INFLUENCE OF DOSE AND ROUTE OF ANTIGEN INJECTION ON THE IMMUNOLOGICAL INDUCTION OF T CELLS*

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If it were possible to promote or suspend the formation of specifically sensitized lymphocytes (activated T cells), the problems of achieving a sustained attack on tumor cells and microbial parasites, or of preventing graft rejection, might be largely overcome. Methods for manipulating the immune response for such purposes have been proposed from time to time (1–3), but progress has been slow because so little is known of how cell-mediated immunity is normally regulated. Although much has been learned about the allied problem of what controls the formation of antibodies (4), almost nothing is known about the mechanism that regulates the production and function of the cells which mediate delayed-type hypersensitivity (DTH).[1]

While studying the tumor-suppressive activity of *Mycobacterium bovis* BCG it was observed that lymphoid tissues which were under the stimulatory influence of a BCG infection were capable of a much more vigorous response to a second antigen (5). Both cellular and humoral immunity to sheep red blood cells (SRBC) were augmented, as evidenced by higher and more sustained levels (DTH) and increased numbers of plaque-forming cells (PFC) in responding lymph nodes. Since DTH does not usually appear unless special conditions of immunization are used, these findings suggested that the formation of activated T cells is normally restricted by an inhibitory mechanism that does not operate properly in lymphoid tissues infected with BCG.

Miller et al. (5) have shown that mice given a subcutaneous injection of SRBC in saline develop a poorly sustained state of hypersensitivity which conforms to all of the established criteria by which DTH is recognized, including its mediation by θ-bearing lymphocytes. It was therefore possible to study the mechanism which regulates T-cell activity in the absence of any influence from adjuvants such as were used by Nelson and Mildenhall (6) when they, too, showed that mice develop classical DTH in response to SRBC.

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[1] *Abbreviations used in this paper*: DTH, delayed-type hypersensitivity; PFC, plaque-forming cells; SRBC, sheep red blood cells.

Materials and Methods

Animals.—Specific pathogen-free, outbred mice (CD-1 strain from Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were used at 4–6 wk of age. They were maintained on Sanicel bedding (R & E Feed Co., Troy, N. Y.), fed a vitamin-supplemented diet (Agway, Inc., Country Foods Div., Syracuse, N. Y.), and were segregated under Isocaps (Lab Cages, Inc., Kennett Square, Pa.).

Antigen.—SRBC were obtained from one animal which was bled at weekly intervals. Blood was collected into Alsever's solution, and was stored at 4°C. The cells were washed three times before use and were finally suspended at the desired concentration in normal saline.

Measurement of Lymphoproliferative Responses in the Popliteal Lymph Node.—The rate of cell division in popliteal lymph nodes responding to immunization by footpad inoculation of SRBC was measured in terms of thymidine incorporation into DNA. The method has been described previously (5).

Test for Delayed-Type Hypersensitivity.—Hypersensitivity was measured as the increase in footpad thickness 24 h after injecting an eliciting dose of 1×10^8 washed SRBC in a volume of 40 μl. The characteristics of the hypersensitivity measured in this way have also been described (5). As before, reactions were conventionally recorded against the day when the test dose of antigen was injected, rather than the day upon which the reaction was measured.

Assay for Plaque-Forming Cells.—PFC were assayed by local hemolysis in gel (5).

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.

RESULTS

The Effect of Antigen Dose on the Induction of DTH.—It was known from the early studies of Salvin (7) and of Uhr et al. (8) that dose and route of injection have a marked effect upon whether or not an immunological stimulus gives rise to delayed-type hypersensitivity (9). When varying doses of SRBC were injected intravenously or into one hind footpad, two different but highly reproducible patterns of response were found (Fig. 1). For reasons that will become apparent, hypersensitivity was measured on day 4 in the intravenously sensitized mice and on day 5 in animals sensitized by footpad inoculation. Maximum DTH was achieved with a dose of 10^5 SRBC in intravenously immunized mice. This was 100-fold less than the dose which gave maximum sensitization by footpad inoculation. Moreover, DTH was sharply reduced in animals receiving more than the optimal intravenous dose. This suggested that production or function of activated T cells might be blocked by excess antigen. The concept of "desensitization" is often invoked to explain loss of DTH in animals injected repeatedly with antigen. It implies that the mediators of DTH can be neutralized by antigen. The experiment recorded in Fig. 2 denies this as a possible explanation: Animals which had been immunized simultaneously by intravenous and footpad injections of 10^8 SRBC displayed almost no DTH when tested on day 5 (group C); but animals which received only the footpad injection of SRBC (group A) lost none of their hypersensitivity as a result of receiving an intravenous injection of 10^8 SRBC on day 4, one full day before testing (group D). Indeed, the desensitized animals gave slightly greater reac-

