抗免疫治疗

CURRENT STATUS OF MODERN THERAPY: VOLUME 7

Immunosuppressive Therapy

Edited by

J. R. Salaman

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Consultant Editor's Note

CURRENT STATUS OF MODERN THERAPY

The *Current Status of Modern Therapy* is a major series with the purpose of providing a definitive view of modern therapeutic practice in those areas of clinical medicine in which important changes are occurring. The series consists of monographs specially commissioned under the individual editorship of internationally recognized experts in their fields. Their selection of a panel of contributors from many countries ensures an international perspective on developments in therapy.

The series aims to review the growth areas of clinical pharmacology and therapeutics in a systematic way. It is a continuing series in which the same subject areas will be covered by revised editions as advances make this desirable.

It can truly be said that the field of immunosuppressive therapy is one in which important and major changes are occurring. Indeed the whole field is only 20 years old. The choice of immunosuppression for the *Current Status* series was thus a natural one.

John Salaman, who is himself well known for his work in transplant surgery, has collected a group of experts from centres in five countries to help him develop the new ideas. The topics that he has selected are of practical human application and interest. This will ensure that this volume, like previous ones in the *Current Status of Modern Therapy* series, will be widely welcomed.

J. MARKS
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Series Editor

Preface

In this volume I have attempted to cover the more important aspects of immunosuppressive therapy. In doing this, I have been very fortunate in securing the help of the distinguished authors who have contributed the chapters that follow and I am grateful to them for providing such thorough and up-to-date accounts of their subjects. Immunosuppression can be mediated by many hundreds of agents and it has been difficult to strike a balance between the 'small print' of drug action in animals and the usefulness or otherwise of drugs and other agents for inducing immunosuppression in patients. The first six chapters deal mainly with the experimental aspects of immunosuppression and include a full discussion of total lymphoid irradiation and cyclosporin-A. Both these agents have shown great promise recently as forms of immunosuppression in animals and undoubtedly will come to be used more and more in patients receiving transplants of one form or another. The last four chapters are devoted to the use of the more traditional immunosuppressive agents for specific clinical conditions. In her chapter on renal transplantation, Dr McGeown describes in great detail not only the immunosuppressive regime she employs but the general management of transplant patients. The clinical results have been so good in her unit that I felt her account should include these other aspects of treatment since they would seem to be just as important as the immunosuppressive regime she uses. In a like manner the chapter by Dr Jamieson and his colleagues from Stanford on cardiac transplantation covers more than just the administration of immunosuppressive drugs to patients with heart grafts. As a transplant surgeon, I was unaware, until recently, of how frequently immunosuppressive agents are used for treating medical diseases, and I am indebted to Dr Hodgson for his summary of those medical conditions where immunosuppressive treatment has been tried.

Our knowledge of the immune system is increasing very rapidly and it seems very likely that more refined methods of immunosuppression will become available to us in the future. Nonetheless we still have a vast range of agents to choose from, and I hope the reader will find this volume helpful when faced with a condition requiring immunosuppressive therapy.

JOHN R. SALAMAN

November 1980

Immunosuppressive Agents

1

Pharmacological immunosuppressive agents

J. R. Salaman

INTRODUCTION

The history of immunosuppressive drugs started in 1959 when Schwartz and Dameshek showed that when the purine analogue, 6-mercaptopurine, was given to rabbits, together with an antigen (human serum albumin) antibody formation was totally suppressed[1]. This exciting finding was followed up by Calne in Great Britain and Zukoski in the United States who independently found that 6-mercaptopurine when given to dogs would prolong the survival of renal allografts[2,3]. Shortly after this, the imidazolyl derivative of 6-mercaptopurine, azathioprine, came to be used in patients undergoing kidney transplantation, and although many powerful immunosuppressive drugs have been discovered since then, azathioprine has not been supplanted as one of the most important drugs in clinical transplantation. Under very favourable conditions, such as following a kidney transplant from a living related donor, azathioprine can be used on its own to suppress rejection[4] but this is seldom done since immunosuppression with azathioprine alone is not very effective. It is much more usual to administer azathioprine together with steroids, and this combination is without doubt highly immunosuppressive in man. It was introduced in 1962 although at that time there was no convincing evidence that such a drug combination was effective in prolonging graft survival in dogs or other animals. Even when fairly high doses of steroids are used, rejection is by no means always suppressed in man, and the chances of a cadaver kidney graft surviving for a year after transplantation are usually no more than 50%.

