Immunosuppressive agents, with special reference to antilymphocytic serum

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1. Introduction

'Immunosuppressive agents' are precisely what their name implies: agents that weaken or abolish the immunological response. The term itself promises more than any agent has in fact achieved. Strictly speaking, an immunosuppressive drug or treatment should be one that inhibits the immune response and no other. A central theme of this lecture is that only antilymphocytic serum (hereafter ALS) comes anywhere near fulfilling the requirements of this definition. The immunosuppressive action of all other agents in common use is a byproduct of some much more general toxic or inhibitory influence which happens to affect, amongst many others, the cells that transact the immunological response. For this reason immunosuppressive agents have not yet begun to do for immunology what specific metabolic inhibitors have done for the analysis of cellular metabolism—to resolve a complex biological performance into separate episodes or cellular events. Our knowledge of how they work is purely empirical; it has been pieced together from the evidence of practical experience, rather than founded on a prior theoretical understanding of how they work.

Immunosuppressive agents owe their importance to the sheer pressure of medical necessity. Diseases or disabilities that are due to immunological failure or insufficiency—e.g. to a congenital insufficiency of blood proteins of the class to which antibodies belong—are less common and less perplexing than those which can be attributed to a miscarriage or abnormal manifestation of the immunological response. Hay fever, asthma, urticaria and the allergies generally, including drug and bacterial allergies; anaphylaxis and serum sickness; haemolytic disease of the newborn; blood transfusion incompatibilities and that rather different form of incompatibility which prohibits the grafting of tissues from one individual to another; the so-called 'auto-immune' diseases, whether primary or secondary, including auto-immune thyroiditis and some of the so-called collagen diseases—all these are, to a greater or lesser degree, miscarriages or misadventures of the immunological response. There can therefore be no question of the strength of the practical incentive to bringing the immunological response under control.
Any complete or systematic treatment of the inhibition of the immune response should begin with the use of antigen and antibody as immunosuppressive agents. To anyone but an immunologist such an enterprise sounds merely paradoxical: surely antigens are, by definition, substances that excite immunity and antibodies the agents that give it effect? It has in fact been known for about twenty years that an antigen can be administered in such a form or dosage, or by such a route or at such an age that, instead of exciting an immune response, it withdraws that response from the organism’s repertoire. The antigen now not only fails to excite immunity, but affects the organism in such a way that it no longer responds to the antigen even when, on some later occasion, it is presented in a form that is known to be immunogenic. This is the phenomenon of ‘immunological tolerance’ or ‘immunological paralysis’. Thanks mainly to the analyses of D. W. Dresser and N. A. Mitchison (Dresser & Mitchison 1968) the term ‘antigen’ is now used to refer to substances that are either immunogenic or ‘tolerogenic’ or ‘paralytogenic’ (equivalent terms whose ugliness or etymological malformation is fully in keeping with the rest of the immunological vocabulary).

It has been known even longer that if a particular antibody is passively injected into an animal before the antigen that would otherwise have excited its formation, the organism’s active response to the antigen may be weakened or delayed. In the context of transplantation immunity this phenomenon is known as ‘enhancement’ (Kaliss 1958, 1965); it is a complex in nature, and no interpretation in terms of an action on afferent, central or efferent sectors of the immune response is singly sufficient.*

Although the ultimate goal of all immunosuppressive procedures should be to achieve a state of immunological tolerance (see below), I shall not count antigen and antibody as immunosuppressive agents for the purpose of this lecture. Generally speaking, the induction of tolerance in adult animals—i.e. the ad hoc induction of tolerance when the occasion calls for it—depends on the combined use of antigen and immunosuppressive agents in the conventional sense. This generalization applies with special force to transplantation immunity, with which I shall be specially concerned. Only in ‘weak’ histocompatibility systems—those in which the donors of grafts are not far removed from their recipients in terms of antigenic make-up—will the administration of antigen alone, in realistic dosages, induce a state of tolerance (Medawar 1963).†

Immunosuppressive agents are so various in character and diverse in their mode of action that no rational classification is yet possible. This lecture is not

* For the combined use of specific antigen and specific antibody to induce immunological non-reactivity of a kind ostensibly similar to immunological tolerance, refer to the striking observations of Axelrad & Rowley (1968) and Stuart, Saitoh & Fitch (1968).

