A STABLE FORM OF DELAYED-TYPE HYPERSENSITIVITY*

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Delayed-type hypersensitivity (DTH)[1] develops naturally in the course of many infections (1) but is seldom a feature of artificial immunization unless the antigen is given in a dose too small to provoke an antibody response (2–4), has been chemically modified (5–7), or is administered in an emulsion containing mycobacteria (8). The state of DTH produced without a mycobacterial adjuvant is usually short-lived (3) and apt to disappear on renewed exposure to antigen, a skin test dose of antigen being enough to abolish reactivity in some cases (9). The DTH associated with infection, on the other hand, tends to be stable and long lasting. It is virtually impossible, for example, to desensitize tuberculin-sensitive guinea pigs by injections of old tuberculin (10). The DTH produced with Freund's complete adjuvant (FCA) is similar, for it is difficult to abolish. It is true that a large dose of antigen may cause a temporary depression of DTH (10), but reactivity soon returns (11).

The important quality of persistence thus seems to be characteristic of naturally occurring forms of cell-mediated immunity and those produced with an adjuvant containing mycobacteria. This may be due, however, to persistence of the antigenic stimulus itself. An active or latent infection, or a depot of emulsified antigen, could serve to prolong the immune response and maintain the production of specifically sensitized lymphocytes. The concept that a prolonged immune response causes persistence of DTH in animals immunized with antigen in FCA (12) is supported by the finding that mediator cells are delivered to thoracic duct lymph throughout a prolonged period of continuous drainage (13). There is evidence, however, to support the opposing view that the cells which mediate tuberculin sensitivity have a long life-span (14). This would allow them to function for extended periods without the necessity for constant renewal. Which of these alternatives explains the persistence of naturally occurring DTH and what makes it resistant to desensitization? Can this stable and enduring

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[1] *Abbreviations used in this paper:* AS4, absorbed 4-day serum; CY, cyclophosphamide; DTH, delayed-type hypersensitivity; FCA, Freund's complete adjuvant.

form of DTH be produced by immunizing procedures that do not make use of impractical adjuvants such as FCA? These questions have been examined in mice which were immunized under the modulating influences of cyclophosphamide (15) and BCG (16), which markedly enhance the T-cell response to sheep red blood cells (SRBC).

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 transfer was performed between syngeneic hosts. The strains used were CH-T_6F_1 ♀ (Balb/c × CBA/H-T_6) and B6H-T_6F_1 ♀ (C57BL/6 × CBA/H-T_6).

Antigen. SRBC from the same animal were obtained twice weekly. They were collected and stored in Alsever's solution, and were washed three times before suspending to a known density in normal saline.

BCG. The Pasteur strain of *Mycobacterium bovis* BCG (TMC 1011) was obtained from the Trudeau Mycobacterial Collection (17). The organisms were grown as dispersed cultures in Middlebrook's 7H9 medium containing Tween 80. After 5–7 days incubation, the cultures were frozen slowly to −70°C and were stored at this temperature (18). Dosage was based upon viable counts performed by plating on Middlebrook's 7H10 medium.

Immunization. Different routes and doses of SRBC were used for different purposes. But except when specified, primary immunization was by intravenous or foot pad inoculation of 10^8 SRBC. Modulation of the primary immune response was achieved with cyclophosphamide (CY) (donated by Mead Johnson and Co., Evansville, Ind.) or by BCG. The drug, in a dose of 200 μg/kg, was given 2 days before intravenous immunization and concurrently with subcutaneous immunization (15). BCG, on the other hand, was always injected 14 days before specific immunization with SRBC. Both BCG (10^7 viable U) and SRBC (10^8) were given intravenously. The same timing was adhered to when both agents were used to modulate the immune response. BCG was not used in these experiments to modulate responses in peripheral lymphoid tissue (16, 19). Secondary immunization was performed in most instances by the intravenous injection of 10^8 SRBC.

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 absorption with SRBC, as previously described (20).

Test for DTH. DTH was measured in the right hind foot pad with an eliciting dose of 10^8 SRBC (19).

Spleen cell transfer. Spleen cells for transfer to syngeneic recipients were prepared as in a previous study (21). The cells were pooled, washed three times, and enumerated in a hemacytometer. Viability, as assessed by dye exclusion with trypan blue, was not less than 90%.

Vinblastine. Vinblastine sulfate (donated by Eli Lilly & Co., Indianapolis, Ind.) was freshly dissolved in sterile saline. An intravenous dose of 100 μg was given 15–17 h before the harvesting of spleen cells.

Results

Persistence of DTH after an Unmodulated Primary Response. The DTH that develops in response to intravenous immunization of mice with SRBC does not persist for long, even after the optimum dose of antigen (4). Fig. 1 shows that the time-course of the DTH generated by unmodulated responses to intravenous immunization varies with dose of SRBC. The peak level of DTH increased as the dose was raised from 10^3 to 10^5 SRBC; but at higher doses DTH decayed with increasing rapidity, and could not be detected at any time in mice immunized

with 10^9 SRBC. A dose of 10^4 SRBC, which does not provoke an antibody response (20), gave the highest persisting level of DTH at 24 days (Fig. 1).

