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THE SELECTIVE ACTION OF ANTILYMPHOCYTE SERUM ON RECIRCULATING LYMPHOCYTES:

A REVIEW OF THE EVIDENCE AND ALTERNATIVES

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INTRODUCTION

The purpose of this article is to examine in summary six lines of evidence which support the hypothesis that ALS achieves its immunosuppressive effect through a selective depletion of the recirculating pool of lymphoctyes and discuss briefly alternative possibilities. When this hypothesis was first advanced (Lance, 1968a) it was based solely upon functional and morphological data. Since that time new facts have emerged which render the probability of being correct more likely.

No general review is intended here and the interested reader is referred to the several excellent ones already extant (Woodruff, 1967; James, 1967a, 1969; Medawar, 1968; Denman, 1969; Sell, 1969). Nor is it possible to acknowledge adequately all who have contributed to the formulation of the various hypotheses under discussion. Special mention, however, must be made of the observations of Levey & Medawar (1967b) who were the first to point out the particular efficacy of ALS in opposing reactions in the periphery, the elegant labelling studies of Denman (Denman & Frenkel, 1968b; Denman *et al.*, 1968a, b) and Taub (1969b, c) who showed that the residual population in ALS treated animals were predominantly rapid turning over, i.e. short lived lymphocytes; and studies by Martin & Miller (1967) and Leuchars, Wallis & Davies (1968) for indicating the selective effect on antigen sensitive cells.

SELECTIVE ACTION ON RECIRCULATING LYMPHOCYTES

1. *Functional*

ALS may depress both humoral and cell mediated immune reactions but is very much more effective when opposing the latter (Lance, 1968a, 1969a; Lance & Batchelor, 1968). Discernible prolongation of skin homograft survival in mice can be obtained even with

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extremely small doses, i.e. $\frac{1}{16}$ ml doses (Lance & Medawar, unpublished observations; Levey & Medawar, 1966a). An already established rejection process can be reversed (Levey & Medawar, 1966a). The second set response is rather easily opposed and with high doses immunological memory can be completely erased (Levey & Medawar, 1966b; Lance, 1968b). Furthermore, the efficacy of immunosuppression is little influenced by the nature of the antigenic differences to be bridged making it possible to carry out hetero-transplantation with an ease which would have been considered remarkable for homotransplants prior to the advent of ALS (Monaco *et al.*, 1966a; Levey & Medawar 1966b; Lance & Medawar 1968; Lance Levey & Medawar, 1969; Shaffer, 1969). With respect to humoral immunities, ALS will suppress the primary response but must be given prior to antigen and very small doses are ineffective (James, 1967b; Berenbaum, 1967; Lance, 1969d). Furthermore, the immunosuppressive effect of a given dose of ALS can be considerably offset or indeed completely overcome by boosting the dose of antigen (Lance, 1969d). An analogous situation has not been identified with respect to cell mediated processes (Lance & Medawar, 1969). The difference in susceptibility of these two types of reactions is vividly seen in sensitized animals where ALS is virtually impotent to offset the secondary humoral antibody response let alone impair immunological memory (Gray, Monaco & Russel, 1964; Monaco *et al.*, 1966a; Lance, 1967, 1968b, 1969a; James & Anderson, 1967).

The most striking evidence derives from parallel assays of cell mediated and humoral antibody responsiveness (Lance, 1968a; Lance & Batchelor, 1968). At a dosage level which completely inhibits rejection of skin homografts across an H–2 antigenic barrier the primary humoral antibody response to a variety of antigens remained intact. The differential susceptibility of these two types of immune responses in parallel study of sensitized animals has also been documented (Lance, 1969a). However, there is not universal agreement on this point (see James & Anderson, 1968; James, Pullar & James, 1969a; Monaco & Franco, 1969).

Recently it has become clear that a requirement for the humoral antibody response to certain antigens is co-operation between thymus and bone marrow derived cells (Claman, Chaperon & Triplett, 1966; Davies *et al.*, 1966, 1967; Miller & Mitchell, 1968). The studies of Martin & Miller (1968) with sheep red blood cells and Mitchison (1969) with bovine serum albumin clearly establish that ALS interferes with this response by a selective depression of the thymus derived component.

