SITE OF ACTION OF SERUM FACTORS THAT BLOCK DELAYED-TYPE HYPERSENSITIVITY IN MICE*

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Delayed-type hypersensitivity (DTH)[1] develops without adjuvant if the dose of SRBC given is appropriate to the route of inoculation (1). Increasing the amount of antigen reduces and eventually abolishes all evidence of DTH. Animals blocked by an excessive dose of SRBC cannot be subsequently sensitized, even when an optimal dose of antigen is given subcutaneously, the most reliable route for inducing DTH in the mouse. DTH does develop, however, when a massive intravenous dose of antigen is given to splenectomized mice, or mice that have received cyclophosphamide before immunization (2). The suppression of DTH cannot therefore be due to excess antigen, as such. The serum of DTH-suppressed mice will partially inhibit the induction and expression of DTH in normal recipients, an effect which is substantially increased by partial absorption of the serum with specific antigen (3). In contrast to a high dose of antigen (4), absorbed serum does not inhibit the proliferative response or the formation of plaque-forming cells (PFC) in lymph nodes draining the site of antigen injection. Moreover, the specific antibody titer is increased by absorbed blocking serum even though the induction of DTH is totally suppressed by it.

In light of indications that the products of the interaction between antigen and antibody depress the activated T cells which mediate DTH, and insofar as pretreatment with specific IGM antibody has been reported to enhance the antibody response (5, 6), an important question arises concerning the functional relationship between the mediators of DTH and the T cells responsible for helper activity in the antibody response. Obviously, a successful antibody response depends upon cooperation between the various cellular and humoral elements involved (7). It is possible that this can be accomplished efficiently only in certain sites, and that the local enactment of a DTH reaction is the process whereby the participating elements are assembled (8). After intravenous immunization, the spleen could be the locus of this coordinated series of events. Evidence in support of this concept is presented in the present report,

which deals with the site of blocking by immune serum of the cells that mediate DTH to sheep erythrocytes (SRBC) in mice.

Materials and Methods

Animals. Except when cell transfers were made, the mice used were specific-pathogen-free mice of the outbred CD-1 strain (Charles River Breeding Laboratories, Inc., Wilmington, Mass.). Adoptive sensitization by spleen cell or lymph node cell transfer was performed syngeneically, using strains B6H-T_6 F_1 (C57Bl/6 × CBA H-T6) or C57Bl/6 mice from the breeding facilities of the Trudeau and Pasteur Institutes. Mice were used at 5 or 8 wk of age.

Antigens. SRBC were obtained from the same animal once weekly. Cells were collected and stored in Alsever's solution at 4°C. Chicken erythrocytes (CRBC) were obtained and stored in the same way. Cells were washed three times with normal saline and suspended to known density by hemocytometer count.

Cyclophosphamide (CY). CY was donated by Mead Johnson & Co. (Evansville, Ind.) and by Lab. Lucien (Colombes, France). A single dose (200 mg/kg) was given intravenously before immunization (2), though intraperitoneal or subcutaneous routes gave identical results.

Immunization. Except when specified, 10^8 SRBC were used for primary immunization by intravenous or by foot-pad inoculation. For modulation of the primary response, cyclophosphamide was administered 2 days before intravenous immunization or concurrently with subcutaneous immunization.

Test for DTH. DTH was measured after 24 h as the increase in foot-pad thickness elicited by an injection of 10^8 SRBC in a 40-μl volume (1).

Splenectomy. Splenectomized mice were prepared 3 wk before an experiment by removing the spleen through a flank incision. The spleen was dissected from its pedicle and excised without attempting to prevent bleeding. The wound was closed by Mischel clips.

Blocking Serum. Serum containing specific inhibitors of activated T cells was prepared from mice bled 4 days after intravenous immunization with 10^9 SRBC. Its blocking activity was increased by partial absorption with packed SRBC as previously described (3). Hereafter, 4-day serum and absorbed 4-day serum are referred to as S4 and AS4, respectively.