The Anamnestic Response after an Unmodulated Primary Response. Since 10^4 SRBC gave the highest sustained level of DTH after intravenous immunization, this dose and route were used for both primary and secondary immunization. The secondary stimulus was applied after 14 and 28 days. Fig. 2 A shows that despite a significant level of DTH after the primary stimulus, animals failed

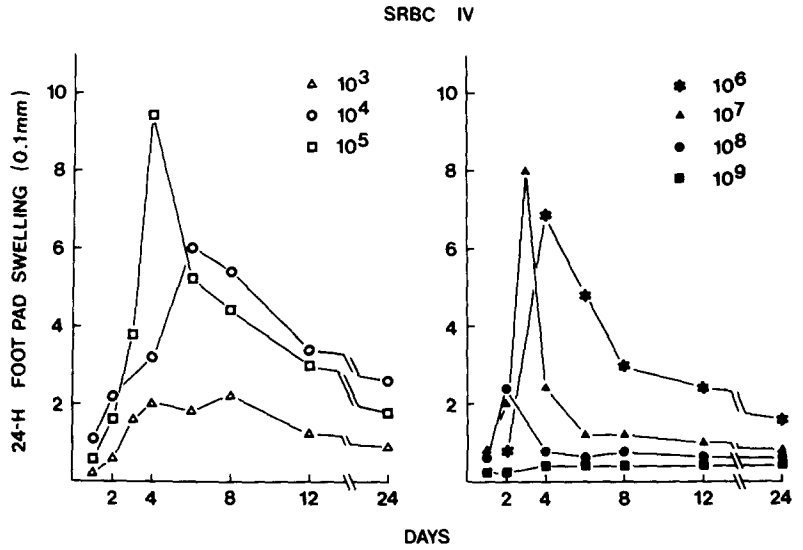

FIG. 1. Development and decay of DTH in groups of mice immunized intravenously with varying doses of SRBC. Tests for DTH to SRBC were performed on the days indicated and were read 24 h later. Means of 5.

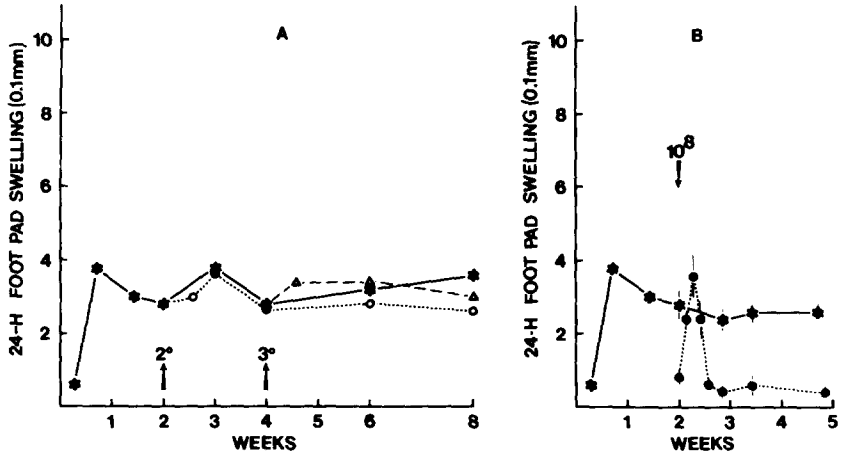

FIG. 2. (A) Levels of DTH found in mice immunized intravenously with 10^4 SRBC (—) and in mice which had received a second (O---O) or third (▲---▲) dose of 10^4 SRBC 14 and 28 days after the primary stimulus. Means of 5. (B) Levels of DTH found in mice immunized intravenously with 10^4 SRBC (* — *) and in a subgroup which received a secondary intravenous stimulus of 10^8 SRBC 14 days after the primary stimulus (●---●). Means of 5 ± SEM.

to respond to the second or third stimulus with any detectable rise in DTH; but neither was there any loss of DTH. However, when the secondary dose of SRBC was raised to a level (10^8) that caused rapid quenching of T-cell activity during the primary response (Fig. 1), DTH disappeared completely (Fig. 2 B). This confirms that the DTH resulting from minimal antigenic stimulation is abolished by secondary immunization (9).

The Anamnestic Response after a Modulated Primary Response. CY raises the level of DTH by delaying antibody production, thereby postponing the onset of feedback inhibition of the T-cell response (15); BCG has a similar effect, but it is achieved without interfering with antibody production (16). It was important, therefore, to observe the anamnestic T-cell responses of animals with residual DTH resulting from sensitization under the modulating influence of CY, BCG, or both.