This dissociation of effect may well explain the observed resistance of ALS-treated animals to infection by common bacterial pathogens. Indeed this clinical observation first suggested that ALS might have a selective action (see Medawar, 1968). Support comes also from the studies of ALS and viral infections where the cellular response is markedly reduced while titres of circulating antibodies may not be at all affected (Hirsch & Murphy, 1968).

2. *Morphological*

Turk & Willoughby (1967), Parrott (1967), Lance (1968a) and Taub & Lance (1968b) have emphasized the selective morphological alterations found in ALS-treated animals. The characteristic triad consists of: depletion of small lymphocytes from the paracortical area (thymus dependent) of the lymph node with preservation of the true cortex, germinal centres and medullary areas; depletion of the small lymphocytes in the periarteriolar areas of the spleen with preservation of the follicular areas, and finally little, if any, alteration in the gross or histological appearance of the thymus. The changes in lymph nodes become

evident after even a single dose of ALS while those in the spleen are usually not seen until after several doses. After chronic administration of ALS, the depletion in the spleen and lymph nodes may be masked by a secondary hyperplasia and hypertrophy of the germinal centres, medullary and follicular zones (Taub & Lance, 1968b). This is most likely a response to immunization with foreign protein and can be considerably curtailed if ALS IgG is given to animal preparalysed with IgG. Sera which contain high titres of anti-red cell or other toxic antibodies may produce extensive and generalized depletion of lymphocytes from the spleen and lymph node and thymus. However, these changes are extraneous to the immunosuppressive action of antilymphocyte serum.

A good correlation exists between the functional and morphologic findings: namely the greatest morphologic changes are found in those areas most closely identified with cell mediated immune responsiveness while those areas associated with humoral antibody formation are relatively well preserved.

3. *The fate of the lymphocyte affected by ALS*

Taub & Lance (1968a) have made use of the fact that lymph node cells tend to home to lymphoid organs after i.v. injection into syngeneic recipients and the distribution can be easily quantitated by use of a Chromium 51 label. After exposure to ALS *in vitro* they lose this capacity and the bulk of radioactivity is recovered in the liver (see also Martin & Miller 1967). Lymphocytes introduced into the circulation of mice after ALS treatment are also prevented from reaching lymphoid organs in normal proportion and are cleared from the circulation by the liver. ALS given after the completion of migration, is very much less effective. Therefore, granted access to the lymphocytes ALS causes divagation of cells from lymphoid organs and into the liver.

Cells from animals chronically treated with ALS showed an altered pattern of migration: the uptake in lymph nodes was considerably reduced whereas that in the spleen was normal. This suggested that there had been a selective removal from the lymph node of cells belonging to the recirculating pool of mobilizable lymphocytes. (For the evidence documenting the propensity of recirculating lymphocytes to home to the lymph nodes see Lance & Taub, 1969).

4. *The natural history of the relevant antibody*

Antilymphocytic antibody is known to persist in recipients for relatively brief periods (Pichlmayr, Brendel & Zenker, 1967; Taub & Ruszkiewicz, personal communication), penetrating lymphoid tissue poorly (Hintz & Webber, 1956; Denman & Frenkel, 1968a).

By absorbing ALS IgG onto thymocyte membrane preparations and subsequently eluting with acid a subfraction is recovered (approximately 1% of the original IgG) which possesses all the characteristic properties of ALS but is very much more potent (Lance, 1969b). Coupling this enriched material with a radioactive label allows the fate of the antibody to be followed. Three chief points emerged from these studies relevant to the action of antilymphocyte serum. Although the half life of rabbit IgG in mice is of the order of 5–6 days, antilymphocytic antibody is eliminated from recipient mice extremely rapidly. Eighty per cent is cleared within 24 hr and by 72 hr only 5% of the label remains. Four hours after a subcutaneous injection, the highest amount of radioactivity (5%) is found in the blood stream. The liver and kidney also have high uptakes while the activity at any time in the thymus is extremely low. Lymph nodes and spleen collect very little radioactivity although on

a weight-for-weight basis a higher percentage of the label can be found in these organs than in, for instance, an equal quantity of skeletal muscle. The pattern of *in vivo* localization strongly suggests that some factor other than antigenic specificity must influence the distribution of antilymphocytic antibodies *in vivo*.