Immune Cell Transfer. Spleen cells for transfer to syngeneic recipients were prepared as in a previous study (9). The cells were pooled, washed twice, and enumerated in a hemocytometer. Viability, as assessed by dye exclusion with trypan blue, was not less than 90%. For the systemic cell transfer, mice received 0.5 ml of spleen cell suspension intravenously. Recipients were tested immediately for DTH by foot-pad injection of 10^8 SRBC. For the local transfer of DTH, the method of Metaxas and Metaxas-Bühler (10) was modified slightly; the specifically sensitized lymphocytes and antigen were mixed and then introduced into the foot-pad of the normal or treated recipients.

Results

Adoptive Transfer of DTH by Spleen Cells from Anergic Mice. Hypersensitivity to SRBC can be transferred adoptively with isolated spleen cells from immunized donors. In the mouse, the active cells bear the alloantigen Thy-1 (11). Those mediating specific sensitivity to SRBC perform well in normal recipients, where they survive with an apparent half-life of about 45 days (12). It has been observed, however, that reactive cells confer no DTH upon recipients which have received 10^9 SRBC 4 days before cell transfer (3). Similarly, DTH did not appear, and a regional antibody response did not develop in mice given an optimal foot-pad dose of SRBC within 3 days of receiving a large (10^9) intravenous dose of SRBC (1). On the other hand, serum of such mice, partially absorbed with SRBC, blocked the induction and expression of DTH (3), but did not inhibit the proliferative response or PFC production in the draining node. It is evident, therefore, that the effects of SRBC and AS4 on the production and circulation of activated T cells are significantly different. As a first step towards explaining this difference, a study was made of the capacity of spleen cells from
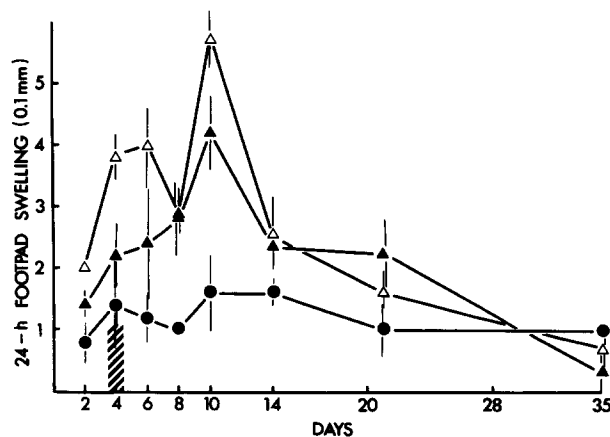
Fig. 1. Local and systemic transfer of DTH with spleen cells from animals in which DTH was blocked by intravenous immunization with a large dose of SRBC. Donor mice were immunized intravenously with 10^9 SRBC on day 0. Representatives were tested for DTH at intervals during the next 35 days (●). At each time point, one spleen equivalent of cells from similar mice was transferred to a normal recipient. Two groups of recipients were used: one received spleen cells by a systemic cell transfer (▲), the other received a mixture of spleen cells and SRBC injected locally into the test foot-pad (△). The reactions elicited in the donors did not differ significantly at any time from those of the unimmunized controls, which were tested on day 4 only (hatched column). Mean of five mice ± SEM.

blocked mice to mediate DTH by local and systemic transfer. It was reasoned that local transfer would test the intrinsic capacity of donor T cells to mediate a DTH reaction, whereas systemic transfer should reveal, in addition, their ability to reach the site of antigen deposition.

Using this approach, the kinetics of the blocking action of an intravenous inoculum of 10^9 SRBC was studied by adoptive transfer of spleen cells harvested at varying intervals after immunization. The purpose of the experiment was to determine whether reactive cells are produced at all, or whether they are produced and subsequently inhibited. The results (Fig. 1) show that although the cell donors were completely anergic, cell recipients gave reactions which were highly significant after both local and systemic transfer of cells harvested on days 8 and 10 ($P < 0.002$). The local transfer was usually more effective, even though the number of cells transferred was less. The difference between the DTH reactions elicited in donors and recipients of locally transferred cells was statistically significant ($P < 0.05$) at all times through day 14. It is concluded, therefore, that the mediators of DTH are present in the spleens of suppressed mice. It is apparent that they are either inhibited in situ or are prevented from leaving the spleen. In either case, the evidence of a previous experiment suggests that the mechanism involved is cyclophosphamide-sensitive (2). The next experiment was designed to examine the first of these possibilities by testing the capacity of blocking serum to interfere with specifically sensitized lymphocytes at the site of a local cell transfer.

