness and hirsutism.

Nephrotoxicity, undoubtedly the most troublesome complication, appears to be dose-related and is reversible if CSA is discontinued.12,14-25 The latter observation was encountered in the Oxford trial6 of CSA in renal transplantation where patients were converted to AZA and prednisone 3 months after transplantation, which resulted in rapid and significant improvement in renal function. In addition, the incidence of lymphoma, both in renal and cardiac allograft recipients, may be no greater than expected in recipients on conventional immunosuppressive therapy.10

The effect of blood transfusion

Recent data indicate that intentional pre-operative blood transfusion significantly improves graft survival in renal allograft recipients regardless of HLA matching of donor or recipient.28-31 Blood transfusion before transplantation creates a major problem because potential recipients may become sensitized to HLA antigens, thus generating lymphocytotoxic antibodies.28 The production of HLA antibodies could thereby potentially decrease the chance of finding a donor of suitable cross-match. At present, although most transplant surgeons agree despite the drawbacks that pretransplant blood transfusions improve graft survival, they are not unanimous on the volume that must be transfused. Opelz and Terasaki26,27 and Opelz25 suggested that at least 5 pretransplant transfusions are required at intervals of 2 - 3 months, while others have shown a long-lasting effect after a single transfusion.29 To complicate matters, animal experiments have indicated that platelet transfusions were as effective as whole blood transfusion in improving renal allograft survival. Results from centres specializing in transplantation research have shown that the effect of pre-operative blood transfusion is less apparent in group O recipients.5

The mechanism of action is unknown but it may be attributed to the induction of nonspecific or perhaps specific immunosuppression.26 Accumulated evidence suggests that blood transfusion evokes production in the recipient of alpha-macroglobulin,
anti-idiotypic antibody, enhancing antibody and suppressor leucocytes. Blood transfusion presumably suppresses the mitogen response and it has been suggested that an HLA-independent aspecific suppression cell might play a role. On the other hand, anti-idiotypic antibodies that have been isolated, which inactivate T-cell clones, might result in cell-mediated lympholysis (CML) non-responsive against the specific kidney donor. It seems as though both aspecific CML-restricted and specific CML-restricted mechanisms may play a role.

Antilymphocyte serum (ALS)

The work of Starzl et al., Najarian et al. and many others has clearly established that ALS is an effective immunosuppressive agent in man. Heterologous ALS and, in particular, the globulin fraction raised against lymphocytes (ALG) and thymocytes (ATG) are potent immunosuppressive agents. ALS acts by selectively eliminating circulating T cells. Daily T-cell monitoring with the sheep RBC rosette test may be used as a guide to determine the ATG dose, thus preventing over- or under-immunosuppression of patients. The ALG or ATG used in clinical practice has been produced in horses, goats, rabbits and cows. One of the disadvantages of ALS has been the differing potency and purity of products so produced. ALG or ATG has been used prophylactically and for treatment during acute rejection episodes in conjunction with conventional immunosuppressive agents. Good results have been reported in cardiac allograft recipients requiring active treatment during first, second and third rejection episodes.

Complications because of ALS therapy may be related to toxic reactions to immunoglobulins or to the immunosuppressed state of the patient. Common side-effects include chills, fever, anaphylactic response, thrombocytopenia, arthralgia, and serum sickness.

In a few series an increased incidence of lymphoma has been reported, particularly in over-immunosuppressed patients. To avoid this phenomenon extreme care must be exercised when ATG is given in combination with CSA.

Thoracic duct drainage (TDD)

The use of TDD is now of historical interest since the introduction of conventional immunosuppression, ATG, irradiation and CSA into clinical practice. Gowans provided the rationale for using TDD as a form of immunosuppression. TDD was first applied clinically by Newton in 1965. Machleider and Paulus demonstrated the profound immunosuppressive consequences following 1-3-month lymphocyte depletion by TDD. To achieve sufficient immunosuppressive potency clinically drainage in excess of 4 weeks is required. Utilizing monoclonal antibodies (MCAs), which characterize specific human T-lymphocyte subpopulations, a decreased population of T lymphocytes has been confirmed. The use of MCAs also showed a reduction in helper T to suppressor T/cytotoxic cell ratio after TDD. Extended hospital surveillance of the patient over a period of several months makes TDD an impractical method of immunosuppression.

Monoclonal antibodies (MCAs)

ALG and ATG have proved effective in reversing acute renal allograft rejection in man. Largely because of this experience, it has been suggested that MCAs against T-lymphocyte subsets might be useful immunosuppressive agents. Theoretically, immunosuppression with MCAs would be more specific and antibodies against selected T-cell subsets could be used to circumvent rejection. In addition, those antibodies would have the added advantage of better consistency among batches, greater ease in monitoring serum levels, and administration of less foreign protein.

Recently, MCA therapy with three different antibodies has been attempted. In monkeys, OKT3 and OKT4 have proved effective immunosuppressive reagents. In contrast, OKT 8 has failed to prolong allograft survival in monkeys. Both OKT3 and OKT4 appear to function by diminishing circulatory T cells.

The mode of action of MCA includes removal of effector or precursor T cells from the circulation by complement-mediated lysis or removal by the reticulo-endothelial system (RES), cell surface modulation of antigens by antibodies or coating of cell surface antigens.

Recently the anti-PAN T cell MCA OKT3 was introduced into practice to treat acute rejection crises in recipients of renal allografts. OKT3 has been administered alone but in most series in conjunction with conventional immunosuppression. In the French study conducted by Chattenoud et al., patients given OKT3 underwent sequential monitoring of cellular and humoral parameters including peripheral T-cell subsets (as defined by OKT3, OKT4 and OKT6) and OKT3 serum levels, as well as the appearance of anti-OKT3 IgG antibodies. Serum examination 1 hour after OKT3 administration was characterized by total disappearance of OKT3+, OKT4+ and OKT8+ lymphocytes confirming the high immunosuppressive capacity of OKT3. By the 2nd-5th day, after the initial and dramatic depletion of all peripheral T cells, OKT4+ and OKT8+ cells were again detectable in the absence of OKT3+ cells.