Autoradiographic studies revealed antibody coated lymphocytes in the circulation initially which were subsequently cleared from the blood stream. Labelled lymphocytes could be found after 4 hr, phagocytized by the Kupffer cells of the liver. Within lymphoid organs the pattern of grain distribution corresponded precisely to that of the traffic pattern of the recirculating cells. The highest grain concentrations were in the paracortical area of the lymph nodes while the cortex and medulla were very lightly labelled.

5. *Kinetic data*

A prompt and drastic fall in peripheral blood lymphocytes follows a single dose of ALS. Recovery occurs rather rapidly and the presence of large numbers of lymphocytes in the circulation when animals are still immunologically unresponsive seems difficult to reconcile with a mode of action requiring depletion of circulating lymphocytes. However, peripheral blood lymphocytes are known to be a heterogeneous population (Everett, Caffrey & Rieke, 1964). A prediction of this hypothesis is that the administration of ALS should be followed by a rapid decline in the output of cells from the thoracic duct, and that the numbers of cells in the thoracic duct compartment should be drastically reduced after a course of ALS treatment. Direct studies on the thoracic duct output in ALS-treated animals by Agnew (1968), Tyler, Everett & Schwarz, (1968) and Lance & Gowans (report in Lance, 1969c) show a rapid and profound fall in the output of small lymphocytes in the thoracic duct which persists at a time when the lymphocyte count in the peripheral blood has already recovered.

6. *Antigenic*

The possibility exists that the antigen theta may be a marker for thymus derived lymphocytes. This at least is the interpretation drawn by Schlesinger & Yron (1969) who found that the lymph node cells in ALS treated animals became refractory to the cytotoxic action of anti-theta but were fully responsive to anti-H-2 antisera. The reduction in the representation of theta antigen in the residual lymph node and spleen population after ALS treatment has been confirmed by Raff & Nehlsen (Raff, 1969 and personal communication).

Based upon the foregoing lines of evidence we contend that ALS achieves its effect by eliminating preferentially, albeit not exclusively, the population of long lived recirculating lymphocytes. The sequence of events is probably as follows:

After an injection of ALS relatively high titres of specific antibody are built up in the blood stream where they come in contact with and coat lymphocytes. These cell-antibody complexes bind complement which renders them susceptible to phagocytosis by the reticuloendothelial cells in the liver. (Some cells may be killed outright as the result of the direct action of antibody and complement.) As cells are removed from the blood stream, they are replaced by new members of the recirculating pool leaving the lymph nodes. This sequence occurs as long as the antibody titre remains above a certain critical level. Because the turnover time of the recirculating pool is relatively rapid, titres of circulating antibodies would not have to be long maintained to produce a drastic depletion of this lymphocyte population. Since the majority of the members of this pool are long lived, slowly regenerating lympho-

cytes, the defect in cell numbers would be very slowly repaired. This implies that the immunosuppressive effects of antilymphocyte serum will long outlast its own metabolic lifetime within the recipient.

Cells which are relatively sessile will be protected because ALS poorly penetrates lymphoid organs. Hence, the preservation of the germinal centre and medullary areas as well as the relative inefficiency of antilymphocyte serum in opposing humoral reactions are explained. Furthermore, the reappearance of lymphocytes in the peripheral blood would be due to the short lived and rapidly turning over population of lymphocytes (Caffrey, Rieke & Everett, 1962; Everett *et al.*, 1694). As soon as the titre of antilymphocyte serum has fallen, this cell population could rapidly reappear.