Combinations of three or more drugs have been tried such as are used for the treatment of malignant disease but these regimes have not been very successful. Antilymphocyte globulin has often been employed in this way but the results have been extremely variable, with some centres showing

improved results of kidney transplantation and others finding no improvement whatsoever[5]. Cyclophosphamide has also been added to azathioprine and prednisone in the treatment of patients with cadaveric kidney transplants since this triple drug combination was effective in prolonging kidney-graft survival in rabbits[6]. The extreme toxicity of this therapy in man, however, has precluded its use in this way[7].

The disadvantage of all pharmacological immunosuppressive agents to date has been the non-specific way in which immunity is depressed. In an environment teeming with potentially pathogenic micro-organisms, man requires his immune system as a defence, and any impairment will obviously increase his susceptibility to infection. Fortunately it is often possible with conventional drugs to reach a half-way situation where rejection is suppressed, yet the patient's ability to fight infections still retained. Just how this is accomplished at an immunological level is not well understood for the interaction of drugs on the various mediators of the immune response is incredibly complex[8]. It would seem that under an umbrella of non-specific immunosuppression the immune system becomes 'adapted' to accept the transplant thereby allowing the doses of the drugs to be reduced. Such 'adaptation' is essential since without it high doses of drugs would be required indefinitely and their severe toxic effects would be inevitable. Although some form of 'adaptation' undoubtedly occurs in successfully transplanted patients, drug therapy, even at very low doses, is still required, and this will be discussed later.

THE IMMUNE SYSTEM

It had been observed over the centuries that tissues exchanged between animals or individuals would be destroyed after a few weeks. The reason remained a mystery until 1944 when Medawar clearly defined for the first time the immune response[9], and it took another twelve years before the cells of the lymphoid system were identified as the mediators of this response. Although lymphocytes appear under the microscope as rather uniform, uninteresting cells, they have been discovered to have a whole range of properties and functions. Following their generation in the bone marrow from stem cells, they can develop into monocytes or lymphocytes (Figure 1). The lymphocytes in turn can develop under the influence of the thymus into T-cells or under the influence of the bursar equivalent into B-cells which produce antibodies. Antibody production however is to a great extent influenced by T-cells through the agency of T-helper and suppressor cells. It can be appreciated that a drug acting on the immune system could produce a variety of different effects, depending on which population of cells is affected. Thus, a drug that inhibits suppressor-cell activity will actually enhance the immune response.

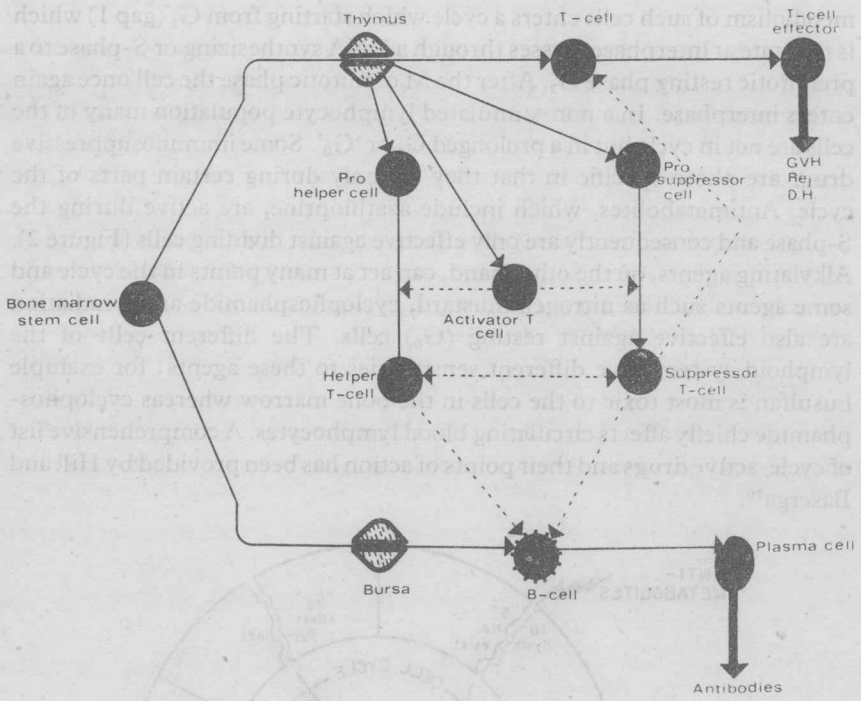

Figure 1 Generation and interaction of immunocompetent cells. Both T- and B-cells are under the influence of suppressor T-cells (dotted lines). Suppressor and helper T-cells can interact with one another and both require the assistance of an activator T-cell for their development