Mice which had been treated with CY at the time of sensitizing with a foot pad inoculation of 10^8 SRBC had a DTH level of 9.8 when tested 21 days later (Fig. 3 A). At this time they were challenged intravenously with a secondary dose of 10^8

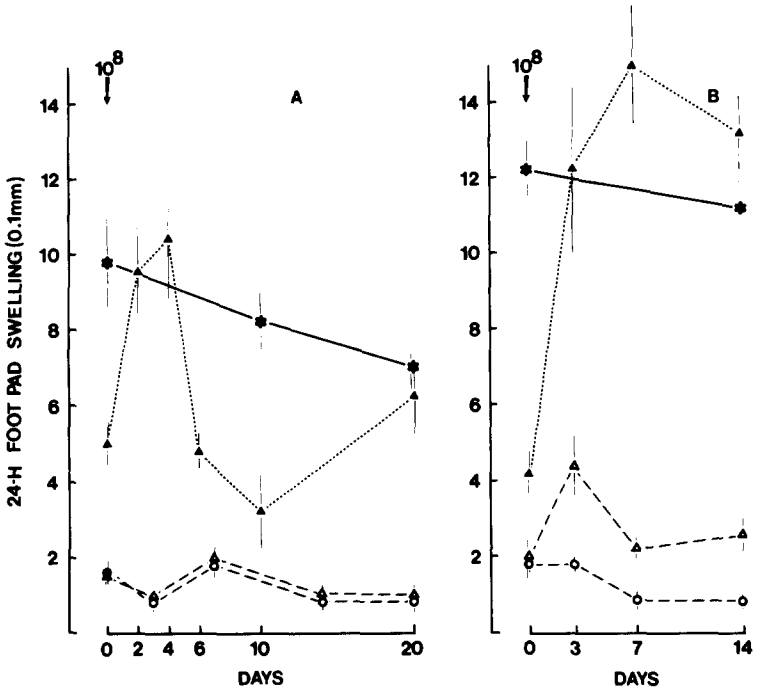

FIG. 3. (A) Level of DTH prevailing from day 21 onward in mice which had been treated with CY and sensitized concurrently in the foot pad with 10^8 SRBC (* — *). A secondary intravenous stimulus of 10^8 SRBC (arrow) caused an immediate fall in DTH and a subsequent trough of desensitization (▲---▲). Control mice were not given the primary dose of SRBC; but one group was treated with CY (O---O), the other was not (Δ---Δ). Means of 5 ± SEM. (B) Level of DTH prevailing from day 21 onward in mice which had been sensitized intravenously with 10^9 SRBC 14 days after intravenous priming with 10^7 BCG (* — *). A secondary intravenous stimulus of 10^8 SRBC (arrow) caused immediate loss of DTH from which recovery was prompt and permanent (▲ · · ▲). One group of controls received BCG but no primary dose of SRBC (Δ---Δ), the other received only the secondary dose of SRBC (O---O). Means of 5 ± SEM.

SRBC, a dose that erased the DTH resulting from an unmodulated primary response (Fig. 2 B). In this experiment it caused an immediate loss of DTH, presumably because of temporary trapping of reactive cells in the spleen (22, 23), and a phase of desensitization from which recovery was slow and incomplete (Fig. 3 A).

In an experiment of similar design, mice were immunized intravenously with 10^8 SRBC 14 days after intravenous injection of 10^7 viable BCG. They had a comparable level of DTH on day 21 (Fig. 3 B). When challenged intravenously with the same secondary dose (10^8) of SRBC, they also showed an immediate loss and rapid recovery of DTH. But unlike the secondary response in mice with CY-modulated immunity (Fig. 3 A), this one did not show a trough of desensitization. Mice of one control group in the preceding experiment had been given BCG but not the primary antigenic stimulus. They responded with a modest enhancement of DTH in response to the secondary antigenic stimulus (Fig. 3 B). Since this was probably due to the modulating influence of the BCG infection (now 5 wk old), tests for anamnestic responsiveness were performed in the next experiment at 3 wk and again at 16 wk when the effects of BCG would have long passed (16).

In the next experiment, DTH was induced under the modulating influence of both BCG and CY. Secondary antigenic stimuli were given at 3 and 16 wk. They both provoked strong anamnestic responses (Fig. 4) which were devoid of the episode of desensitization that characterized the anamnestic responses of animals which had not been infected with BCG (Figs. 2 and 3 A). It was apparent, therefore, that DTH developed under the influence of BCG leaves the capacity to respond to a secondary stimulus intact, but obviates the episode of desensitization that normally accompanies it. In view of these findings, it became necessary to test animals immunized in different ways for the presence or absence of feedback inhibition during a secondary immune response. Since CY had been shown to cause temporary abolition of feedback inhibition during a primary response (15), it was used here in the expectation that it would have a similar effect on the secondary response.

Influence of Feedback Inhibition on Anamnestic Responses. Two groups of mice were prepared. Each possessed DTH resulting from a primary response to 10^8 SRBC. The primary response was modulated by CY in mice of one group and by both BCG and CY in the other. Mice of both groups were subdivided 3 wk later so that half of them could be given CY 2 days before the secondary antigenic stimulus (10^8 SRBC), which was also given intravenously.