It can be concluded from recent data that OKT3 PAN MCA in man has a major immunosuppressive impact but low toxicity, does not induce tolerance, and its effect is limited by its immunogenicity as witnessed by the development of anti-idiotypic antibodies to MCA. Clinical use of MCAs is still experimental but allows for more precise immunotherapy although their use may be limited because of immunogenicity.

Donor-specific immunosuppression

Despite the success so far achieved in preventing organ allograft rejection, immunosuppressive regimes used at present unfortunately result in generalized immunosuppression. The ultimate aim of transplantation surgeons is to suppress organ rejection with potent but non-toxic drugs, thus enabling transplant to be performed regularly and safely. This may be achieved through donor-specific immunosuppression, i.e. immunosuppression directed only at that part of the recipient's immune system responsible for rejection of the organ graft.

Fabre and Morris have defined specific immunosuppression which directly or indirectly suppresses the action of those lymphocyte clones specifically reactive to donor histocompatibility antigens and responsible for the rejection phase. This may be achieved by antigen-induced suppression and is induced by treating the recipient with preparations containing donor histocompatibility antigens. On the other hand, passive enhancement referring to antibody-induced suppression is achieved by challenging the re-
I onizing irradiation as an immunosuppressive agent

Recent studies have shown that the use of irradiation, a powerful immunosuppressive agent, can significantly prolong survival of various organ allografts including the heart, kidney and liver in a number of experimental species. Total lymphoid irradiation (TLI) has proved immunosuppressive in mice, dogs, primates and man and the potential applicability to clinical transplantation has been reviewed. A conditioning regimen previously reported by the Stellenbosch group in a primate model gave encouraging results and is similar to that reported by the University of the Witwatersrand. This entails fractionated, subtotal marrow irradiation, amounting to near total body irradiation without bone marrow reconstitution. Photons from a 6 MV linear accelerator (Siemens Corp., Mevatron, Iselin, New Jersey, USA) are administered at 215 cm source-skin distance delivered at a radiation rate of 2.857 rad/h and exposure rate of 50 rad (0.5 Gy)/min. A total dose of 800 rad (8 Gy) - 1000 rad (10 Gy) administered in a dose of 200 rad (2 Gy) weekly has proved effective.

Irradiation cell death is due to DNA base damage and/or single-stranded breaks in DNA resulting in death during attempted mitotic divisions. In particular the small resting lymphocytes which are highly radiosensitive are destroyed during interphase. Patients with Hodgkin's disease subjected to fractionated irradiation develop T-cell lymphocytopenia, β-cell lymphocytosis, depression of responsiveness in mixed lymphocyte reaction and phytohaemagglutinin mitogenesis. In man, maximal depression of T cells is achieved after administration of 2000 rad (20 Gy). Loss of responsiveness to allogenic and mitogenic stimulation occurs. The function of remnant T cells is affected by TLI and a possible explanation may be attributed to greater depletion of helper cells creating a larger donor immune cells are employed which are selectively cultured at room temperature before transplantation and the recipient is immunosuppressed with donor immune cells are employed which are selectively cultured in a high oxygen concentration before transplantation. Donor immune cells are killed by ultraviolet irradiation before treatment; dendritic cells in culture are eliminated by pretreatment with antidendritic antibody; peripheral blood cells are treated with TLI and complement before transfusion into the recipient; and blood is pretreated by ultraviolet irradiation before transfusion into the recipient.

The same authors have further divided specific immunosuppression into an induction phase during which the state of unresponsiveness is produced, and a maintenance phase when the interactions between host and graft have stabilized.

Immunoregulation by tissue treatment (immune alteration)

Generalized immunosuppression, using conventional agents and CSA, has resulted in significant prolongation of organ allografts in experimental models and man. Despite their use, rejection of transplanted islets of Langerhans is still a formidable problem. Recent studies have indicated that prolonged survival of islets is possible by pretreating the graft before transplantation. The unique ability of this cellular graft to be manipulated in vitro provides a distinct advantage of isolated islet tissue over segmental pancreatic grafts. Further studies have demonstrated that the concept of immune alteration originally suggested by Snell in 1957 is applicable to islet transplantation. Immune alteration approaches are essentially concerned with finding a selective method of eliminating the passenger lymphoid cells, and dendritic cells, from the graft before transplantation. This has been partially accomplished in experimental rat models but not in man.

Seven methods have been developed for successful immune alteration of islet cell grafts before transplantation: (i) the islets are cultured at room temperature before transplantation and the recipient is immunosuppressed with ALS; (ii) islets are cultured in a high oxygen concentration before transplantation; (iii) dendritic cells in the graft are eliminated by culturing islets in a medium containing MCA and complement; (iv) donor immune cells are employed which are selectively killed by ultraviolet irradiation before treatment; (v) dendritic cells in culture are eliminated by pretreatment with antidendritic antibody; (vi) peripheral blood cells are treated with TLI and complement before transfusion into the recipient; and (vii) blood is pretreated by ultraviolet irradiation before transfusion into the recipient.

These remarkable advances in islet tissue isolation and immune alteration have brought transplantation immunologists to the threshold of potential clinical trials. From the foregoing, we can expect to see a move away from the present reliance on immunosuppression with greater emphasis on techniques that may be used to reduce immunogenicity of the tissue being transplanted. This dramatic shift away from conventional practice may have profound effects on clinical transplantation in the future.

REFERENCES