† An important exception to this rule of thumb may have to be made in respect of observations such as Owen’s (1968; see also Owen, Slome & Waterston 1968). On the face of it, the weakening of the immune response to homografts and even heterografts brought about by the repeated injection of very low doses of liver extracts looks like a realization, in terms of grafting, of Mitchison’s induction of tolerance with bovine serum albumin in a low
intended to be either systematic or exhaustive, and to keep it within reasonable bounds I shall confine my attention to immunosuppressive agents of three kinds: antiproliferative and cytotoxic drugs or physical treatments; steroids of the cortisol family (e.g. prednisone and hydrocortisone itself); and antilymphocyte serum.* As already indicated, I shall confine my attention almost exclusively to transplantation immunity.

2. Cytotoxic and antiproliferative agents

This highly heterogeneous category of immunosuppressive agents includes ionizing radiations, DNA base analogues, biological alkylating agents, folic acid antagonists, methylhydrazine derivatives, and miscellaneous antibiotics.

(1) Ionizing radiations, especially X-irradiation, the immunosuppressive properties of which have been recognized for about 60 years. As a method of controlling the immune response in clinical practice, ionizing radiation has lost ground in recent years in spite of certain advantages (e.g. the degree to which it lends itself to precise local application).

(2) Purine and pyrimidine analogues. The former class, considered as immunosuppressive agents, are now dominated by 6-mercaptopurine and the derivative azathioprine† of which it represents the active ingredient. The latter class, less generally useful for immunosuppressive purposes, is exemplified by, for example, 5-bromouracil, a thymine analogue, and its deoxyribonucleoside, an analogue of thymidine.

(3) Biological alkylating agents, a large and internally diverse group including nitrogen and (for their historic interest) sulphur mustards, but better represented by a variety of important cancer chemotherapeutic drugs, especially cyclophosphamide and chlorambucil.

(4) Folic acid antagonists, especially aminopterin and a closely related methyl derivative methotrexate, now with a history of more than 20 years of use in the treatment of acute lymphoblastic leukaemia.

zone of dosage (1964; see also Ada & Parish 1968). At present, however, other explanations have not been ruled out. One of the ambitions of transplantation research is to make homografts acceptable by applying the principle of 'low zone tolerance', and there are many otherwise unexplained phenomena in the literature of transplantation which look as if they might yield to an interpretation in these terms.

* The properties and, so far as they are known, the modes of action of immunosuppressive agents generally, have been discussed by Schwartz (1965), Humphrey (1965), Makinodan, Albright, Perkins & Nettesheim (1965), Berenbaum (1965). Gabrielsen & Good (1967); see also WHO Conference on use of antimetabolites in disease associated with abnormal immune responses (in 5th International Symposium on Immunopathology (eds. P. A. Miescher and P. Grabar), Basel: Schwabe 1968). In addition, special reference should be made to the immunosuppressive possibilities of ribonucleases (see Boylston, Mowbray & Ackermann 1968; Mowbray 1963; Mowbray & Hargrave 1966).

† The following synonymy of commonly used immunosuppressive agents may be useful: azathioprine = Imuran, BW 57322; methotrexate = Amethopterin; aminopterin = antifolic acid; cyclophosphamide = Endoxan, Cytoxan; chlorambucil = Leukeran.
(5) Methylhydrazine derivatives, introduced by Bollag & Grunberg (1963) and applied to the study of transplantation immunity by Floersheim (1965, 1967).

(6) A miscellaneous collection of ‘natural’ products, especially antibiotics and plant alkaloids, which have shown various degrees of promise in cancer chemotherapy; e.g. actinomycin, mitomycin, puromycin, and chloramphenicol; and the Vinca alkaloids extracted from the Madagascar periwinkle.