tions (compare groups A and D). Animals of the other two groups in Fig. 2 demonstrate again that an intravenous dose of 10^8 SRBC produced very little hypersensitivity (group B), and that unsensitized mice did not react to the eliciting dose of SRBC (group E).

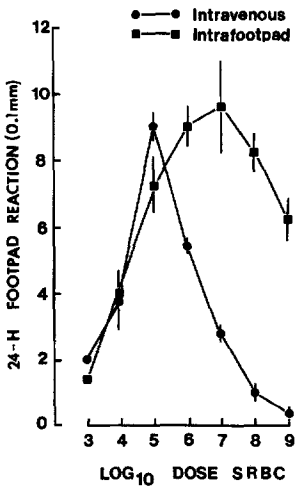

FIG. 1. Levels of delayed-type hypersensitivity generated in response to varying doses of SRBC. Antigen was administered either intravenously or into one hind footpad, and reactions were measured on days 4 and 5, respectively. Means of 5 ± SE.

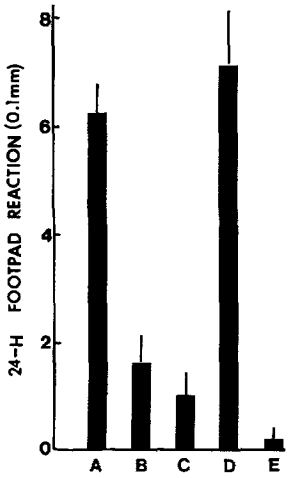

FIG. 2. Levels of DTH elicited in the left hind footpad of mice immunized with 10^8 SRBC by inoculation of the right hind footpad only (group A), by intravenous injection only (group B), by footpad and intravenous injection (group C), or by footpad inoculation followed by an intravenous injection of 10^8 SRBC on day 4 (group D). Animals of group E represent unsensitized controls. Footpad reactions were elicited on day 5 and read on day 6. Means of 5 ± SE.

Kinetics of the Immune Response to SRBC.—As desensitization did not seem to offer an explanation for the low levels of hypersensitivity displayed by heavily immunized animals, it had to be considered possible that excess antigen inhibits DTH by suppressing the proliferation of T cells. To examine this question, cellular proliferation rates were measured in popliteal lymph nodes responding to varying doses of SRBC, the resulting levels of DTH being followed in parallel. Sensitizing and eliciting injections of SRBC were given in opposite hind paws, and separate groups of mice were used on successive days to measure DTH and thymidine incorporation into DNA.

Fig. 3 shows a dose-dependent increase in the rate of thymidine incorporation into DNA in lymph nodes responding to varying doses of SRBC. Regardless of dose, the rates of DNA synthesis increased to a maximum on day 4. With the highest dose, hypersensitivity and the rate of DNA synthesis reached coincident peaks, but with lower doses hypersensitivity developed more slowly. The levels reached, however, were almost equal to those achieved more quickly with the highest dose of SRBC.