According to this hypothesis, the explanation for the incompetence of the cells obtained from the lymph nodes or spleen of ALS-treated animals to perform in graft versus host responses, (Boak, Fox & Wilson, 1967, 1968; Brent, Courtenay & Gowland, 1967, 1968; Levey & Medawar, 1967a; Ledney & Van Bekkum, 1968) or to adoptively transfer the ability to reject skin grafts (Levey & Medawar, 1967a) is due to the fact that the population of cells which mediates this function has been selectively ablated. The residual cell population is, however, by no means composed of entirely incompetent cells, since they are perfectly able to adoptively transfer the secondary humoral antibody response (James, 1968; James *et al.*, 1969b; Lance, 1969a). It is known that the ability to respond to PHA *in vitro* by blast cell transformation is a property of the long-lived recirculating lymphocyte (Metcalf & Osmond, 1966), and it has also been found that the lymphocytes in the blood after ALS treatment do not so respond (Taub, 1967). It seems likely that, if ALS eliminated the population of recirculating lymphocytes, regeneration should take place slowly since these cells are replaced at a very slow rate (1–2% per day), (Gowans, 1959; Caffey *et al.*, 1962) and since it is also known that other procedures which deplete this population do not appear to give rise to an adoptive acceleration in the rate of cell addition to the pool (Gowans, 1959; Gowans & Knight, 1964). Since the presence of ALS is evanescent, there is no reason why a rapidly turning over population of cells should not be quickly replaced. Therefore, it seems likely that the blood lymphocytes found in the initial phases of recovery from ALS belong to this category, and the labelling experiments of Taub (1969c), Denman, *et al.* (1968a, b) and Denman & Frenkel (1968b) may be viewed as evidence for this contention. The recovery of both peripheral lymphocytes and immuno-competence after cessation of ALS treatment is widely known.

After a series of ALS injections administered over a period of several weeks or months, recovery of competence takes place slowly between 40 and 50 days (Lance, 1968a; Lance & Medawar, 1969). Furthermore, Taub & Lance (1968b) have shown that the recovery of normal lymphocyte numbers in the peripheral blood after several injections of ALS in mice takes place slowly over a period of several weeks, and restoration of normal numbers of small lymphocytes in the paracortical area of lymph nodes, after cessation of chronic ALS treatment in mice, may not be entirely completed for as long as 1 month. Russell & Monaco (1967) have shown that the recovery of lymph node histology correlates well with the recovery of immunological competence. This observation is also consistent with the notion that the long-lived recirculating pool of lymphocytes is replaced very slowly. Thymectomy in association with ALS retards the rate of recovery of competence (Jeejeebhoy, 1965a, b; Monaco, Wood & Russell, 1965a, b). Since the thymus is believed by many to be essential for the generation or regeneration of the cells belonging to the mobilizable pool (see Miller,

1967; Taylor, 1965; Cooper, Peterson & Good, 1965; Miller & Osoba, 1967), it is not surprising that this should be the case.

One objection to this hypothesis is that it does not account for the fact that ALS is so much more efficient in producing immunosuppression than other procedures, e.g. thoracic duct drainage, which also deplete this pool of cells (Woodruff & Anderson, 1963, 1964). However, even the most efficient form of thoracic drainage must be very inefficient when compared to ALS, for whereas there are many escape routes for a lymphocyte to avoid being exteriorized via a thoracic duct fistula, there will be no place to hide from the effects of ALS. Moreover, Chanana *et al.* (1966) have shown that in calves a posteriorly placed skin graft will survive throughout the period of thoracic duct drainage. Therefore, we believe the differences observed between these two modalities is merely a quantitative one.