Absence of a Direct Effect of Blocking Serum on the Mediators of DTH. Since it has been reported that immune complexes can bind to activated T cells (13), and that some T cells have receptors for IgM (14-16), an experiment was

designed to test whether AS4 is adsorbed to activated T cells, thus rendering them functionally inactive, as suggested by Sinclair et al. (17).

Spleen cell suspensions from intravenously immunized mice were washed twice in medium 199. The pellets of pooled spleen cells were resuspended in AS4 or in normal serum in the proportion of 1 ml of serum per spleen equivalent. The suspensions were incubated for 1 h at 37°C in a humidified atmosphere containing 5% CO_2. The spleen cell preparations were then washed three times in medium containing 1% fetal calf serum at 4°C. The supernates after the first wash were kept and subsequently tested for blocking activity. The serum-treated cells were injected intravenously into normal recipients which were tested immediately for DTH by foot-pad inoculation. The supernates (post-absorption serum) were transferred into normal mice that had also received an aliquot of immune cells which had been treated with absorbed normal serum.

Fig. 2 shows, as expected, that the blocking factors in AS4 inhibited DTH to SRBC ($P < 0.001$), and that blocking was specific since immune serum absorbed with CRBC did not block the reaction in SRBC-sensitized mice ($P = 0.4$). Although not recorded in Fig. 2, the inverse was also true: AS4 (CRBC) blocked DTH to CRBC but not to SRBC. However, spleen cells incubated with homologous blocking serum showed no change in viability and the specifically reactive component remained intact, as shown by their undiminished capacity to confer DTH upon normal recipients. Moreover, the supernate (post-absorption serum) had not lost its T-cell inhibition as a result of absorption ($P < 0.005$) (Fig. 2). These results suggest that specifically sensitized lymphocytes are not inhibited, at least in vitro, by direct interaction with specific blocking factors in AS4.

Indirect Effect of AS4 on the Mediators of DTH. The preceding experiment did not exclude the possibility that union between AS4 and activated T cells is unstable, and that treated cells could regain functional activity after transfer. This would be less likely to happen if AS4 were mixed with the specifically sensitized cells and introduced directly into the test-site, as in the Metaxas and Metaxas-Bühler technique for transferring DTH (10). This possibility was examined in groups of mice that were adoptively immunized by either systemic or local cell transfer.

As described in the legend of Fig. 3, AS4 blocked DTH only when given intravenously to recipients immunized by systemic cell transfer ($P < 0.001$); it failed, almost completely, to prevent DTH when it was introduced locally with the sensitizing cells. Conversely, AS4 given intravenously did not block the local transfer of DTH, but did affect systemic transfer to some extent when given subcutaneously. This seems to say that there is a geographic restriction to the blocking action of AS4. It can be concluded, in fact, that AS4 actively blocks DTH only when AS4 is present in the systemic circulation with the specific mediators of DTH.

It should be noted, too, that AS4 given intravenously did not interfere with the local transfer of DTH. This means that blocking serum does not affect the migratory behaviour of the accessory cells (monocytes) which are necessary participants in a DTH reaction. This appears to implicate the T cell as the victim of the blocking reaction.

In an additional experiment, SRBC and immune serum (S4) were mixed as for the production of blocking serum (AS4), and injected into the foot-pads of