The six classes of agent listed above are immunosuppressive only, so to speak, by second intention; they are not immunosuppressive in any acceptably specific sense. They were, without exception, introduced into medicine or experimental biology for some purpose other than their action on the immune response (almost always for cancer chemotherapy); and, without exception, their power to inhibit the immune response is a byproduct of some much more general toxic action on cells. None of them exercises its action on any cellular process that is distinctive of the immunological response.

This last point was first made clear by Brent’s and my lengthy analysis of the normal lymphocyte transfer reaction, a reaction which (inspite of many drawbacks) has advantages not shared by other test systems, one being the insight it gives into the early and characteristically immunological episodes of the response to antigens, before the response has been amplified by cell division or by some other equivalent means (Brent & Medawar 1966a, b; summary, 1967).

A normal lymphocyte transfer (NLT) reaction is aroused when lymphoid cells from one normal adult guinea pig are injected into the skin of another, and it takes the form of a small inflamed spot which is perceptible 5 to 8 h after injection and reaches a first peak at about 24 h. Genetical analysis along what are now quite conventional lines shows that the inflammatory lesion is the outward sign of a homograft reaction in reverse, i.e. a reaction of the injected cells against the tissues of the animal into which they are injected. From all that is known of the tempo of lymphocyte activation, we can be reasonably sure that cell division plays very little part in the early episodes of the NLT reaction; the inflammation we observe is therefore taken to be the outward sign of the first engagement of lymphoid cells with antigenic matter, before cell division or some other unknown process amplifies the immunological response from a local to a systemic scale.

The effect of conventional immunosuppressive agents on the NLT reaction in these early stages is easy to summarize: they have no discernible effect; and this generalization holds good whether the immunosuppressive agents are applied to the lymphoid cell donor before the cells are removed for transfer, to the cells in vitro on the way from donor to recipient, or to the recipient itself. Evidently they do not interfere with any peculiarly immunological event that may accompany the confrontation of a lymphoid cell with antigen.

When the NLT reaction is carried out in the way described above, it normally fades out between the second and third days. This is because the transferred cells themselves excite a conventional immune response from the recipient which causes them to be destroyed. If the recipient is exposed to from 600 r to 1500 r
whole-body irradiation a day before cell transfer, the recipient can no longer
counter-attack the injected cells, and the NLT reaction therefore flares up from
the third or fourth day after transfer to form a severe inflammatory lesion to
which cell division presumably makes an important contribution. All conventional
immunosuppressive agents weaken or abolish this second inflammatory episode;
it is the *amplification* of the immune response they suppress, not the response
itself.

Exactly the same applies to a specially informative variant of the NLT
reaction in which the lymphoid cells transferred from donor to recipient have
been taken from a guinea pig that has been deliberately presensitized against the
tissues of their future recipient. It has been shown that the reaction excited by
sensitized cells is merely a scaled-up version of the NLT reaction, with exactly
the same time-course and quantitative pattern, but transposed into a higher key.
Thus the essential immunological performance of 'presensitized' cells—more
correctly, of cells in sensitized lymphoid populations—is no more vulnerable
than that of 'normal' lymphoid cells to the action of immunosuppressive
agents.

The inference we have drawn from the study of NLT reactions is that conven­
tional immunosuppressive agents are in no sense specific inhibitors of the immune
response; lymphoid cells are vulnerable to their action only during the ampliative
stage of the immune response, and then only by virtue of properties they have
in common with other dividing cells.

Although future analysis will certainly uncover a fundamental affinity between
them, it is at present operationally sound to distinguish between immunological
reactions mediated through circulating antibodies and those that are transacted
by the direct engagement of antigens with lymphoid cells.* Most forms of anti­
bacterial and anti-viral immunity belong to the former class, and transplantation
immunity belongs to the latter. A second generalization that probably holds
good of all immunosuppressive agents imported into immunology from cancer
chemotherapy is that they inhibit humoral immunity more strongly than they
inhibit cell mediated responses. This is just what we should expect if their action
is essentially anti-proliferative in character, for humoral (unlike cellular) immunity
depends unconditionally upon a proliferative or ampliative episode if it is to
reach a systemic level of effectiveness. This property has serious clinical conse­
quences. When conventional immunosuppressive agents are used to control the
rejection of organ homografts, the surgeon is walking a tightrope: give too much,
and the patient will be deprived of most of his natural defences against infectious
illness; give too little, and the graft will be thrown off.