This theory offers no explanation for the observed increased production of lymphocytes, as measured by the increased incorporation of DNA precursors into the cells of the thymus and other lymphoid organs. A feedback mechanism is rendered less likely by the finding of Metcalf (1964) that lymphopoeisis in the thymus is autonomous and unaffected by the resection of lymphoid tissue or the presence of extra thymic tissue. This analogy may not be entirely apt since the degree of lymphoid depletion brought about by ALS is not comparable quantitatively to that studied by Metcalf. Moreover, it remains to be demonstrated that increased lymphopoiesis is relevant to the immunosuppressive action of ALS. If this should turn out to be the case, then more credence will have to be given to a twofold mechanism of action with some variant of sterile activation supplementing the depletion of the recirculating pool. Since the functional deficit in ALS-treated animals can be adequately explained without invoking sterile activation, it would seem wiser at present to stick to the simpler version.

BLINDFOLDING

Levey & Medawar (1966a) suggested that ALS might preempt the function of lymphocytes by coating surface receptors and preventing the recognition of antigen. The evidence in favour of such an hypothesis is that ALS is known to coat lymphocytes *in vitro* and *in vivo* (Levey & Medawar, 1966a; Lance, 1969b). Support was provided by Brent *et al.* (1967, 1968), who claimed to be able to restore competence to lymphocytes from ALS treated donors by exposing them *in vitro* to trypsin, which presumably stripped away the protein (antibody) coat. Lance and Medawar were unable to duplicate these findings (Lance, 1969d).

This hypothesis was subsequently rejected by its proposers after they observed that immunocompetence persisted in a situation where repeated cell division was expected to occur (Levey & Medawar, 1967a). Furthermore, the rapid elimination of specific antibody from the lymphoid tissues and the body of recipients is inconsistent with the notion of coating, as the functional deficit extends well beyond the period of potential coating (Lance, 1969b). In addition, coating of central lymphocytes 48 hr after ALS treatment could not be demonstrated by either an indirect or direct cytotoxicity assay (Lance, 1968c, d).

ENHANCEMENT

Enhancement (peripheral), can interfere with coagnate phenomena by covering up antigens on the target (Kaliss, 1965). Guttman and his colleagues (1967a, b) have proposed that the protection of rat renal homografts may result from binding of antibody to renal tissue itself.

Levey & Medawar (1966b) found anti-epidermal serum to prolong skin graft survival suggesting an enhancing mechanism, and the recent demonstration of the ability of an isologous antiserum to protect skin homografts in mice may also be construed as a manifestation of enhancement (Taub, 1969a).

However, the idea that the general immunosuppressive properties of heterologous ALS can be attributed to this mechanism can be readily dismissed. The majority of studies suggest that ALS is largely species specific (Besredka, 1900; Cruickshank, 1941; Abaza & Woodruff, 1966; Gray *et al.*, 1964, 1966). Yet, ALS can promote the survival of heterografted tissue in mice from guinea-pigs, rabbits, rats and man (Monaco *et al.*, 1966a; Levey & Medawar, 1966a; Lance & Medawar, 1968). Furthermore, enhancement could not possibly apply to protein or bacterial antigens (Lance, 1967; James & Anderson, 1967).

The possibility that enhancement may play some role for skin homografts has not been entirely ruled out. Raju, Grogan & Hardy (1969) reported prolongation of skin homografts after *in vitro* incubation with ALS. However, rabbit anti-A-strain lymphocyte serum is no more effective in prolonging the survival of A-strain skin on CBA mice than is rabbit anti-CBA lymphocyte serum (Levey & Medawar, 1967b). Tail skin homografts taken from ALS-treated donors and transplanted to untreated recipients are rejected as rapidly as untreated skin grafts in the same donor-recipient combination (Jooste, 1968, personal communication).

STERILE ACTIVATION

This hypothesis, an alternative to cell death, would account for the persistence of immunoincompetence throughout a period when large numbers of lymphocytes were present in the circulation, lymphoid tissues which often appear hypertrophic and hyperplastic rather than atrophic and hypoplastic (Levey & Medawar, 1966b), transformation of lymphocytes *in vitro* (Grasbeck, Nordman & de la Chapelle, 1963, 1964; Holt, Ling & Stanworth, 1966; Sell, Row & Gell, 1965; Ling *et al.*, 1967) and the presence of transformed (blast) cells in the tissues of ALS-treated animals (Levey & Medawar, 1966b). The proposition is that ALS causes lymphocytes to transform and pursue a line of replication without a specific immunological object, thus precluding reaction towards a specific immunogen. According to this view the lymphoid depletion which is known to occur after ALS treatment is incidental and not essential to its action.