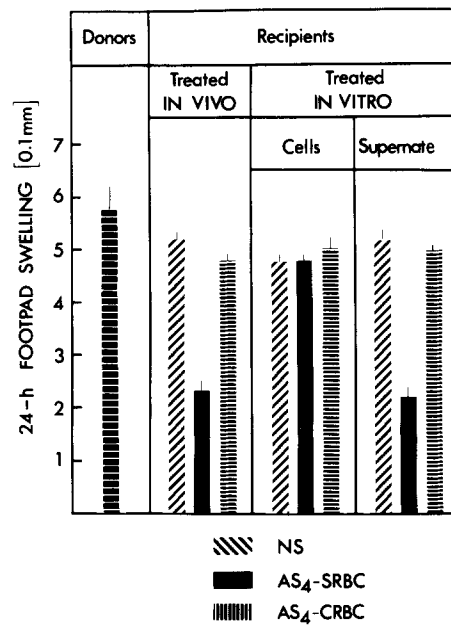|	NS
■	AS₄-SRBC
▤	AS₄-CRBC

Fig. 2. Specificity of blocking and absence of direct effect of blocking serum on the mediators of DTH. Donor mice were immunized intravenously with 10^8 SRBC 2 days after treatment with CY. 5 days later the spleens from these sensitized mice were used to prepare cell suspensions for immediate transfer to recipients, or for pretreatment with normal serum, or specific or nonspecific immune serum in vitro. Normal serum (NS), and the absorbed sera from SRBC-immunized (AS4-SRBC) or CRBC-immunized mice (AS4-CRBC) were injected intravenously into recipients of SRBC-sensitized cells. The DTH reactions elicited in the in vivo treated mice demonstrate a high degree of specificity in the blocking reaction.

The aliquots of sensitized cells treated in vitro with NS, AS4-SRBC, or AS4-CRBC were centrifuged, and the supernates were used to test for blocking activity, while the cells were washed and tested for DTH activity by adoptive transfer. The reactions elicited in recipients of in vitro treated cells showed that the cells were functionally intact. Moreover, blocking activity of the supernatant serum was undiminished by absorption with immune spleen cells (supernate). Means of 5 ± SEM.

hypersensitive mice. The mixture elicited reactions greater than those produced with antigen alone, probably because the SRBC were more effectively retained at the inoculation site. The experiment showed again that mediator cells are not directly inhibited by factors present in blocking serum. It was interesting to find, however, that AS4 failed to elicit a DTH reaction even though it provoked a PFC response in the regional node (P. H. Lagrange, unpublished observations). This suggests that free antigen is needed for eliciting a DTH reaction but not for the induction of an antibody response, at least in hypersensitive mice.

Localization of Site of Action of AS4. We have shown that DTH develops in splenectomized mice even when blocking doses of antigen are administered (1). The same conditions prevail in animals given a dose of CY sufficient to prevent antibody formation. Being the major organ of both antibody formation (18) and the regulation of some T-dependent immune responses (19), the spleen thus seemed a likely anatomical site for the event that results in blocking of DTH. This possibility was tested in a composite experiment in which blocking by AS4
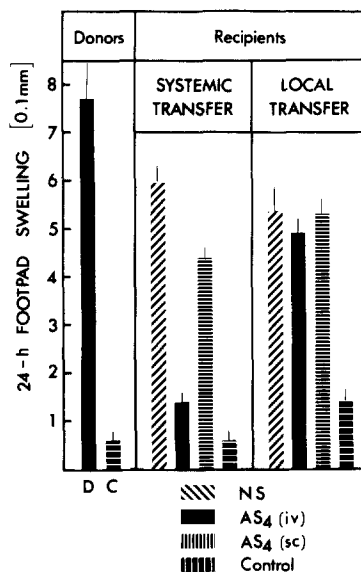
FIG. 3. In an experiment of design similar to that of Fig. 2, the blocking of DTH was shown to be geographically restricted to cells in the systemic circulation. The sensitized donors (D) and a group of unimmunized controls (C) were tested for DTH on day 5. The remaining donors were used to prepare suspensions of normal or sensitized spleen cells for systemic or local transfer to normal recipients. The systemic recipients received one spleen equivalent of normal (C) or sensitized cells intravenously. Before testing for DTH, mice of separate groups were given 0.2 ml of normal serum (NS) or AS4. The former was given intravenously, and the latter intravenously (iv) or subcutaneously (sc) in the neck. Even when administered subcutaneously, AS4 depressed the foot-pad reaction in systemically sensitized mice. With local transfer of sensitized cells, the effect was different. Whether given intravenously or into the reaction site with spleen cells and antigen, AS4 caused no significant depression of DTH. Groups of 5 ± SEM.

was compared in normal, splenectomized, and CY-treated recipients of SRBC-sensitized spleen cells.