In practice, of course, the danger is no longer so great as I have represented
it to be. In the most favourable cases, an immunosuppressive regimen need not

* For a succinct recent review, see 'Delayed type hypersensitivity: Specific cell mediated
immunity', *Br. med. Bull.* 23, no. 1, 1967; see also the specially authoritative reviews by
be continued indefinitely (though drugs may continue to be given in token dosages). During their period of administration these immunosuppressive agents will tend to abet the induction of immunological tolerance, i.e. a specific non-reactivity which in effect cancels the immunogenic action of the graft; and, what is probably more important in the context of organ transplantation, they will give time for the graft to undergo some process of adaptation to its host.* Moreover, the secondary or collateral damage caused by immunosuppressive agents—and notoriously by X-irradiation, which destroys blood-forming cells—must not be exaggerated beyond reason. There is no reason why conventional agents should not be found which are to a fairly high degree specific to the lymphoid system; and to whatever degree they are so, they must also be specifically immunosuppressive, for the lymphoid system has no known function other than to transact immune responses. Azathioprine, introduced into transplantation practice by Professor R. Y. Calne (Calne 1967), answers reasonably well to this requirement, and it is the combined use of azathioprine with adrenal corticosteroids that has led to the quite remarkable success of modern kidney transplantation when donors and recipients are antigenically well matched.

3. Steroids

Adrenal corticosteroids of the cortisone family (glucocorticoids) have certain distinctive immunosuppressive properties, not all of which have yet been turned to clinical use. The most striking, and the one that distinguishes them most sharply from the agents discussed in §2, is the power of cortisone to annul a pre-existing state of sensitivity if given over a sufficient length of time. This property was discovered and analysed by Krohn (1954a), and rediscovered independently by transplantation surgeons; steroids such as prednisone are now in fact used in clinical practice to reverse an incipient process of rejection; i.e. to maintain, rather than to institute, a state of non-reactivity, because as primary immunosuppressive agents the glucocorticoids are rather weak (Billingham, Krohn & Medawar 1951a; Krohn 1954b; Medawar & Sparrow 1956). A second property, which will come into prominence when skin is for the first time grafted from one human being to another with the intention that it should remain alive, is that cortisone can exercise its action locally on a skin graft, and at dosages which have little effect when given by a systemic route (Billingham, Krohn & Medawar 1951b). (Presumably this effect will be exercised much more strongly by fluoro-substituted prednisone derivatives administered in an oily base.) A third property

* The importance of adaptation was first insisted upon by Professor M. F. A. Woodruff (see Woodruff 1960). Many experimental immunologists including myself discounted the phenomenon, because it seemed to imply that the cells of the graft underwent an antigenic adaptation. The question of antigenic ‘modulation’ in metazoan cells is too big to be gone into here; it need only be said that ‘adaptation’ in Woodruff’s sense probably comprehends a whole variety of processes (antibody-coating, endothelial relining, enhancement, etc.) which might help to shield a graft from the immunological opposition of its host.
which deserves special mention is the remarkable power of hydrocortisone to prolong the state of non-reactivity engendered by treatment with antilymphocytic serum (Levey & Medawar 1966; see below, and figure 1).