The results in Fig. 5 A show that the animals in which the primary response had been modulated only by CY again showed a phase of partial desensitization, whereas those which received a second dose of CY 2 days before the secondary antigenic stimulus showed only an acute and transient depression of DTH from damage done by CY to the nonspecific accessory cells (monocytes) needed for the expression of DTH (15). Mice which had initially responded under the joint influence of BCG and CY behaved quite differently (Fig. 5 B). Although the nonspecific effect of CY on accessory cells was again in evidence in the subgroup which received the second dose of CY, the phase of desensitization, which is immunologically specific (unpublished results), was absent in both groups. Thus,

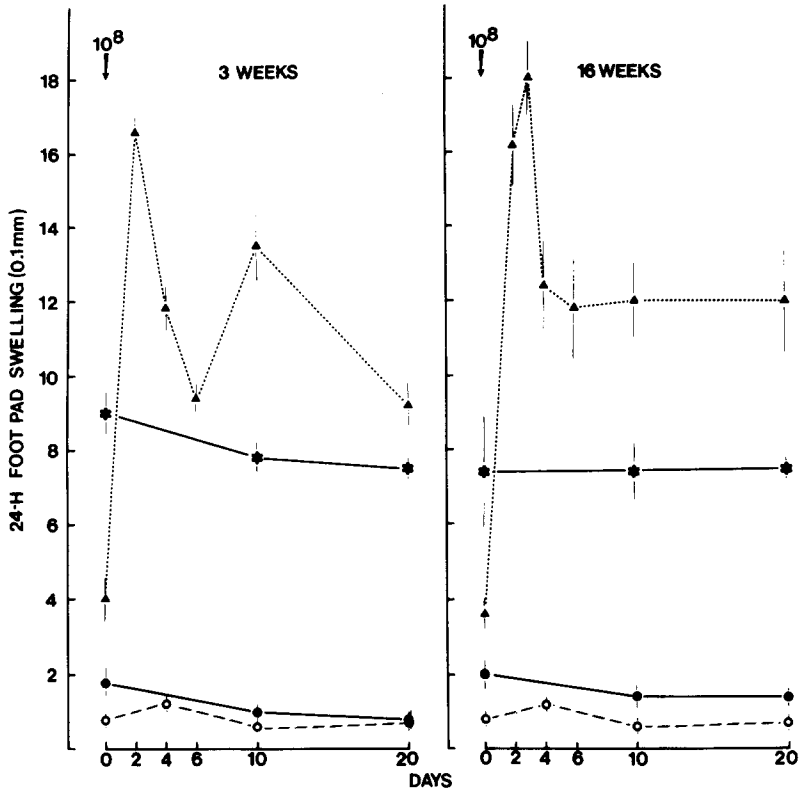

FIG. 4. Anamnestic T-cell responses in mice injected with 10^8 SRBC intravenously, 3 or 16 wk after primary intravenous immunization with 10^9 SRBC which was given under the modulating influence of both BCG and CY (▲···▲). The background level of DTH in the absence of challenge remained almost constant throughout (* — *). Untreated control mice received both primary and secondary doses (O---O) or only the primary dose of SRBC (●—●); they were devoid of hypersensitivity. The low levels of DTH recorded in these controls reflect a trend that occurs spontaneously as mice grow old. Means of 5 ± SEM.

when the primary response occurred under the influence of BCG, CY had nothing but a delaying influence on the secondary T-cell response. It seems, therefore, that BCG-modulated immunity is not subject to feedback inhibition. If it were, CY would have enhanced the T-cell response as it does during a primary response (15) or the secondary response in CY-modulated immunity (Fig. 5 A). This finding focuses attention on the properties of the specifically sensitized lymphocytes created under the influence of BCG. Their longevity and susceptibility to blocking by immune complexes become questions of immediate interest.

Properties of Specifically Sensitized Lymphocytes. Continuous production of specifically sensitized lymphocytes was raised at the outset as a possible explanation for the persistence of DTH after immunization with FCA. If the cells that mediate DTH were produced continuously under the influence of BCG, they would remain susceptible to a mitotic poison such as vinblastine (13, 24).

A transfer system was used to test the specific mediators of DTH for their vulnerability to vinblastine because specifically sensitized lymphocytes are not