The results of the experiment described in Fig. 4 show the reactivity to SRBC in the donor mice and in recipients given varying numbers of immune spleen cells intravenously. It can be seen that undiminished reactions were elicited in splenectomized or CY-treated recipients of 10^8 immune spleen cells that had been treated with normal mouse serum. However, in groups of similar recipients that had received AS4 instead of normal mouse serum, significant blocking of DTH occurred only in normal recipients. The effect of AS4, which was almost abolished by splenectomy or CY alone, was completely abolished in animals that were both splenectomized and treated with CY. It will be noted from Fig. 4 that the dose of AS4 administered was enough to reduce the apparent level of T-cell activity in normal recipients by a factor of 10^3. Thus, it seems that blocking factors in AS4 interfere with the function of the mediators of DTH through an event occurring in the spleen and involving CY-sensitive cells. Trapping of activated T cells in the spleen suggests itself as a likely mechanism.

Discussion

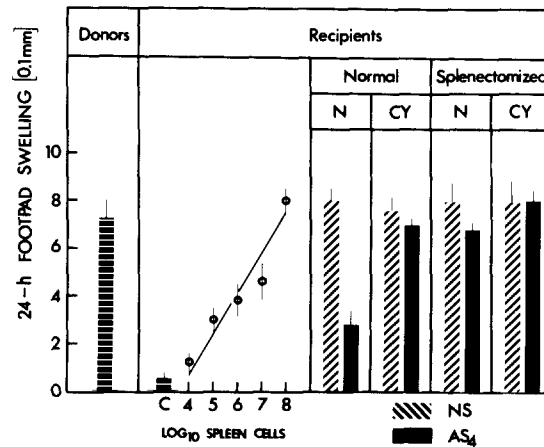The present experiments begin by demonstrating that the absence of DTH

FIG. 4. Donor mice were immunized intravenously with 5×10^7 SRBC 2 days after treatment with CY. On day 6, representative donor mice were tested for DTH. The rest were used to prepare spleen cell suspensions for transfer to various groups of normal or splenectomized recipients. An additional group of 25 untreated recipients was used to obtain the curve which shows the relationship between the intravenous dose of spleen cells and the level of DTH conferred. Half of the remaining normal or splenectomized recipients were given CY (200 mg/kg) 24 h before receiving the standard intravenous dose of 10^8 viable nucleated spleen cells. 1 h before cell transfer, the recipient mice were given 0.2 ml of either normal mouse serum (NS) or AS4 intravenously. Immediately after cell transfer, mice of all groups were tested for DTH by foot-pad inoculation with 10^8 SRBC. Splenectomy performed 3 wk earlier and CY given 24 h beforehand were both effective in preventing the blocking action of AS4. Means of 5 mice $\pm$ SEM.

after immunization with an excessive dose of antigen cannot be due to the absence of sensitized cells, because cell transfer showed that reactive cells were present in the spleens of anergic mice. It was then shown in several ways that the blocking factors which appear in serum do not interfere directly with the function of mediator cells. In fact, blocking could be demonstrated only when blocking factors and reactive T cells were present together in the systemic circulation. It must be emphasized, however, that this statement applies only to T cells in a recently activated state. In effect, this means mitotically active cells, because it was demonstrated previously that only vinblastine-sensitive cells are susceptible to blocking by AS4 (13).

The finding that blocking factors are specific for homologously reactive T cells, and can function only in the presence of an intact spleen, implies that suppression of DTH is due to an immunologically specific event involving the spleen. The most likely role for the spleen in blocking DTH is that of selective trapping of mediator cells. Experiments to demonstrate specific trapping of T cells have been reported (17, 20, 21), but they have relevance to the inductive phase of an immune response rather than to the mediators of DTH. The present experiments demonstrate quite clearly that the spleen has a part to play in the suppression of DTH which is observed after administration of large quantities of antigen. They also show that the underlying mechanism is ablated by prior treatment with cyclophosphamide. This implies the participation of a drug-sensitive population of cells. Four cell types deserve consideration.