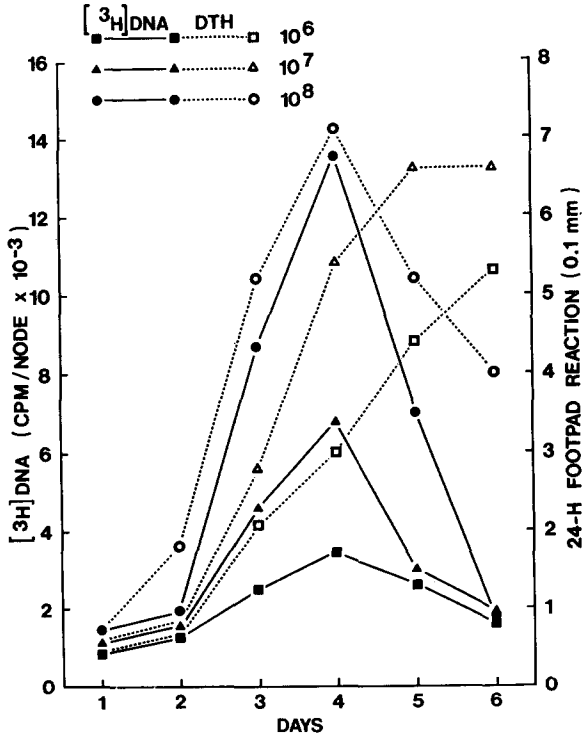

FIG. 3. Rates of thymidine incorporation into DNA by popliteal lymph nodes of mice inoculated in the footpad with varying doses of SRBC. The interrupted lines represent the levels of DTH measured in the contralateral footpad at the corresponding times. Plots of thymidine incorporation represent means of 10 individual popliteal lymph nodes from five mice injected in both footpads. Plots of DTH represent the means of five mice per group.

The pattern of DNA synthesis in responding nodes showed that a critical event occurs on day 4 of the immune response to SRBC. The sudden cessation of cell proliferation at that time stands in contrast to the much more protracted cellular response in nodes or spleens stimulated with living BCG (10) or virulent tubercle bacilli (11). The proliferative response to living mycobacteria continues to mount until immunity brings an end to bacterial growth and kills most of the infecting organisms. Withdrawal of the antigenic stimulus is not, however, a likely explanation for the sudden interruption of cell proliferation that occurred coincidentally in nodes responding to a 1,000-fold variation in antigenic dose. It seems much more probable that cell proliferation is actively inhibited by a product of the immune response. An inhibitory agent that prevents cell proliferation was therefore sought in the following way.

Effect of Intravenous Immunization on the Cellular Response in a Regional Lymph Node.—Mice were inoculated intravenously and in one hind footpad with 10^9 and 10^8 SRBC, respectively; the two injections were given at different times relative to each other. The proliferative responses in the popliteal lymph nodes were followed at daily intervals. They are recorded, together with the levels of DTH and peak numbers of PFC, in Fig. 4.

As in the observations reported by Radovich and Talmage (12), antigen given systemically 1 day in advance of the footpad stimulus (T_{-1}) interfered with cell

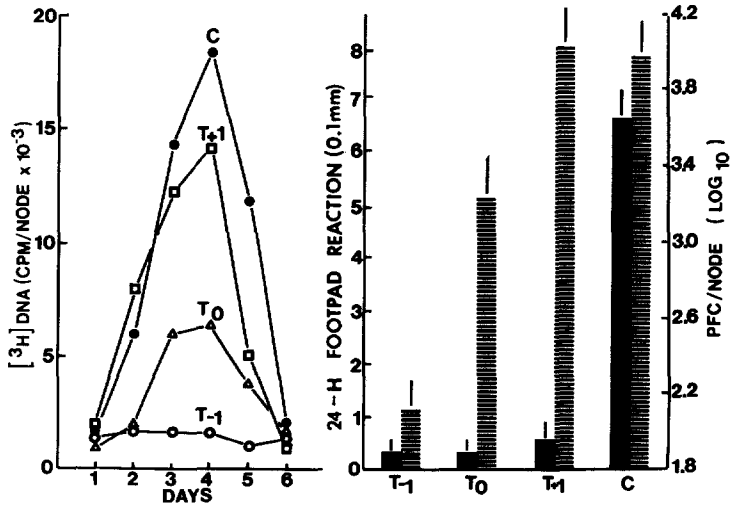

FIG. 4. (A) Levels of thymidine incorporation by popliteal lymph nodes in response to footpad inoculation of 10^8 SRBC in mice receiving 10^9 SRBC intravenously at different times relative to the footpad inoculation. The curve marked control (C) represents an animal which did not receive the intravenous injection of SRBC. (B) Corresponding levels of DTH (black) and numbers of PFC (hatched) in popliteal lymph nodes on day 4 (time of the peak) in mice of the groups represented in A. Control animals (C) received only the sensitizing footpad inoculum of SRBC. Means of 5 ± SE.