Transplant rejection starts with the recognition of foreign material by the immune system, and it is thought that macrophages have an important role in this step. Certainly agents that immobilize macrophages such as gold, silica and anti-macrophage serum, can help to prolong the survival of tissue allografts. Soon other members of the lymphoid system are involved, with proliferation of those cells with specificities for the foreign antigens. B-cells develop into plasma cells which secrete antibody, and T-cells (cytotoxic, helper and suppressor), B-cells, K-cells and macrophages invade the graft to bring about its destruction. It is because cell proliferation forms part of the immune response that many cytotoxic drugs are immunosuppressive. The action of these drugs is better understood if one considers the metabolic events that occur in cell division.

THE CELL CYCLE

Lymphoid cells, like other cells in the body, replicate by cell division. The

metabolism of such cells enters a cycle which starting from G_1 (gap 1) which is the state at interphase, passes through a DNA synthesizing or S-phase to a premitotic resting phase G_2. After the M or mitotic phase the cell once again enters interphase. In a non-stimulated lymphocyte population many of the cells are not in cycle but in a prolonged G_1 or 'G_0'. Some immunosuppressive drugs are phase-specific in that they act only during certain parts of the cycle. Antimetabolites, which include azathioprine, are active during the S-phase and consequently are only effective against dividing cells (Figure 2). Alkylating agents, on the other hand, can act at many points in the cycle and some agents such as nitrogen mustard, cyclophosphamide and irradiation are also effective against resting (G_0) cells. The different cells of the lymphoid system have different sensitivities to these agents; for example busulfan is most toxic to the cells in the bone marrow whereas cyclophosphamide chiefly affects circulating blood lymphocytes. A comprehensive list of cycle-active drugs and their points of action has been provided by Hill and Baserga[10].

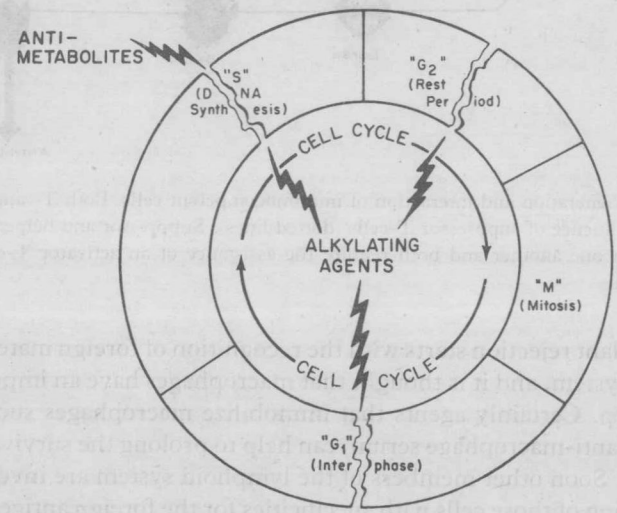

Figure 2 The cell cycle (from Hurd[24] with kind permission). Antimetabolites are only active during the S-phase of the cycle, whereas alkylating agents can arrest cell division by acting at different phases

ANTI-INFLAMMATORY AND OTHER EFFECTS

The immune process that brings about rejection of a foreign tissue is usually accompanied by a local inflammatory response. Lymphocytes, macrophages and polymorphs are attracted to the area in a non-specific way. Therefore, drugs which are known to act as anti-inflammatory agents will also depress,