It will not rank as a feat of clairvoyance to suggest that, in addition to their known collateral properties (e.g. their anti-inflammatory action), the glucocorticoids will be found to affect the recruitment or maturation of lymphocytes or, alternatively, their release into or recapture from the peripheral circulation. Adrenal cortical hormones or the lack of them were known to have a profound effect on the lymphoid system long before the immunological performance of lymphoid cells was fully recognized, and the involution of the thymus of weanling rats was used as a method of assay for corticotrophin and cortisone long before the work of Miller and others began to uncover its immunological function (Loraine & Bell 1966). Unfortunately the attempts made 15 or 20 years ago to make sense of the functional relationship between the adrenals and the lymphoid system must be judged to have foundered; we knew almost nothing in those days about the immunological functions of the lymphoid system as a whole, so it would have been almost a miracle if the enterprise had not failed. It is now high time that the question was reopened in the light of fundamental new discoveries about the population dynamics of lymphoid cells. Among the most important are these:

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**Figure 1.** Survival of A-strain tail skin homografts on adult male CBA mice. All mice received ALS 0.5 ml + 3 × 0.25 ml, starting 2 days and ending 11 days after grafting, and group B received no other treatment. Group A received hydrocortisone acetate 0.5 mg/week from the 15th to the 150th day after grafting, and group C were adrenalectomized 15 days after grafting. The injection of hydrocortisone greatly prolongs the effects of ALS; adrenalectomy, if anything, curtails it (Levey & Medawar 1966).
(a) The proof by Gowans (1957, 1959, 1966) of the continuous recirculation of small lymphocytes, with the corollary that some lymphocytes at least are not short-lived cells.

(b) The distinction drawn by Everett and his colleagues (Caffrey, Rieke & Everett 1962; Everett, Caffrey & Rieke 1964) between long-lived and short-lived populations of lymphocytes, the former now known to make up the bulk of the recirculating pool.

(c) A highly important complex of discoveries relating to the thymus: the role of the thymus in the development* and continued performance† of immunological functions, especially those concerned with reactions against homografts; the recognition of a specifically thymus-dependent population of lymphocytes which is also the long-lived moiety (Rieke & Schwarz 1966); and the discovery that the thymus-dependent population is anatomically associated with the paracortical areas of lymph nodes (Parrott, de Sousa & East 1966), i.e. the areas specially implicated in homograft reactions (Seethorne & McGregor 1965) and delayed-type hypersensitivities (Oort & Turk 1965) that are stations along the pathway of lymphocytic circulation (Gowans & Knight 1964).

(d) More recently, evidence has come to light which suggests that humoral antibody formation is a cooperative venture between thymus-derived and marrow-derived cells, the former perhaps responsible for antigenic recognition, the latter for the synthesis of immunoglobulin.‡

(e) Some mention should also be made of the concept of ‘peripheral sensitization’ which has come to the fore in a number of recent experimental studies.§ The underlying idea (Medawar 1958, 1965) is that the antigens which give to delayed hypersensitivity reactions, including the antigens of solid tissue grafts, are characteristically sessile or locally anchored, so that they sensitize through engagement with blood-borne lymphocytes and not through a percolation of cell-free antigenic matter into the regional nodes. If this interpretation is correct, it follows that quite short-lived stimuli which release lymphocytes into or, alternatively, withhold them from the peripheral circulation could have a profound effect on the inception of sensitivity. It may be through some such mechanism as this that steroids exercise some of their direct and indirect effects on cell-mediated immunities.

A systematic analysis of adrenal-thymic-lymphoid relationships stands a


† Miller (1962b); Claman & Talmage (1963); Miller, Doak & Cross (1963); Taylor (1965); Metcalfe (1965); Miller (1965).

‡ Claman, Chaperon & Triplett (1966a, b); Davies, Leuchars, Wallis, Marchant & Elliott (1967); Mitchell & Miller (1968); Taylor (1968).