proliferation and PFC production in a peripheral lymph node. Since many of the cells that proliferate in response to SRBC are T cells (13–15), it was not surprising that animals which showed no increase in thymidine incorporation failed to develop DTH, but neither did animals given intravenous SRBC 1 day after the sensitizing footpad injection, yet cell proliferation in this group (T_{+1}) was substantially normal. This raised again the possibility that antigen injected intravenously can intercept and inhibit activated T cells that reach the circulation from nodes that are engaged in a seemingly normal response (cf. Fig. 3). The data of Fig. 2 made it quite clear, however, that mediator cells were not blocked by antigen given intravenously 1 day before testing for hypersensitivity. It seemed possible, therefore, that induction and expression of DTH were blocked by different mechanisms. In any event, blocking of cell-mediated immunity by intravenous antigen was clearly not an instantaneous process, but one requiring time in which to respond to the blocking stimulus.

Effect of Splenectomy on the Activation of T Cells.—The mechanism which interferes so profoundly with the activation of T cells is obviously touched off more quickly by intravenous than by subcutaneous immunization. Since the spleen is almost exclusively involved in the immune response when SRBC are administered intravenously (16), it was reasonable to think that the spleen would play a decisive role in the mechanism responsible for systemic inhibition of T cells. The data of Fig. 5 show that splenectomy, performed 3 wk before immunization, had a profound effect upon the response of mice to intravenous injection of varying doses of SRBC. Whereas a small dose caused DTH in normal mice, it failed completely to sensitize the splenectomized animal; and

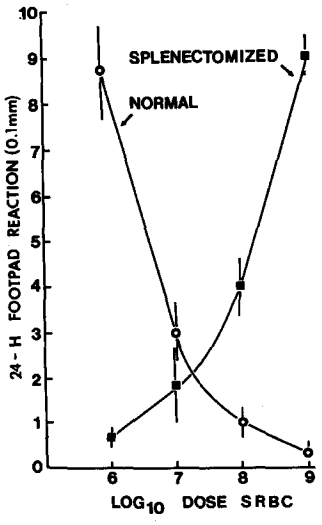

FIG. 5. Levels of DTH measured 4 days after intravenous inoculation of normal (○) and splenectomized (■) mice with the indicated doses of SRBC. Means of 6 ± SE.

large doses which blocked the intact animal were strongly sensitizing in splenectomized mice. The symmetry of this reciprocal relationship probably has no special significance, even though it is reproducible; it does show, however, that the spleen is at once the major source of the cells which mediate, and of the factors which block, the hypersensitivity produced by intravenous immunization. The data also establish that SRBC in the intravascular compartment can interact inductively with T cells in the absence of the spleen, but that the process is highly inefficient since it requires a stimulus almost 1,000-fold greater than suffices in the intact animal. Evidently, SRBC in the vascular compartment do not have ready access to lymphoreticular tissues other than in the spleen.

Effect of One Regional Response Upon Another.—The spleen would seem, then, to be the major cause of the abrupt interruption of T-cell activation in animals immunized intravenously. The experiment reported in Fig. 6 shows, however, that the products of the immunological response in one lymph node can depress responsiveness in the contralateral lymph node. To show this effect to best advantage, mice were stimulated in the left footpad with one of two different doses of SRBC, 10^6 or 10^8. 6 days later, mice of both groups re-