Table 1 Chemical immunosuppressive agents

ALKYLATING AGENTS	Nitrogen mustard, L-phenylalanine mustard, chlorambucil, busulfan, tri-ethylene-thiophosphoramide, uracil mustard, cyclophosphamide
ANTIMETABOLITES	*Purine antagonists:* 6-mercaptopurine, azathioprine, 6-thioguanine *Pyrimidine antagonists:* 5-fluorouracil, 5-fluorodeoxyuridine, bromodeoxyuridine, cytosine arabinoside, iododeoxyuridine, 5-trifluoromethyldeoxyuridine, 6-azauridine, 6-azauridine triacetate, azarabine *Folic acid antagonists:* Methotrexate
ANTIBIOTICS	Actinomycin C and D, puromycin, mitomycin C, rubidomycin, bleomycin, thiamphenicol, chloramphenicol, adriamycin, rifampicin, distamycin A, alanosin, ovalacin
HORMONES	Prednisone, prednisolone, oestrogens, melengestrol acetate, medroxyprogesterone, the Pill, hydrocortisone, progesterone, 17α-oestradiol, 5-α-dihydrotestosterone
ENZYMES	L-asparaginase, L-glutaminase, papain, adenosine deaminase inhibitors
VINCA ALKALOIDS	Vincristine, vinblastine
METHYL HYDRAZINE	Procarbazine
ANTIFUNGAL AGENTS	Griseofulvin
ANTI-INFLAMMATORY AGENTS	Aspirin, indomethacin, phenylbutazone, gold salts, aldoferac, penicillamine, colchicine, phenylene dialkane carboxylic acid
IMIDAZOLE AND BENZIMIDAZOLES	Miconazole, mebendazole, nocodazole, frentizole, DTIC, oxibendazole, parbendazole, albendazole, cambendazole, cyclobendazole, econazole, flubendazole, fenbendazole, ketoconazole, niridazole, metronidazole, clotrimazole, tinidazole
OTHER KNOWN IMMUNOSUPPRESSANTS	Serotonin, 5-hydroxytryptophan, iproniazid, hydroxyurea, diphenylhydantoin, phenobarbital, chlorpromazine, valium, halothane, chloroquine, cycloheximide cinnamates, bredinin, heparin, dicoumarol, α-carrageenan, cyproheptadine, prostaglandin E, tilorone, oxisaran, fentirin, linoleic acid, concanavalin A, cigarette smoke, cinanserin, disodium chromoglycate, quinine sulphate, 9-tetrahydrocannabinol, ethylenimine, epichlorohydrin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), di-n-octyltindichloride (DOTC), di-n-butyltindichloride (DBTC), polychlorinated biphenyls, cyclic AMP, dioxane, 6-(2,4-dinitrophenyl)-mercaptopurine, histamine, lidocaine, lindane, chondroitin sulphate

to some extent, the expression of an immune response without necessarily modifying the specific lymphocyte sensitization which underlies it. The anti-inflammatory agents indomethacin and aspirin which inhibit prostaglandin synthesis are thought to be mildly immunosuppressive but in all probability it is merely the inflammatory responses that they reduce. Steroids are both anti-inflammatory and immunosuppressive and this will be discussed later. Antihistamines are weakly immunosuppressive[11] and inhibitors of histidine decarboxylase have been shown to prolong graft survival in animals[12]. The H-2 antagonist cimetidine has been reported to do the same[13]. The immunosuppressive effect of cimetidine is extremely weak however and paradoxically cimetidine behaves as an immunostimulant *in vitro*[14,15]. Fibrin deposition and thrombosis in small arteries are common

features of rejection especially when there is antibody-mediated damage. Unfortunately anticoagulants have not been helpful in preventing this and in one clinical trial there was no benefit to a group of patients who received warfarin in addition to conventional immunosuppressive drugs[16]. Anti-platelet agents have looked more promising, for cyproheptadine has been found to markedly prolong the survival of kidney allografts in rats[17] and dogs[18]. Nonetheless no benefit was seen in a controlled clinical trial of patients with renal transplants[19] and in another trial in which dipyridamole was administered with warfarin, there was again no worthwhile improvement in results[20].

IMMUNOSUPPRESSIVE COMPOUNDS

A vast range of different compounds seem capable of depressing the immune response and these are listed in Table 1. It has been particularly disappointing that so few of these have shown potential as immunosuppressive agents in man (Figure 3). The action of many has been to suppress lymphocyte reactivity *in vitro* and of those that were capable of prolonging graft survival in animals in addition, just a handful have been found to be safe and effective in man. It is not intended in this chapter to discuss in great detail the properties of most of these compounds since many excellent reviews already exist[21–25], rather I would wish to concentrate on those drugs that have proved useful as immunosuppressive agents in man.