§ Strober & Gowans (1965); Brent & Medawar (1967); Barker & Billingham (1968); Macher & Chase (1969a, b). For a critical discussion, see Wilson & Billingham (1967).
reasonable chance of success if it is carried out with this newer knowledge of the population dynamics of lymphoid cells in mind, but it is not at all likely steroid hormones will be found to exercise their action in only one way.*

4. Antilymphocytic Serum

'Antilymphocytic serum' (ALS) stands in a class apart—in a class of which it is, in fact, the only known member. An ALS was first devised by Elie Metchnikoff in 1899, and first used as a specific immunosuppressive agent by J. H. Humphrey and his colleagues at the National Institute for Medical Research (cf. Inderbitzin 1956). Modern research on the subject was, however, inspired by the striking results reported by Woodruff & Anderson (1963), followed shortly after by those of Gray, Monaco & Russell (1964) at the Massachusetts General Hospital. Today twenty or thirty research groups throughout the world are busy with the problems raised by its preparation and mode of action, and ALS is being prepared on a scale extending all the way from a major commercial enterprise to a cottage industry.

ALS is normally a 'heterologous' antiserum, i.e. is raised in one species against lymphoid cells taken from another. An ALS intended for use in human beings will be raised by injecting human lymphoid cells into, for example, horses, and for use in mice by injecting murine lymphoid cells into, for example, rabbits. The active principle is an antibody or complex of antibodies directed against antigens present in (but not necessarily peculiar to) lymphoid tissue, and the consensus of opinion is that it acts through a complement-dependent mechanism on lymphocytes themselves. Containing as it does a complex of foreign proteins, ALS is strongly antigenic—much more antigenic, in fact, than the normal serum of the animal in which it was prepared—but Lance & Dresser (1967) have shown that its immunosuppressive action in no way depends upon its power to excite immunity.

Against the background of what has been said about conventional immunosuppressive agents (§2), the distinctive properties of ALS are quite easy to summarize. In what follows, I shall be drawing mainly upon the evidence of our work at Mill Hill.

(1) ALS is an immunosuppressive agent 'by first intention'. At present there is no evidence that any other systemic action necessarily accompanies its inhibition of the immune response. Among all the immunosuppressive agents that have been investigated with that property in mind, only ALS extinguishes the NLT reaction (Levey & Medawar 1966, and see above). As ordinarily prepared, ALS may of course have damaging collateral actions, e.g. by virtue of its own antigenicity (which can be avoided) or through contamination by toxic antibodies.

* Due weight should be given to the possibility that steroids interfere with the cytotoxic action that presumably represents the final common pathway of all cell-mediated immunities: refer to Rosenau & Moon (1962), and compare Weissmann & Dingle (1961), Weissmann & Thomas (1964).
irrelevant to its action (which either should not be allowed to form in the first place or should be removed).

(2) At dosages sufficient and necessary to suspend the reaction against grafts of foreign tissue, ALS has comparatively little effect on humoral immunity, e.g. on the protective antibody responses excited by bacterial or viral antigens. ALS, like the agents discussed in §2, is therefore a differential inhibitor of cellular and humoral immunity, but in precisely the opposite sense: it acts more effectively on the cellular than on the humoral response. This point has repeatedly been emphasized at the level of clinical observation (cf. Levey & Medawar 1967b) and has been on a concrete basis by the observations of Lance (1967b, 1969), Lance & Batchelor (1968) and Möller & Zukoski (1968).* The differential inhibition of homograft community has very practical implications, for it means that foreign tissue grafts may be kept alive without prostrating the orthodox defensive immunological reactions of their hosts.

(3) Animals which have already rejected a foreign graft will reject a second graft from an antigenically related source very much more quickly. This is the so-called ‘second-set response’ (not to be confused with the ‘secondary response’ of conventional immunology). ALS does not merely weaken the second-set response: at high dosage levels it can restore the animal to the level of reactivity it would have enjoyed if it had never been exposed to a foreign tissue before (Levey & Medawar 1966; Lance 1968b). There are even hints that presensitized mice may be more vulnerable than ‘virgin’ mice to the immunosuppressive action of high doses of ALS (Lance & Medawar 1969, and see figure 2).