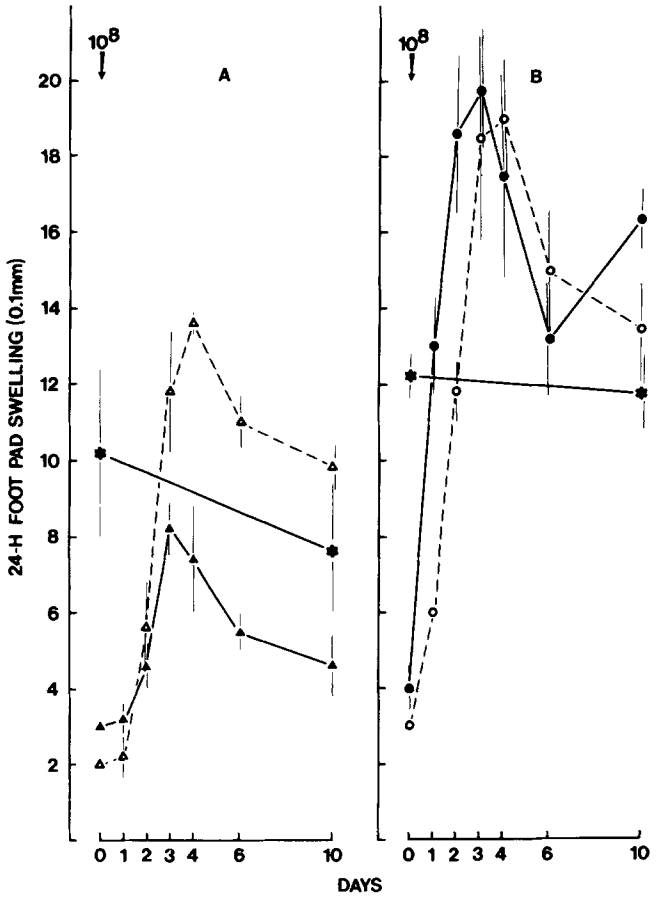

FIG. 5. (A) Influence of CY on the anamnestic T-cell response of mice in which the primary response had been modulated with CY to give a high and well sustained level of DTH (* — *). When the secondary challenge of 10^8 SRBC was given (arrow) DTH levels fell after challenge, but rose steeply before the onset of a phase of partial desensitization (▲—▲). In mice given CY (200 mg/kg) 2 days before challenge DTH was restored more rapidly and reached higher levels than in unchallenged mice (Δ---Δ). Means of 5 ± SEM. (B) An experiment of similar design was performed in mice in which primary immunization with 10^9 SRBC was modulated with both BCG and CY. In this case CY given at the time of challenge did not potentiate the secondary T-cell response, though it did cause a definite delay in its onset. Means of 5 ± SEM.

the only relevant cells that are potentially susceptible to this drug (15, 25). Donor mice were treated with CY 2 days before intravenous immunization with 10^8 SRBC. Groups of prospective donors were treated at intervals with vinblastine and were sacrificed 15–17 h later for spleen cell transfer. An equal number of untreated donors were used at each time point. Recipients were challenged immediately after cell transfer with an eliciting dose of SRBC.

Fig. 6 shows the development of DTH in a group of animals immunized under the influence of CY, and the level of DTH transferred to recipients by one spleen equivalent of cells from similarly immunized donors which had or had not been treated with vinblastine 15–17 h before cell transfer. Two distinguishable

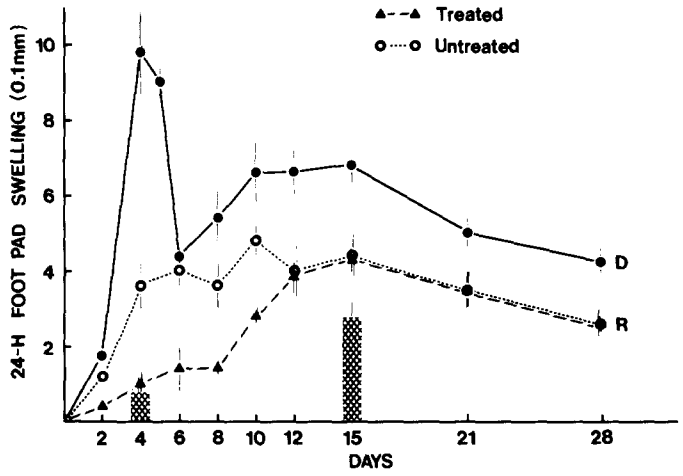

FIG. 6. Susceptibility of specifically sensitized spleen cells to vinblastine. Donor mice (D) were immunized intravenously with 10^8 SRBC 2 days after treatment with CY. Representatives were tested for DTH at intervals during the next 28 days (●—●). At each time point one spleen equivalent of cells were transferred to normal recipients (R) which were then tested for DTH. Two groups of donors were used: one was untreated and the other was given vinblastine (100 μg/mouse) 15–17 h before cell transfer. The levels of DTH are recorded for recipients from untreated (O · · · O) and vinblastine-treated donors (▲---▲). Means of 5 ± SEM. 1 h before the transfers were made on days 4 and 15, five additional recipients were each given 0.2 ml of blocking serum (AS4). The DTH found in these mice is represented by the hatched columns. Means of 5 ± SEM.

populations of specifically sensitized lymphocytes were revealed: one, produced during the first 8–10 days of the immune response, which was inactivated by vinblastine; the other, which emerged more slowly, was not. Hence, the DTH transferred by one spleen equivalent of cells harvested beyond day 10 of the immune response was undiminished by treating the donors with vinblastine. In the much more highly sensitized animals which had been immunized under the joint influence of BCG and CY a similar pattern of susceptibility to vinblastine was observed except that mediator cells remained sensitive to vinblastine over a much longer period (Fig. 7). In either case, the results indicate that sooner or later DTH becomes vested in cells that cease to replicate. Since DTH persists despite evidence that production of the mediators has ceased, the responsible cells would be expected to survive well in syngeneic recipients.