B-Cell Precursors. It has been shown by several investigators that cyclo-

phosphamide depresses antibody production preferentially when given before antigenic stimulation (2, 22–26). It is likely, therefore, that after cyclophosphamide the capacity to produce antibodies would have been depressed in animals that had been adoptively sensitized with SRBC-specific T cells and given blocking serum intravenously. Since blocking serum contains specific antibody, it is obvious that blocking is not due to antibody per se. Hence, by inference, the absence of blocking in CY-treated animals cannot be simply due to the prevention of antibody production by the transferred cells. This makes it difficult to directly implicate B cells in the blocking process.

Monocyte Precursors. The monocyte precursor in bone marrow is radio-sensitive (27) and susceptible to cyclophosphamide (2, 28, 29). It is well known that monocytes are essential to the enactment of a DTH reaction in the skin (30) or foot-pad (27). It is conceivable, therefore, that the uptake of antigen by the spleen after intravenous immunization would serve to elicit the equivalent of a DTH reaction in that organ, with a resulting retention of specifically reactive lymphocytes and an accompanying accumulation of monocytes. Such an event would obviously limit the availability of cells in circulation for a reaction in the foot-pad, for example. It was observed, however, that a local transfer of DTH was possible in systemically blocked mice (Fig. 3). Obviously, retention of monocytes by the spleen cannot explain the blocking phenomenon.

Suppressor Cells. There is substantial evidence for the existence of suppressor T cells. They are usually detected through their capacity to interfere with antibody production (31). It has been shown more recently, however, that T cells with a capacity to suppress cell-mediated hypersensitivity also appear in the spleen under the conditions that lead to suppression of DTH (32). Therefore, it must be admitted that a suppressor cell, which is sensitive to cyclophosphamide, could be involved in the blocking of DTH (33). If so, it must be assumed that blocking factors actually induce the formation of the suppressor cells and that the inductive process is very rapid because blocking is complete and instantaneous in adoptively sensitized animals. Moreover, it can be deduced from the present experiments that a suppressor cell, if it exists, is not available in the circulation to exert its suppressive effect on a DTH reaction elicited in the foot-pad. The results of experiments designed to evaluate the role of suppressor cells in the blocking of DTH will be reported in a subsequent paper.

Dendritic Cells. The dendritic cell, which has a known capacity to trap antigen-antibody complexes at the cell surface (34), is very sensitive to radiation (35). It might therefore be sensitive to a radiomimetic drug such as CY, although no evidence for this has been reported. Since a low intravenous dose of SRBC (10^5–10^6) causes T-cell activation and DTH without causing antibodies to be formed (including an absence of PFC), whereas larger doses cause antibody production without DTH even though the activated T cells are present in the spleen (Fig. 1), it seems plausible that the association between activated T cells and antibody-forming B cells may be more than circumstantial: it may represent a true physical relationship which could involve the dendritic macrophage as a focus for the cellular cooperation that results in antibody production. It may be, in fact, that the germinal center is a morphological structure created to satisfy the necessity for cooperation between cell types. According to this hypothesis, blocking factors, which are believed to be a complex of antigen and IgM, would

become associated with dendritic macrophages, thus creating the attractive focus that prevents the release of activated T cells from the spleens of massively immunized mice, or causes them to become trapped in that organ after the passive administration of AS4 to adoptively sensitized subjects. If such a mechanism exists, the molecular mechanism and the relevant receptors involved in cell-cell binding have yet to be elucidated.

There is a long history to the close functional relationship that seems to exist between the phenomenon of DTH and the mechanism of antibody formation (8, 36). It has been suggested, in fact, that when a DTH reaction is observed in the spleen it is a displaced expression of a physiological process that normally occurs in lymphoid tissue during the inductive phase of an antibody response. Viewed in this way, the blocking of DTH in animals immunized with a large intravenous dose of antigen cannot be thought of as suppression of a particular form of immunological reactivity, but rather as the redirection of cells (37) from one possible role as the mediators of DTH to an alternative role as helper cells in antibody formation. This, however, may be far too simplistic an explanation for the observed effects of serum factors on the expression of DTH in mice. It is consistent, however, with the frequent coexistence of specific anergy and hypergammaglobulinemia.