(4) ALS is less sensitive than conventional immunosuppressive agents to antigenic differences between the donor and the recipient of a graft (Levey & Medawar 1966). Everyone who studies transplantation immunity knows that some grafting combinations are immunologically easy, and others difficult, in accordance with the strength of the reactions that the grafts excite. Conventional immunosuppressive agents work much better when the grafts are immunologically easy. With ALS it makes very much less difference; grafts in easy combinations last for a shorter time, and in difficult combinations much longer, than one would have predicted with only the experience of conventional immunosuppressive agents to go on. The effect of this is that ALS now brings it within the realm of practical possibility to use xenografts (heterografts), in which the donor and recipient belong to different species. ALS treatment alone will sustain human skin on mice (Lance & Medawar 1968), and it is even possible to secure tolerance of rat grafts in adult mice (Lance, Levey, Medawar & Ruszkiewicz 1969). These experiments show—what could not otherwise have been assumed—that the opposition to some heterografts in some donor-recipient combinations is wholly immunological in nature.

* This view has been questioned by James & Anderson (1967, 1968). In the light of modern ideas about how ALS works (below, p. 166) it may turn out to be more exact to say that ALS discriminates between thymus-dependent and thymus-independent immune responses rather than between cellular and humoral immunity.
ALS shares at least two important properties with other immunosuppressive agents: the power to abet the induction of immunological tolerance (Monaco, Wood & Russell 1966; Lance & Medawar 1969, and see figure 2); and the fact that its action may be potentiated and prolonged by the removal of the thymus gland (Monaco, Wood & Russell 1965; Jeejeebhoy 1965). In the induction of tolerance, as Lance and I have shown, the very non-toxicity of ALS puts it at something of a disadvantage vis-à-vis, for example, irradiation. The administration of ALS inflicts no lasting damage on the lymphoid system of the host; the descendants of the donor lymphoid cells that were used to induce tolerance do not therefore enjoy a permanent selective advantage. Sooner or later they are ousted by regenerating host cells, and normal reactivity returns. The combination of ALS with doses of irradiation too small to be more than trivially effective in themselves has a dramatic effect, however; by this means we have secured average graft survival times well above 400 days. The effect of whole-body irradiation shortly before the injection of the tolerance-conferring dose of cells is no doubt partly to make room for the proliferation of donor cells, but partly also, perhaps, to affect the recipient's lymphoid system in such a way that the donor cells and their descendants retain a permanent selective advantage.

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**Figure 2.** $A \rightarrow CBA$ grafts, as figure 1: illustrating the induction of tolerance in normal mice (solid lines) and in mice which had been presensitized by the prior rejection of an $A$-strain graft (broken lines). All mice received a total of 2.3 ml ALS beginning 4 days before and ending 8 days after grafting; in addition, to induce tolerance, two sets of mice (as indicated) received an intravenous injection of $10^8$ ($CBA \times A$) splenic cells on the 14th day after grafting. The cellular injection prolonged the median survival time of the grafts from about 40 to about 140 days in the normal (non-sensitized) mice. Note that some measure of tolerance is also induced in presensitized mice, and that ALS, acting alone, was on this occasion more effective in presensitized mice than in normal (Lance & Medawar 1969).
Figures 1 to 3 with their respective captions illustrate some of the functional properties of ALS discussed above. Figure 1 is an up-to-date record of the experiment that was still in progress when published by Levey & Medawar in 1966, and figures 2 and 3 represent the later stages of experiments already described by Lance & Medawar (1969). All three figures are cast in the form of life-tables, i.e. of day-to-day records of the number of homografts surviving on mice exposed to ALS or to various combinations of ALS with other treatments.

![Figure 3](image)

**Figure 3.** $A \rightarrow CBA$ grafts, as in figures 1 and 2. Induction of tolerance, illustrating the potentiation of ALS by sublethal whole-body X-irradiation and by cyclophosphamide. All mice received 2.25 ml ALS in 7 pulses ending 3 days after grafting, followed on the 7th day by an intravenous injection of $30 \times 10^6$ splenic cells from ALS-treated $A$-strain donors. One group received no other treatment; a second was exposed to 450 r whole-body irradiation on day 6, and a third received 50 mg/kg cyclophosphamide on the 4th and 6th days. The median survival time of the grafts in the irradiated mice has not been reached after one year (Lance & Medawar 1969).