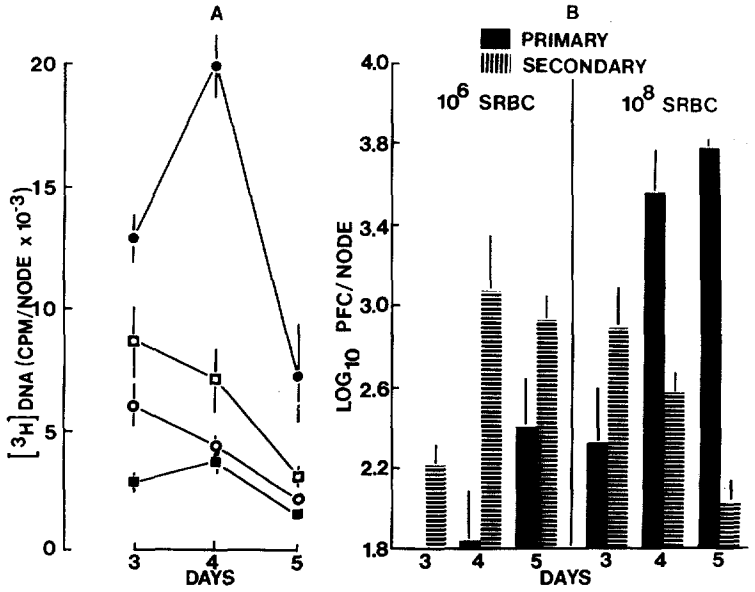

FIG. 6. (A) Rates of thymidine incorporation into DNA in popliteal lymph nodes on days 3, 4, and 5 of a primary response to footpad inoculation with 10^6 SRBC (■) or 10^8 SRBC (●). Incorporation rates were also measured at the same times in contralateral nodes responding to a secondary stimulus of 10^8 SRBC (□, ○) given 6 days after the primary stimulus. Separate groups of five mice were used for each time point. Means of 5 ± SE. (B) Histogram showing the corresponding numbers of direct PFC/node on days 3, 4, and 5 of primary responses to 10^6 or 10^8 SRBC, and the secondary responses to 10^8 SRBC in the contralateral lymph nodes which were stimulated 6 days later. Means of 5 ± SE.

ceived a second, large antigenic stimulus (10^8 SRBC) in the contralateral footpad. Rates of thymidine incorporation during primary and secondary responses were followed concurrently in mice which received both the first and second or only the second injection of SRBC.

Fig. 6 A shows that high and low doses of SRBC caused cell proliferation in proportion to the dose (cf. Fig. 3), and had corresponding effects on the secondary response in the contralateral node. This was severely depressed in both groups of mice as indicated not only by thymidine incorporation rates, but even more conspicuously by the relative numbers of PFC detected during primary and secondary responses. Whereas direct PFC production in the popliteal lymph nodes increased progressively in animals responding to both primary doses of SRBC, the numbers reached premature and much diminished peaks in modes responding to the secondary stimulus. The larger primary dose had the greater influence on both parameters of the cellular response.

Although these findings suggest that an immune response in one lymph node can influence a subsequent response in another. the possibility exists that antigen from the footpad inoculum could have reached the spleen and exerted its inhibitory influence from there. This is unlikely in view of the fact that PFC levels do not increase above background in the spleens of animals immunized by footpad inoculation; but to rule out this possibility completely the foregoing experiment was repeated in splenectomized mice with identical results.

The immunological induction of cells is evidently obstructed by something that appears very early in the immune response. The data of Fig. 7 show that the suppressor is produced in proportion to the intensity of the applied stim-

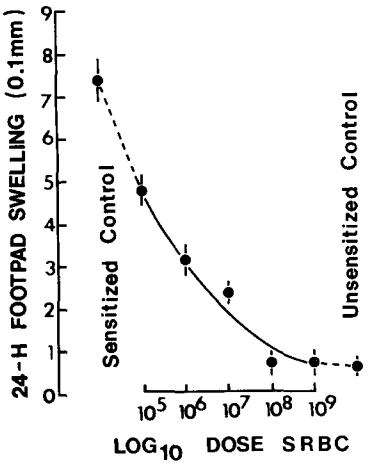

FIG. 7. Levels of DTH measured in the left hind footpad 4 days after a sensitizing inoculum of 10^8 SRBC had been given in the right hind footpad to mice injected 2 days previously with the indicated intravenous doses of SRBC. One group of controls received the sensitizing footpad but not the intravenous injection of SRBC, the other received neither. Means of 10 ± SE.

ulus: the larger the intravenous dose of SRBC the greater the reduction in the levels of DTH generated in response to a sensitizing injection of SRBC (10^8) given 2 days later in one hind footpad. Even the dose (10^5 SRBC) that gives maximum sensitization by intravenous immunization caused partial suppression of the response to a sensitizing injection of SRBC in the footpad. In this experiment hypersensitivity was measured 4 days after the footpad inoculation.