However, the transforming ability of ALS *in vitro* occurs in the absence of complement, and when complement is added, transformation is replaced by cell death (Woodruff *et al.*, 1967). The reintroduction of ALS-coated cells into syngeneic recipients has a similar effect, presumably mediated by the complement of the host (Taub & Lance, 1968a; Martin & Miller, 1967), making it unlikely that contact between ALS and cells *in vivo* leads to transformation. Secondly, transforming ability is shared by a wide variety of agents: PHA, polk weed mitogen, anti-allotype serum and bacterial extracts (Sell *et al.*, 1965), none of which approach the immunosuppression of ALS *in vivo* (Landy & Chessin, 1969). The F(ab)2 fraction prepared from ALS IgG retains transforming ability (Woodruff *et al.*, 1967) but has lost immunosuppressive properties (Anderson *et al.*, 1967, 1968; Lance, 1967; Riethmuller, 1967). Therefore, transformation cannot be a sufficient cause of the action of ALS.

Is transforming ability a necessary property for the action of ALS? While the answer cannot be dogmatically given, I believe it will be negative. The question is not whether transformation can occur *in vitro* but whether it does in fact occur *in vivo*. The evidence

in the affirmative depends largely on the histological observation of increased blast cells in the tissues of ALS-treated animals as compared to *untreated* controls (Levey & Medawar, 1966b; Denman & Frenkel, 1968b; Taub & Lance, 1968b). Reinforcement comes from the studies of Denman *et al.* (1968a), in which it was shown that the lymphoid tissues of ALS treated animals have an increased incorporation of thymidine. However, when the lymphoid tissues of ALS-treated animals are compared to NRS-treated controls, the same numbers of blast cells can be found (Bunting, 1903; Monaco, Wood & Russel, 1967; van der Werf *et al.*, 1968; Taub, 1968; Taub & Lance, 1968b). Taub & Lance (1968b) found a reduction in the lymphoid proliferative response when ALS IgG was given to hosts paralysed to IgG. Denman *et al.* (1968b) observed that ALS given to animals which had been pretreated with pertussis resulted in a marked drop in lymphocyte count in the peripheral blood (from 100,000 to less than 1000). This occurred within a few hours but no evidence of blast transformation was found. The most likely explanation for the blast cells and rapidly dividing cell populations found after ALS treatment is that they are the consequence of immunization. We infer from these findings that blast transformation is not a necessary feature of the action of ALS.

ANTIGEN COMPETITION

The simultaneous presentation of two immunogens to the same animal may result in a reduced response (Adler, 1964). It is not yet clear whether competition occurs at the level of the antigen sensitive cell, whether space is the limiting factor, or if the availability of essential metabolites is involved. The finding that ALS IgG is a powerful immunogen (Clark, James & Woodruff, 1967; Lance & Dresser, 1967; Guttman *et al.*, 1967a) raises the possibility that the action of ALS is related to this property. However, because ALS is equally or more effective in situations where an immune response is proscribed (Lance, 1967; Denman & Frenkel, 1967) this possibility is effectively eliminated.

THE LIACOPOULOS PHENOMENON

Liacopoulos and his co-workers (1964, 1965, 1967, 1968) have described a situation where, after loading of animals with foreign antigens, a state of non-specific immunological unresponsiveness is induced. They have further shown that animals so treated are susceptible to the induction of tolerance to antigens introduced during the period of unresponsiveness. Could ALS act in an analogous manner? Two distinctions are immediately apparent. A unifying feature of Liacopoulos' studies is that, regardless of the substance introduced, enormous quantities are required. ALS, on the other hand, is capable of immunosuppression in minute quantities (Levey & Medawar, 1966a). A second feature of the Liacopoulos effect is that it is limited in time to a finite period beginning and terminating after several weeks despite continued treatment. The immunosuppression induced by ALS takes effect immediately and does not wane even after months of continuous treatment (Lance, 1968a).