There is now fairly general agreement about the way ALS works. At the First International Congress of the Transplantation Society in 1967, Lance (see Lance 1968a) proposed that ALS acts by a selective depletion of lymphocytes belonging to the recirculating, long-lived and thymus-dependent pool, and this view has been upheld or independently arrived at by several authoritative groups of workers, reasoning from a mainly histopathological* or mainly ‘population dynamical’† point of view. Three independent lines of evidence (Levey & Medawar

* Turk & Willoughby (1967); Parrott (1967); Taub (1968); Taub & Lance (1968a).
† Martin & Miller (1967, 1968); Leuchars, Wallis & Davies (1968); Denman, Denman & Embling (1968); Denman, Denman & Holborow (1968); Denman & Frenkel (1968); Tyler, Everett & Schwarz (1968).
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1967a; Taub & Lance 1968b) point to the peripheral lymphocyte as the immediate
target of ALS (as opposed to lymphocytes housed within the central lymphoid
organs). Precisely what ALS does to the cells it acts upon has been the subject
of a lot of speculation, but since its action is complement-dependent (Reithmüller
1967; Jooste, Lance, Levey, Medawar, Ruszkiewicz, Sharman & Taub 1968) there
is now little inducement to believe in the idea of ‘blindfolding’ or sterile activation
(Levey & Medawar 1967a). Presumably ALS acts by immune cytolysis or by
opsonization (Greaves, Tursi, Playfair, Torrigiani, Zamir & Roitt 1969).

The formidable difficulties involved in scaling up the production of ALS from
a laboratory level to the volume and degree of purity required for clinical trials
in human beings have not yet been fully overcome, but I should like to express
my personal appreciation of the tireless and determined way in which the Wellcome
Research Laboratories are facing them.

Although the properties of ALS approximate to those which we might specify
a priori for an inhibitor of cell-mediated immune responses, ALS itself has too
many inherent disadvantages to be regarded as the definitive solution of the
problems an ideal agent must overcome. The importance of ALS is to show that
immunosuppressive agents do exist, or can be devised, which suppress the cell-
mediated response without exercising a generalized toxic action. What ALS can
do well, newly devised synthetic agents will one day be found to do better, but
they will not be found unless they are looked for—and that means abandoning the
practice of raiding cancer chemotherapy for compounds chosen originally for
their antiproliferative properties, and not placing too much reliance on screening
systems based on the inhibition of the humoral antibody response. The normal
lymphocyte transfer reaction suggests itself as an alternative screening system
(see above, §2), but here too we must hope that even simpler tests can be devised.

Finally, it is worth emphasizing the very special reasons why the use of any
immunosuppressive agent should have as its goal the institution of immunological
tolerance, so that the non-reactive state outlives the immunosuppressive stimulus
and the organism’s immunological performance is specifically, not generally
impaired.

The first reason is that although the protective functions of cell-mediated
immune reactions are not yet fully understood, there is little doubt that they
contribute to the control of mycobacterial and at least some viral infections.

The second reason is this. We can now take it as firmly established that some
tumours, whether chemically or virally induced, excite a specific defensive
reaction which is functionally identical to that which leads to the identification
and rejection of homografts of skin. (It may one day come to be said that the
great importance of laboratory research on skin transplantation immunity was
to have provided most of the conceptual background and the methodology of the
analysis of tumour immunity.) The use of agents like ALS over long periods is
therefore open to the grave charge that it may weaken our natural defences against
malignant growths. This danger is raised in a specially acute form by ALS
because it is a specially effective immunosuppressive agent, not because it has specially undesirable properties of its own. A study of the consequences of lifelong or very lengthy administration of ALS should therefore stand high on the agenda of modern research on immunosuppressive agents. It may turn out that the dangers associated with the prolonged use of ALS have been overestimated, but to depreciate them would be unpardonable. If our worst fears are realized, the least ALS will have achieved will be to have provided the first really critical evidence of the function of cell-mediated immunity in the natural control of malignant disease.

References


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