Persistence of DTH and Response to a Second Sensitizing Stimulus.—An eliciting dose of 10^8 SRBC was chosen for use throughout these studies because it represents a good sensitizing dose when injected into the footpad (Fig. 1). It was thus possible to test for responsiveness to reimmunization in an experiment designed to examine the natural decay of DTH. This was done merely by performing a second footpad test 4 days after the first. In the experiment recorded in Fig. 8, mice were immunized intravenously with 10^5 or 10^9 SRBC. The higher dose did not produce DTH at any time, whereas 10^5 SRBC caused hypersensitivity which peaked on day 4 and then decayed exponentially. The levels of hypersensitivity found in mice retested 4 days later are indicated by the interrupted line in Fig. 8. Even the animals which were first tested on days 1 and 2 were already refractory to the sensitizing effect of a test dose of SRBC. The reactions elicited in them on days 5 and 6 were actually less than those in mice being tested for the first time (solid line). Though not recorded, the mice immunized with 10^9 SRBC were also retested but failed to react. It should be emphasized that separate groups of mice were

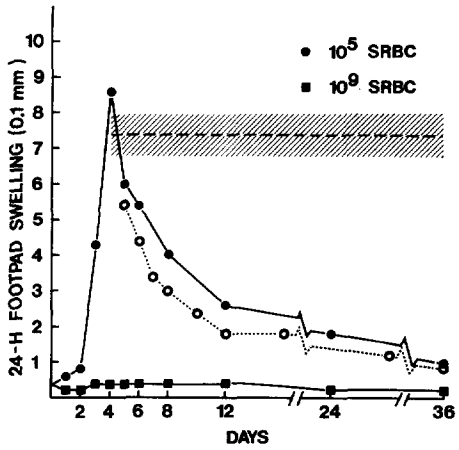

FIG. 8. Development and decay of DTH in mice injected intravenously with 10^5 (●) or 10^9 SRBC (■). The interrupted line represents the levels of hypersensitivity measured upon retest in the contralateral footpad 4 days after the first test for hypersensitivity. The horizontal line and area of hatching represents the mean level of DTH (±SEM) measured on day 4 in mice given only the eliciting dose of 10^8 SRBC. Animals immunized with the higher dose of SRBC (10^9) were also retested but showed no evidence of DTH at either test; only the first test is recorded. Means of 5 ± SE.

used at each time point. In a similar experiment, mice were still nonresponsive when tested on days 42 and 56.

An experiment of similar design was performed in splenectomized animals. It showed that hypersensitivity from an intravenous injection of 10^9 SRBC (a highly sensitizing dose in the absence of the spleen) decayed with a normal time-course (black bars, Fig. 9) and left the animal almost nonresponsive, for DTH was not significantly elevated in animals tested for the second time on days 8 and 25 (hatched bars, Fig. 9). On the other hand, 10^6 SRBC produced very little hypersensitivity by day 4 in splenectomized animals, but mice of this group reacted strongly when retested on day 8 (hatched bar, Fig. 9). Obviously the presence of 10^6 SRBC in the body had not compromised the T-cell responsiveness of splenectomized mice as they would an intact mouse (Fig. 7). It was significant, too, that hypersensitivity increased between days 4 and 8, but the trend was not maintained. Tests performed on days 14 and 21 showed that DTH had decayed in the normal way.

The hypersensitivity produced by the smaller dose of SRBC in splenectomized animals possessed another interesting feature, it was not accompanied by unresponsiveness. Animals with a low but significant level of residual hypersensitivity on day 21 gave a normal T-cell response to the eliciting dose of SRBC (hatched bar, day 25, Fig. 9). This shows that activated T cells do not