The Life-Span of Specifically Sensitized Lymphocytes. Specifically sensitized lymphocytes were transferred to syngeneic recipients from donors which had been immunized with a view to producing a high level of hypersensitivity for adoptive transfer, or to permit the behavior of replicating and resting cells to be compared. In the first experiment donor mice were infected intravenously with 10^7 BCG Pasteur. After 12 days they were given CY and 2 days later they were immunized intravenously with 10^9 SRBC. Animals immunized in this way have a stable and high level of DTH by day 21 (reference 16 and Fig. 7). The mean level of DTH at this time in a representative group of five donors was 13.8 (Fig. 8). The level of DTH conferred with one spleen equivalent (about 2×10^8) of pooled

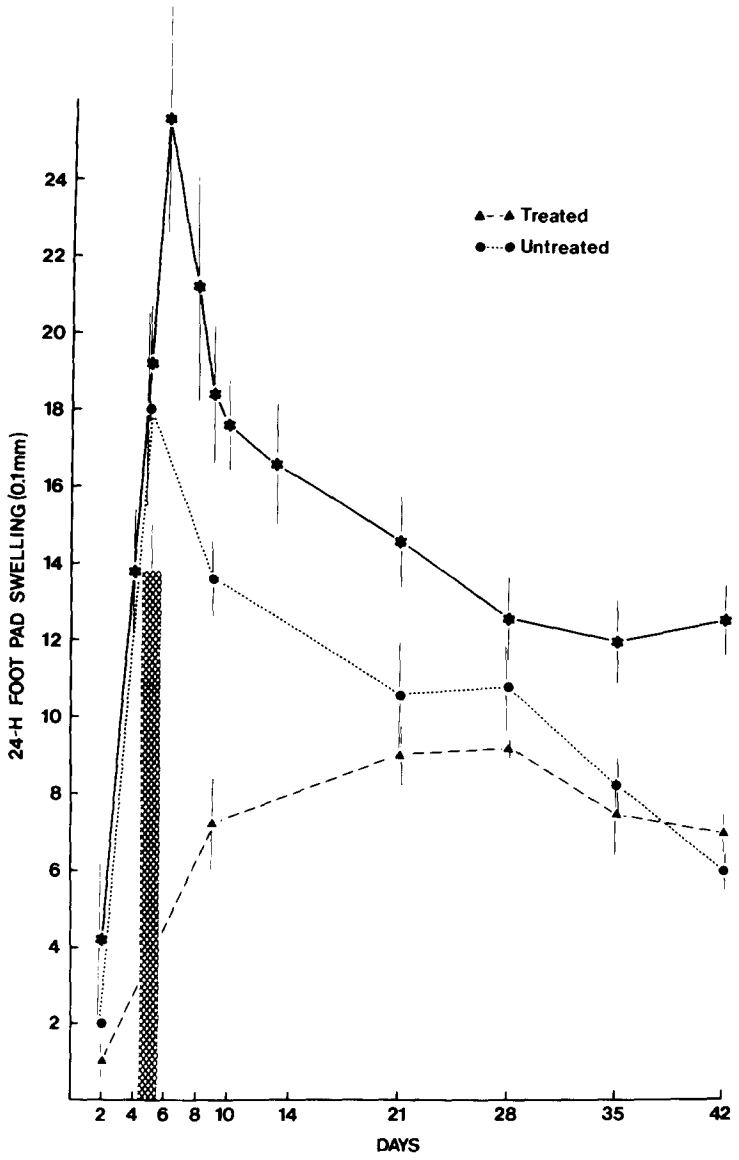

FIG. 7. In an experiment of similar design to that of Fig. 6, donor mice were immunized intravenously with 10^9 SRBC after treatment with BCG and CY. Representative mice were tested for DTH at intervals after immunization (* — *). At each time point one spleen equivalent of cells from normal or vinblastine-treated donors were transferred to normal recipients. The scale used to record the levels of DTH in recipients of untreated (● · · · ●) and vinblastine-treated spleen cells (▲---▲) is the same as that used in Fig. 6. Means of 5 ± SEM. On day 5 a separate group of recipients was given 0.2 ml of AS4 intravenously 1 h before receiving sensitized cells from untreated donors. The level of hypersensitivity in these recipients is depicted by the hatched bar. Mean of 5 ± SEM.

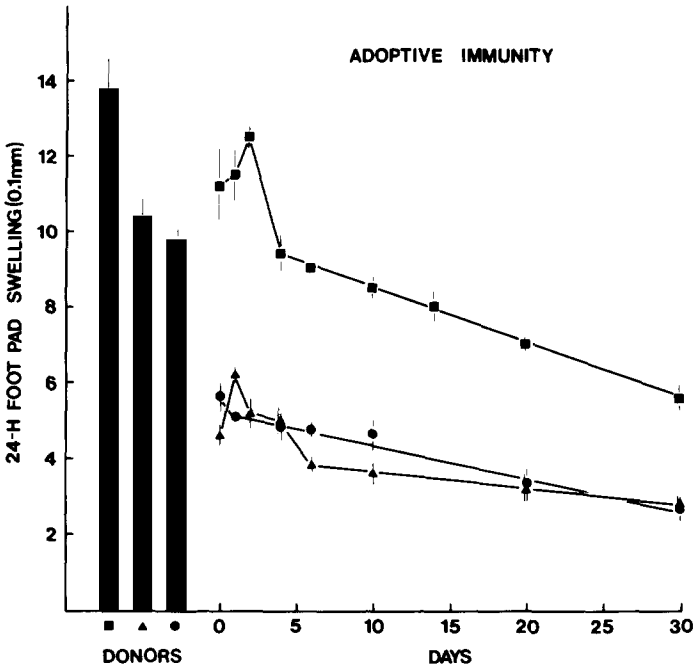

FIG. 8. The persistence of DTH was followed for a period of 30 days in mice immunized adoptively with one spleen equivalent of cells from donors sensitized under the modulating influence of both BCG and CY and used for transfer on day 21 (■) or modulated with CY and used for transfer on day 5 (▲) or day 14 (●). Corresponding symbols are used to show the levels of DTH in the donors and recipients. Means of 5 ± SEM.