Summary

When mice have been rendered anergic by a large intravenous dose of sheep erythrocytes, their inability to mount a delayed-type hypersensitivity (DTH) reaction is not due to an absence of mediator cells, for these can be detected in the spleen by cell transfer. Nor is it due to disappearance of accessory cells (monocytes) from circulation. The serum of anergic mice contains blocking factors which are more abundant after absorption with antigen. Such factors are unable to inactivate the mediators of DTH in vitro, nor do they suppress a DTH reaction when introduced locally into the reaction site. They are active, however, when given intravenously to systemically sensitized mice, provided that the sensitized animal has an intact spleen. If the spleen has been removed or the recipients of sensitized cells have been treated with cyclophosphamide before cell transfer, blocking factors are no longer able to suppress a DTH reaction. Reasons are given for the belief that suppression of DTH in animals undergoing a vigorous antibody response is due to the diversion of reactive cells from circulation to undertake an alternative role in antibody formation in the spleen.

References

1. Lagrange, P. H., G. B. Mackaness, and T. E. Miller. 1974. Influence of dose and route of antigen injection on the immunological induction of T cells. *J. Exp. Med.* **139:**528.
2. Lagrange, P. H., G. B. Mackaness, and T. E. Miller. 1974. Potentiation of T cell mediated immunity by selective suppression of antibody formation with cyclophosphamide. *J. Exp. Med.* **139:**1529.
3. Mackaness, G. B., P. H. Lagrange, T. E. Miller, and T. Ishibashi. 1974. Feedback inhibition of specifically sensitized lymphocytes. *J. Exp. Med.* **139:**543.

4. O'Toole, C. M., and A. J. S. Davies. 1971. Preemption in immunity. *Nature (Lond.)*. 230:187.

5. Henry, C., and N. K. Jerne. 1968. Competition of 19 S and 7 S antigen receptors in the regulation of the primary immune response. *J. Exp. Med.* 128:133.

6. Dennert, G. 1973. Effects of IgM on the *in vivo* and *in vitro* immune response. *Proc. Soc. Exp. Biol. Med.* 143:889.

7. Hanna, M. G., Jr., and R. L. Hunter. 1970. Localization of antigen and immune complexes in lymphatic tissue with special reference to germinal centers. *In* Morphological and Functional Aspects of Immunity. K. Lindahl-Kiessling, F. Alm, and M. G. Hanna, Jr., editors. Plenum Publishing Corporation, New York. 257.

8. Voisin, G. A., and F. Toullet. 1966. Etudes sur l'hypersensibilité. VI. Rapports des différents types d-hypersensibilité entre eux et avec les anticorps circulants. *Ann. Inst. Pasteur (Paris)*. 111:517.

9. Mackaness, G. B. 1969. The influence of immunologically committed lymphoid cells on macrophage activity *in vivo*. *J. Exp. Med.* 129:973.

10. Metaxas, M. N., and M. Metaxas-Bühler. 1948. Passive transfer of local cutaneous hypersensitivity to tuberculin. *Proc. Soc. Exp. Biol. Med.* 69:163.

11. Miller, T. E., G. B. Mackaness, and P. H. Lagrange. 1973. Immunopotentiation with BCG. II. Modulation of the response to sheep red blood cells. *J. Natl. Cancer Inst.* 51:1669.

12. Lagrange, P. H., and G. B. Mackaness. 1975. A stable form of delayed-type hypersensitivity. *J. Exp. Med.* 141:82.

13. Yoshida, T. O., and B. Andersson. 1972. Evidence for a receptor recognizing antigen complexes immunoglobulin on the surface of activated mouse thymus lymphocytes. *Scand. J. Immunol.* 1:401.

14. Webb, S. R., and M. D. Cooper. 1973. T cells can bind antigen via cytophilic IgM antibody made by B cells. *J. Immunol.* 111:275.

15. Ferrarini, M., L. Moretta, M. C. Mingari, P. Tonda, and B. Pernis. 1976. Human T cell receptor for IgM: specificity for the pentameric Fc fragment. *Eur. J. Immunol.* 6:520.

16. Santana, V. 1977. Receptors for IgM on murine lymphoid cells. *Immunology*. 33:273.

17. Sinclair, N. R. St. C., R. K. Lees, S. Abraham, P. L. Chan, G. Fagan, and C. R. Stiller. 1974. Regulation of the immune response. X. Antigen-antibody complex inactivation of cells involved in adoptive transfer. *J. Immunol.* 113:1493.