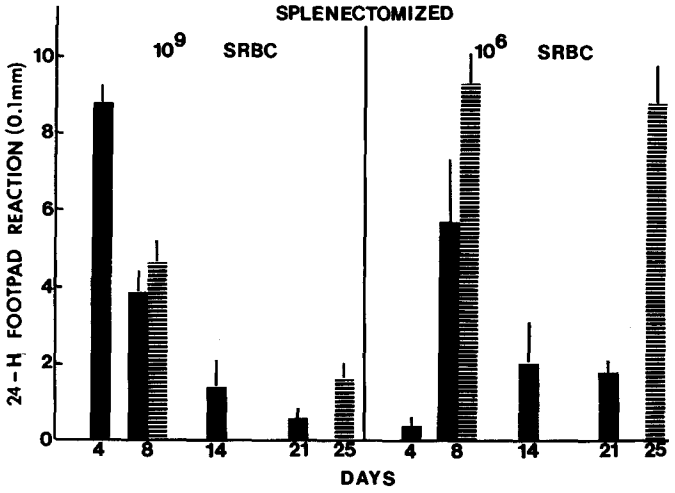

FIG. 9. Levels of hypersensitivity found in splenectomized mice after intravenous sensitization with 10^9 or 10^6 SRBC. The prompt onset and immediate decay of hypersensitivity in animals receiving the larger dose of SRBC is shown on the left (black). The retests performed in this group on days 8 and 25 showed little evidence of responsiveness (hatched). Animals sensitized with the smaller dose (right) developed sensitivity much more slowly (black) but responded vigorously to the eliciting dose of SRBC given on days 4 and 21. This is indicated by the large reactions recorded at retest on days 8 and 25 (hatched). Means of $5 \pm$ SE.

interfere with the further induction of T cells. It also suggests that the mediators of DTH are naturally short-lived cells because hypersensitivity decayed at a normal rate in the apparent absence of the inhibitory mechanism that prevents animals from responding to reimmunization.

The hemagglutinating antibody titers found in mice belonging to the groups represented in Fig. 9 showed no antibody on days 4 or 21 in splenectomized animals immunized with 10^6 SRBC. Mice given 10^9 SRBC had not formed antibody by day 4, but antibody to a titer of 1:512 was present on day 21 in these mice. It was all mercaptoethanol sensitive.

DISCUSSION

Although T cells serve an important ancillary role in the implementation of most humoral response, it is uncommon to find overt evidence that antigenic stimulation has caused activation of T cells. This is certainly so if DTH is taken as the index of T-cell activity. By this criterion, most immune responses would seem to be accomplished without creating significant numbers of specifically sensitized T cells. There are, however, some well known circumstances that give rise consistently to a more or less sustained state of DTH. Infection was such a common cause of this type of hypersensitivity that DTH was once known as bacterial allergy (17, 18). There are, however, some artificial ways of inducing DTH. The addition of mycobacteria to Freund-type adjuvants is, for example, the conventional method of producing DTH (9); but minute doses of antigen, particularly when complexed with antibody, cause DTH of somewhat limited duration (7, 8); and macrophage-associated (19–21) or macrophage-processed (22) antigens have also been found to favor the induction of DTH, sometimes without evidence of antibody formation (22). In contrast to this last finding, an ongoing response to living BCG, like that observed originally in tuberculous animals by Dienes and Schoenheit (23), conditions the immunological apparatus for an accentuated T-cell response despite a marked increase in antibody production (5).

At first sight, there is no obvious link between these diverse conditions that are conducive to the induction of DTH. This is another way of saying that we do not know what regulates the production of activated T cells. However, the present investigation recalls with some emphasis what Uhr, et al. had clearly established (8), namely that the induction of DTH depends critically on antigenic dose and route of immunization. SRBC, in the absence of adjuvant, were found in the present study to produce DTH even when administered intravenously. It appeared rapidly and reached its peak much earlier than when Freund-type adjuvants are used (1). The induction of DTH is exquisitely sensitive to blocking by an overdose of antigen if an adjuvant is not used. Thus an intravenous dose of SRBC in the range that gives maximum antibody responses (24) may produce no peripheral evidence of T-cell activity in the form of DTH (Figs. 1 and 8). Since splenectomy eliminates the blocking effect of

an intravenous injection of antigen, without affecting the T-cell response to SRBC injected into the footpad, it is apparent that splenectomy creates no dearth of competent precursor cells; but it does remove the major source of antibody production (16). This points directly to a role for antibody in regulating the participation of T cells in the immune response to SRBC, as it is known to have in the regulation of antibody formation (4).

There is, however, another possibility to be considered. An intravenous injection of SRBC causes prompt withdrawal of specifically responsive lymphocytes from the recirculating lymphocyte pool (25). As a result, thoracic duct lymph becomes depleted of cells which can render irradiated recipients responsive to SRBC. Though it is not yet certain which cell type is most affected by this process of selective trapping, Rowley et al. (25) give reasons for believing that both T cells and B cells are involved. It may be asked, therefore, whether animals fail to display DTH after an excessive dose of SRBC merely because reactive cells are trapped in a central organ such as the spleen. This would explain one aspect of the observed effect of splenectomy on the induction of DTH. Trapping has also been suggested (23) as an explanation for what O'Toole and Davies (26) have called "pre-emption," a phenomenon in which peripheral lymph nodes fail to give a PFC response 2–4 days after injecting SRBC into the peritoneal cavity. It is clear from Fig. 4 that peripheral nodes can be suppressed even more quickly by an intravenous injection of SRBC, and that cell proliferation is more completely blocked than is PFC production.