ACTION THROUGH A NON-IMMUNOLOGICAL PATHWAY

Most discussions of the action of ALS presume that the relevant feature involves the interaction of an antibody with lymphocytes. However, there is no *a priori* reason to believe that some non-antibody constituent could not be responsible for the effect. The clearest evidence

for the antibody nature of ALS is that the activity may be completely removed by absorption with lymphocytes (Levey & Medawar, 1967b).

ACTION THROUGH THE THYMUS

It has been suggested that ALS may act by interfering with the action of a thymic hormone (Nagaya & Sieker, 1965, 1967; Russe & Crowle, 1965). Antisera raised against thymocytes were found to be more effective than antisera raised against lymph node cells; the former induces a lesser uptake of thymidine in the thymus of recipients than does the latter. *Ab initio* this mechanism applies only to antithymocyte serum, but even in this circumstance it can be discounted. The rapid elimination of antibody eluted from thymocytes implies that any peripheral opposition to a thymic hormone could at best be evanescent (Lance, 1969b). Secondly, Monaco *et al.* (1965a, b), and Jeejeebhoy (1965a, b; Lance, Levey & Medawar, 1969) have found that the effects of ALS and thymectomy are synergistic. This suggests that whether the contribution of the thymus to the immunological apparatus of the host is in the form of cells or a hormone product, anti-thymocyte serum does not in itself completely oppose this contribution. Lastly, antigenic extracts from thymus, e.g. the membrane fraction, are as effective as whole thymocyte preparations in raising a potent ALS (Levey & Medawar, 1966b; Lance *et al.*, 1968), and it is extremely unlikely that any thymic hormone would have remained after the extensive processing involved.

The fact that the immunological deficit and the dynamic changes in lymphocyte kinetics appear to be the same for the various types of antisera, and the demonstration by Levey & Medawar (1966a and unpublished observations; Wood & Vriesendorp, 1969) that anti-lymph node serum is just as effective as anti-thymocyte serum *in vivo* in augmenting skin homograft survival make it unlikely that the target or mode of action differs as a consequence of the type of lymphoid antigen used to raise ALS.

TOLERANCE

To say that ALS achieves its generalized immunosuppressive effect through tolerance is excluded by the definition of tolerance itself. To say that ALS achieves its effect by rendering animals susceptible to the induction of tolerance by antigen is to beg the question.

INDISCRIMINATE LYMPHOCYTE DESTRUCTION

This possibility must be considered because of the known cytotoxic action of ALS on lymphocytes *in vitro* (Reif, 1963; Abaza & Woodruff, 1966; Gray *et al.*, 1966) and because ALS-treated animals may have massive and non-selective ablation of lymphocytes in the circulation and central lymphoid organs (Monaco *et al.*, 1965a, b, 1966a, b, 1967; Gray *et al.*, 1966; Lawson *et al.*, 1967). But this general depletion is not a prerequisite for immunosuppression since equal or greater effects follow the use of sera which cause only a zonal depletion in lymph nodes and spleen, and which do not alter the morphology of the thymus (Levey & Medawar, 1966a, b; Lance, 1968a; Taub & Lance, 1968b).

Sera raised with adjuvants can be highly toxic (Jooste *et al.*, 1968), and a stress effect mediated through the host adrenals is the most likely explanation for the seeming discrepancy in observations. However, it should be mentioned that the effects of ALS do not depend on

adrenal participation, since ALS is effective in adrenalectomized hosts (Levey & Medawar, 1966b).

In summary, a review of alternatives to the hypothesis of selective depletion of recirculating lymphocytes finds them wanting in one respect or another. A dual or multiple mechanism (Turk & Willoughby, 1969) has not been completely excluded but seems unnecessary to account for the essential observations and we would favour the strict application of Occam's razor at this time.

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