Anti-metabolites

These compounds interfere with protein synthesis by competing for and blocking specific receptors. They include the purine antagonist 6-mercaptopurine and azathioprine, the pyrimidine antagonist 5-fluorouracil, cytosine arabinoside, and the folic acid antagonist methotrexate. Since these agents are cycle specific and only effective against proliferating cells, they are most effective when given after, rather than before, the exposure to antigen.

6-Mercaptopurine and azathioprine

6-Mercaptopurine is an analogue of the purine base hypoxanthine in which the 6-hydroxyl group has been replaced by a thiol group. Azathioprine is the same compound with an imidazol group attached to the sulphur atom. It is following ingestion and for this reason the activities of the two compounds are largely the same. Nonetheless, various differences have been described in the actions of the two compounds and these have been summarized by Berenbaum[25]. During the breakdown of 6-mercaptopurine thioinosinic acid is produced which competes with its analogue inosinic acid for the enzyme which converts inosinic acid to xanthylic acid. This latter step is important

Figure 3 The clinical usefulness of immunosuppressive compounds

in the synthesis of DNA, and its inhibition profoundly affects RNA synthesis as well. All immune responses requiring cell proliferation may be inhibited including antibody production, graft rejection and the induction of autoallergic disease. Azathioprine and 6-mercaptopurine also exert a non-specific, anti-inflammatory effect but this is probably not an important part of its immunosuppressive action. As has been mentioned previously the optimum time for administering these drugs is after exposure to antigen and it has been shown that antibody production in man is affected very little if they are given before[26]. Nonetheless 'pretreatment' with azathioprine has

been shown to be effective in prolonging renal transplant survival in dogs[4] and as a result some transplant centres elect to start treatment a few days before transplantation in those patients who are planned to receive a kidney from a living relative. Azathioprine and 6-mercaptopurine have been shown to be capable of prolonging the survival of organ allografts in many experimental animals[22] although the effect varies considerably between species. Rats for example are affected very little by these drugs. Even in human organ transplantation in those patients who are planned to receive a kidney from a living relative. Azathioprine and 6-mercaptopurine have been shown to be capable of prolonging the survival of organ allografts in many experimental animals[22] although the effect varies considerably between species. Rats for example are affected very little by these drugs. Even in human organ transplantation azathioprine is rather ineffective on its own. This was the practice in some kidney transplant centres in the past, but graft survival was on the whole rather poor[27]. Kreis *et al.* described a series of 54 patients in whom only azathioprine was administered after transplantation[28]. Because of a high incidence of early renal failure episodes, 88% of these patients subsequently received steroids during the first week although not all these episodes were likely to have been due to rejection. 6-mercaptopurine and azathioprine exert their main toxic effects on the bone marrow to cause leukopenia, thrombocytopenia and occasionally anaemia. Approximately 20% of kidney transplant patients experience leukopenic episodes, the frequency of which are related to the dose of azathioprine given as well as the degree of function of the transplant[29]. Fortunately the bone marrow usually recovers quickly when the drug is withdrawn or the dosage reduced. Azathioprine is more toxic when administered with allopurinol since the degredation of azathioprine is blocked by the drug. It has been suggested that co-trimoxazole also increases the toxicity of azathioprine[30], but a controlled trial has shown this not to be so[31]. Very occasionally azathioprine can cause liver dysfunction and when this occurs it is common practice to substitute cyclophosphamide for azathioprine.

Methotrexate

Methotrexate is an analogue of folic acid in which a methyl and amino group respectively replace a hydrogen atom and a hydroxyl group. It binds to the enzyme folic reductase which has the effect of blocking the recycling of folic acid derivatives. Since these derivatives are involved in the conversion of deoxyuridine to thymidine, DNA synthesis and cell proliferation are impaired.

Apart from its immunosuppressive activity, the drug is also an inhibitor of inflammation[32] due to the way it can block responses to histamine and other mediators of inflammation.

Like azathioprine, methotrexate is active against dividing cells and is