Despite the unequivocal evidence that cells can be specifically trapped, trapping alone does not explain why overdosed animals show no peripheral evidence of activated T cells. Delayed-type hypersensitivity depends upon activated lymphocytes which can function as mediators only by entering the blood. This means that T cells, though they may become trapped in central or peripheral lymphoid organs during the inductive phase of an immune response, must give rise to effector cells that are subsequently released to the circulation. The present studies show that animals previously sensitized by a footpad injection of SRBC remained fully sensitive for at least 24 h after a massive intravenous injection of SRBC (Fig. 2), yet the phenomenon of selective trapping occurs within 24 h of injecting antigen (25). It can be concluded, therefore, that specifically sensitized lymphocytes, once formed, are not subject to specific trapping by antigen alone. This conclusion is supported by the fact that cells which can support an immune response to SRBC have been shown to reappear and be fully represented in central lymph 4 days after an intravenous injection of SRBC (25). At this time, DTH remains fully suppressed in mice immunized intravenously with a comparable dose of SRBC (Figs. 1, 5, and 8). This means that the trapping which occurs in a primary immune response affects precursor cells only, and is a temporary event which is probably limited to the period of blast transformation and cell division that give rise to specifically sensitized lymphocytes (11). Hall and Morris (27) have

shown that there is, in fact, an initial fall in the output of cells from a regionally stimulated node. It ends with a massive release of blast cells to the efferent lymphatics (28). No doubt this efflux of cells includes the specific mediators of DTH which are known to be represented in central lymph (29). It is pertinent, too, that a subcutaneous injection of SRBC in Freund's complete adjuvant caused the same selective removal of reactive cells from central lymph (25), yet DTH appeared in due course. Obviously, the cells that mediate DTH reactions are not retained for long at the site of the primary immune response whether this occurs centrally or in peripheral lymphoid tissues.

The ease with which a small excess of antigen can interfere with the induction of a T-cell response (Fig. 7) raises a question concerning the interpretation of findings such as those of Pearson and Raffel (22) or of Parish (30, 31). The former showed that as SRBC become degraded after ingestion by macrophages they gain progressively in their ability to induce DTH, but lose activity as a stimulus to antibody production. Parish's observations show that chemical modification of SRBC (30) or flagellin (31) has a similar effect on antigenicity. In light of the present observations, these effects of antigen "processing" may be no more than would be expected from lowering the dose of antigen, or rendering a given dose less antigenic.

It is reported in the following paper (32) that antigen and antibody are both involved in regulating T-cell activity. Antigen which reaches the circulation provides not only a powerful stimulus to antibody formation by the spleen but is immediately available to interact with antibody and create factors that interfere with effector cells which circulate in the vascular compartment. There are, therefore, two reasons why DTH is difficult to induce by immunizing procedures that permit antigen to gain access to the circulation.

SUMMARY

Delayed-type hypersensitivity (DTH) develops in the absence of an adjuvant when mice are injected intravenously or subcutaneously with an appropriate dose of sheep red blood cells (SRBC). The optimal intravenous dose of 10^5 SRBC (in CD-1 mice) produces maximum DTH which decays exponentially from its peak on day 4. Increasing the dose of SRBC reduces and eventually abolishes all evidence of DTH. DTH fails to reappear in respose to secondary stimulation except in splenectomized mice in whom the development of DTH is not suppressed, even by massive doses of SRBC. Hence the suppression cannot be due to antigen as such.

The optimal dose of SRBC for sensitization by footpad inoculation is 100-fold higher (10^7 SRBC in CD-1 mice), but even 10^9 SRBC do not block the induction of DTH by this route of immunization. A blocking dose of SRBC, given intravenously 1 day before footpad inoculation, completely suppresses cell proliferation in the draining lymph node, prevents PFC production there, and blocks the induction of DTH by a sensitizing dose of SRBC. If given 1

day after footpad sensitization, intravenous antigen has little effect on the cellular response in the regional node but DTH is still completely suppressed. Blocking of induction and expression may depend, therefore, on different mechanisms.

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