spleen cells from these donors rose during the first 2 days after cell transfer. This may reflect a continuing replication of mediator cells, as suggested by their partial sensitivity to vinblastine at this time (Fig. 7). After this initial period of equilibration the level of sensitivity in recipients decayed slowly with an apparent half-life of 34 days. A notable feature was the remarkably uniform level of DTH found within the groups of five recipients tested at each time point.

In the second experiment two types of donors were used. Neither had received BCG, thus avoiding the objection to the foregoing experiment that BCG would have been transferred with the spleen cells. The first group of donors provided a high level of DTH during the period of cell proliferation when specifically sensitized cells are vinblastine sensitive. They had been treated with CY 2 days before intravenous immunization with 10^8 SRBC. When transfer was performed on day 5 of the response the donors gave a mean DTH reaction of 10.4. The second group of donors had been treated with CY and sensitized concurrently with a foot pad inoculum of 10^8 SRBC. Spleen cells from these donors were transferred 14 days later when vinblastine sensitivity had passed and the level of DTH had fallen to 9.8. The levels of DTH measured in recipients of one spleen equivalent (about 1×10^8 cells) are recorded in Fig. 8. They show that the DTH conferred by replicating and resting cells decayed at comparable rates, and provide strong presumptive evidence that the early and later mediators of DTH

are lineally related. In this experiment, the apparent half-lives of the reactive cells were longer (about 45 days) than in the previous experiment in which the influence of BCG may have been transmitted to the recipients.

Susceptibility to Blocking by Immune Complexes. In the experiment recorded in Fig. 6, two groups of recipients of the spleen cells harvested on days 4 and 15 were treated with blocking serum. This was prepared by absorbing immune serum with an equal volume of packed SRBC (20). The absorbed 4-day serum (AS4), 0.2 ml of which was given to recipients 1 h before cell transfer, completely abolished DTH in recipients of 4-day immune spleen cells, but was only partially inhibitory for cells harvested on day 15. More significant still was the finding (Fig. 7) that the sensitized cells harvested on day 5 from the BCG/CY-treated donors were also highly resistant to blocking by AS4.

Discussion

Following the precedent of Raff and Cantor (26), the following convention will be used to designate the specific mediators of DTH: The post-thymic precursors of specifically committed lymphocytes will be called T2 cells; the vinblastine-sensitive cells which are found during the proliferative phase of the immune response, and which are so readily inhibited by blocking serum (Fig. 6), will be called T3 cells; while the vinblastine-resistant cells that come later in the immune response, and are relatively resistant to blocking, will be called T4 cells.

The present studies have shown (Fig. 1) that an unmodulated response to SRBC generated little of the T-cell activity represented by DTH. Moreover, the low level of T4-mediated DTH that did result from the most effective dose of SRBC (10^4) could not be built upon by further antigenic stimulation at the same dose level (Fig. 2 A). It was also quite unstable in that a larger dose of antigen extinguished all remaining DTH (Fig. 2 B). It seemed as though all of the T4 cells created by the primary response had been consumed, perhaps in helper function, during the secondary response. Some findings seemed to support this explanation, for when the general level of T-cell activity was raised to a much higher plane by immunizing under the modulating influences of CY (Fig. 3 A) or BCG (Fig. 3 B), DTH was not abolished by a secondary antigenic stimulus of the same magnitude. There was, however, a very notable difference between the secondary responses given by mice in whom the primary responses had been modulated by CY or BCG. In the former the secondary T-cell response was apparently still susceptible to feedback inhibition (Fig. 3 A), an impression that was confirmed by the fact that CY had the same potentiating effect on the T-cell response during secondary antigenic stimulation (Fig. 5 A) as it has during a primary response (15). In BCG-modulated immunity, on the other hand, the secondary T-cell response was apparently protected against feedback inhibition (Fig. 3 B) and could not be potentiated by treating with CY at the time of the secondary antigenic stimulus (Fig. 5 B).

It seemed certain that the observed freedom of BCG-modulated immunity to give an uninhibited secondary T-cell response was not due directly to any persisting effect of active infection with BCG because previous studies had shown that the modulating influence of BCG on a primary response had largely

disappeared within 6 wk (16), yet animals immunized under the modulating influence of BCG plus CY responded to secondary immunization at 16 wk as well as they did at 3 wk (Fig. 4). It should be noted, too, that control mice which had not received the primary antigenic stimulus but had been infected with BCG for 5 wk showed only a minor degree of potentiated T-cell activity when given SRBC at the time of the secondary challenge in the experiment recorded in Fig. 3 B. Evidently the capacity of the primed mice to give an exaggerated secondary T-cell response was due to conditions that originated during the primary response, as modulated by BCG.