18. Rowley, D. A. 1950. The effect of splenectomy on the formation of circulating antibody in the adult male albino rat. *J. Immunol.* 64:389.

19. Grebe, S. C., and J. W. Streilin. 1976. Graft-versus-host reaction: a review. *Adv. Immunol.* 22:119.

20. Emeson, E. E., and D. R. Thursh. 1974. Antigen-induced trapping of immunospecific lymphocytes in the spleen. *J. Immunol.* 113:1575.

21. Sprent, J., and J. F. A. P. Miller. 1974. Effect of recent antigen priming on adoptive immune responses. II. Specific unresponsiveness of circulating lymphocytes from mice primed with heterologous erythrocytes. *J. Exp. Med.* 139:1.

22. Lerman, S. P., and W. P. Weidant. 1970. The effect of cyclophosphamide on the ontogeny of the humoral immune response in chickens. *J. Immunol.* 105:614.

23. Turk, J. L., D. Parker, and L. W. Poulter. 1972. Functional aspects of the selective depletion of lymphoid tissue by cyclophosphamide. *Immunology*. 23:493.

24. Katz, S. I., D. Parker, and J. L. Turck. 1974. B cell suppression of delayed-hypersensitivity reactions. *Nature (Lond.)*. 251:550.

25. Neta, R., and S. B. Salvin. 1976. Autoregulation of delayed-hypersensitivity in the guinea pig. *In* Mitogens in Immunobiology. J. J. Oppenheim and D. L. Rosenstreich, editors. Academic Press, Inc., New York. 573.

26. Kerckhaert, J. A. M. 1974. Influence of cyclophosphamide on the delayed hypersensitivity in the mouse after intraperitoneal injection. *Ann. Inst. Pasteur (Paris).* 125C:559.

27. Volkman, A., and F. M. Collins. 1972. The restorative effect of peritoneal macrophages on delayed hypersensitivity following ionizing radiation. *Cell. Immunol.* 2:552.

28. Dewys, W. D., and N. Knight. 1969. Kinetics of cyclophosphamide damage: sublethal damage repair and cell-cycle related sensitivity. *J. Natl. Cancer Inst.* 42:1955.

29. Tyler, W. S., E. Niskanen, F. Stholman, J. Keane, and D. Howard. 1972. The effect of leutropenia on myeloid growth and the stem cell in a uvo culture system. *Blood.* 40:634.

30. Lubaroff, S. M., and B. H. Waksman. 1968. Bone marrow as a source of cells in reactions of cellular hypersensitivity. 1. Passive transfer of tuberculin sensitivity in syngeneic systems. *J. Exp. Med.* 74:247.

31. Baker, P. J. 1975. Homeostatic control of antibody response: a model based on the recognition of all-associated antibody by regulatory T cells. *Transplant. Rev.* 26:3.

32. Ramshaw, I. A., P. A. Bretscher, and C. R. Parish. 1976. Regulation of the immune response. 1. Suppression of delayed-type hypersensitivity by T cells from mice expressing humoral immunity. *Eur. J. Immunol.* 6:674.

33. Askenase, P. W., B. J. Hayden, and R. K. Gershon. 1975. Augmentation of delayed-type hypersensitivity by doses of cyclophosphamide which do not affect antibody responses. *J. Exp. Med.* 141:697.

34. Hanna, M. G., Jr., and A. K. Szakal. 1968. Localization of ^{125}I-labeled antigen in guminal centers of mouse spleen. Histologic and ultrastructural autoradiographic studies of the secondary immune reaction. *J. Immunol.* 101:949.

35. Steinman, R. M., D. S. Lustig, and Z. A. Cohn. 1974. Identification of a novel cell type in peripheral lymphoid organs of mice. III. Functional properties *in vivo. J. Exp. Med.* 139:1431.

36. Coe, J. E., and S. B. Salvin. 1964. The immune response in the presence of delayed hypersensitivity or circulating antibody. *J. Immunol.* 93:495.

37. Schlossman, S. F., H. A. Levin, R. E. Rocklin, and N. J. David. 1971. The compartmentalization of antigen-reactive lymphocytes in desensitized guinea pigs. *J. Exp. Med.* 134:741.