There are two possible reasons for the abnormal immunological responsiveness of mice with BCG-modulated immunity: either their T4 cells are different or they exist in an altered environment. In either case, the findings seem to explain why a tuberculin test causes enhancement of tuberculin sensitivity (27), even though tuberculin cannot induce tuberculin sensitivity in the first place; and why repeated skin testing should raise the level of hypersensitivity after BCG vaccination in man (28). These observations suggest that T4 cells may differ from T2's, their uninduced precursors, in being more easily engaged by antigen. This, however, would not explain the difference between the secondary responses in BCG- and CY-modulated immunity. Nor, would it explain why the T3 cells produced under the combined influence of BCG and CY were so resistant to blocking by AS4 (Fig. 7) while those formed under the influence of CY alone were completely inhibited by it (Fig. 6). Resistance of activated T cells to blocking by immune complexes no doubt explains why BCG-modulated immunity is not susceptible to feedback inhibition (Figs. 3 B and 5 B) and provides an alternative explanation for the previously reported finding that activated T cells formed under the influence of a BCG infection are resistant to specific blocking serum (16). Unfortunately, the present observations do not indicate whether the cells themselves are physiologically different, or whether they merely behave differently because of conditions created when a primary immune response is enacted in the presence of a BCG infection.

Since the production and function of activated T cells is largely governed by the humoral response (20), the stability of BCG-modulated immunity may depend upon the nature of the humoral response that accompanies it. It has long been known, for example, that FCA favors γ_2-antibody production (29, 30). It may be, therefore, that the antibody produced in a BCG-modulated response is predominantly of a type that cannot form the complexes which block T cells (20). Alternatively, the antibody formed during the secondary response in BCG-modulated immunity might quickly inhibit IgM production (31). And since the complexes formed with IgM have a powerful blocking effect on T3 cells (unpublished results), this could delay the shutdown of the T-cell response and cause the observed rise in the level of T-cell activity after a secondary stimulus (Fig. 4).

This explanation is not consistent, however, with the results of Fig. 7 which show that T3 cells may be resistant to passively administered blocking serum. Since the cell donors in this experiment were treated with CY 2 days before immunization, they would have suffered a delay in the onset of antibody production. But at the time when spleens were taken (day 5) antibody production

could already have resumed and may have included a class of antibody with the ability to cause "unblocking" (32). These are important questions but they will remain unanswered until more studies have been performed.

The correlation observed by Coe et al. (13) between the content of large lymphocytes in thoracic duct lymph and the capacity of its cells to transfer DTH makes it likely that specifically sensitized lymphocytes were still being actively formed at the time chosen by these authors to observe the traffic of specifically reactive cells in central lymph. The protracted survival of functionally active mediator cells in syngeneic recipients (Fig. 8) makes it certain, however, that DTH can persist for an extended period without constant renewal of the mediators. Nevertheless, a discrepancy was noted in the rate of decay of DTH in syngeneic recipients of immune spleen cells. In one case, a half-life of 34 days was computed; in two others it was rather longer (45–50 days). In an unreported experiment, mice sensitized under the influence of BCG and CY were followed for a period of 24 wk without retesting. DTH persisted in them with a half-life of 35 wk. There is, therefore, a possibility that the state of DTH, like that of antibody formation (33), may be driven by residual antigen; and that antigen can be carried into recipients with the spleen cells used for adoptive immunization. This does not contradict the evidence indicating that specifically sensitized lymphocytes have a long life-span which seems to be comparable with that of the long-lived memory cells of rats (34).

Summary

An antigen dose below the level needed to provoke an antibody response produces in mice a persistent, but minor degree of delayed-type hypersensitivity (DTH) to sheep red blood cells. The DTH is unstable. It is erased by larger doses of antigen and cannot be built upon by further antigenic stimulation. The much higher levels of DTH resulting from immunization under the modulating influence of cyclophosphamide (CY) or BCG persist under strong secondary antigenic stimulation, though the former is subject to partial suppression unless CY is used to prevent the secondary humoral response. The DTH produced by a BCG-modulated primary response is not subject to this suppressive effect of a secondary antibody response. In this case the anamnestic T-cell response is very brisk and cannot be potentiated by giving CY at the time of the secondary antigenic stimulus. This effect is not due to the modulating influence of a residual BCG infection. It results from a permanent change induced during the primary response.

The mediator cells formed under the influence of BCG are apparently resistant to inhibition by blocking serum containing immune complexes. Even the actively dividing T cells which are susceptible to vinblastine, and most readily blocked in the absence of BCG, are highly resistant to blocking by immune complexes. It is not clear whether these cells are intrinsically different or whether their insensitivity to blocking results from features peculiar to the humoral response that accompanies a BCG-modulated primary response. The mediator cells produced by both BCG- and CY-modulated responses become vinblastine

resistant, relatively insensitive to humoral blocking factors, and capable of surviving in a functionally active form in syngeneic recipients with an apparent half-life of about 50 days. There were indications, however, that their effective life-span may be greatly extended in some circumstances by persisting antigenic stimulation; and in the case of BCG-modulated immunity the prevailing level of T-cell activity can be greatly augmented by a further antigenic stimulus without the necessity for renewed exposure to BCG.

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