FEEDBACK INHIBITION OF SPECIFICALLY SENSITIZED
LYMPHOCYTES*

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A lymphoproliferative response occurs in the regional lymph node after stimu-
luation with sheep red blood cells (SRBC). The rate of cell division increases
for a period of 4 days and then rapidly subsides (1). One product of this pro-
liferative response is a poorly sustained state of delayed-type hypersensitivity
(DTH) which is mediated by θ-bearing lymphocytes (1), but animals once
stimulated to respond in this way become refractory and cannot again be
rendered hypersensitive (2). This impediment to the reinduction of DTH is not
easily overcome; even Freund's complete adjuvant may fail to establish DTH
in previously immunized animals, a phenomenon known as immune deviation
(3). A mechanism which obstructs or terminates a T-cell response, or which
otherwise interferes with the functional activities of specifically sensitized
lymphocytes, is of obvious importance in transplantation, antimicrobial, and
antitumor immunity because the implementation of these forms of immunity
depends (in varying degree) upon the T-cell component of the immune response.
The present report deals with the mechanism that regulates T-cell activity in
animals which have been immunized without the adjuvants or selective immuno-
suppressants that are commonly used to induce a state of DTH.

Materials and Methods

Animals.—Male and female mice of the CD-1 strain (Charles River Breeding Labora-
tories, Inc., Wilmington, Mass.), from a specific pathogen-free colony, were used at 5 or 6
wk of age.

Antigens.—SRBC were obtained twice weekly, always from the same animal. Cells
collected and stored in Alsever's solution were washed three times with normal saline and sus-
pended to a known density by hemocytometer count.

Serum.—Bleeding was performed by heart puncture of ether-anesthetized mice. Bleedings
were pooled or handled individually. After clotting the serum was separated, sterilized by
membrane filtration, and stored at --20°C. It was inactivated before use by heating for 30
min at 56°C.

Antibody Titration.—Hemagglutinin titers were determined in microtitration trays using

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Abbreviations used in this paper: AS4, absorbed S4; DTH, delayed-type hypersensitivity;
PFC, plaque-forming cells; S4, immune serum drawn on day 4 of the immune response; SRBC,
sheep red blood cells; US4, unabsorbed S4.
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0.1 ml vol of serum diluted serially in isotonic Veronal buffer (4), to which was added an equal volume of 1% packed SRBC. After 30 min at 37°C the trays stood overnight at 4°C. Mercaptoethanol-resistant antibody titers were determined in the same way with serum which had been reduced for ½ h at room temperature with mercaptoethanol at a final concentration of 0.1 M (5).

Other Techniques.—Descriptions have been given of the methods used for measuring delayed-type hypersensitivity in the footpad (1), the lymphoproliferative response in a regional lymph node (6), and the number of plaque-forming cells (PFC) that appear in the popliteal lymph node following footpad inoculation with SRBC (1). The procedure for adoptive sensitization by spleen cell transfer has also been described (7).

RESULTS

It was shown in the preceding paper (2) that the induction of DTH by intravenous immunization with SRBC is critically dependent upon antigen dose. Fig. 1 depicts the results of an experiment in which the total hemagglutinating activity of the serum and corresponding levels of DTH were measured on day 4 in mice which had been immunized intravenously with varying doses of SRBC. As in the preceding study, the peak level of DTH was achieved with a dose of 10⁵ SRBC; but hemagglutinins could not be detected in the pooled serum of mice immunized with this dose of red cells, and were scarcely detectable after a dose of 10⁶. They appeared in increasing amounts, however, as the dose of SRBC was increased above this threshold, and as they increased, the level of DTH decreased proportionally. This inverse relationship between antibody titer and prevailing level of DTH points to a direct inhibitory effect of antibody on the production or function of specifically sensitized lymphocytes. A role for antibody in suppressing the development of DTH had been suspected when

![Graph](#)

**Fig. 1.** Mean levels of DTH measured on day 4 (●—●), and of hemagglutinin titer (△—△) in pooled serum, also obtained on day 4, from mice immunized intravenously with the indicated doses of SRBC. Means of 5.
splenectomized animals, which produce little or no antibody in response to intravenous immunization (8), became hypersensitive with doses of SRBC that were too big to sensitize intact mice (2).

Since the antibodies measured during the initial phase of the immune response (Fig. 1) were completely sensitive to mercaptoethanol it seemed that the induction of T cells might be inhibited by specific IgM antibodies. It was equally possible, however, that activated T cells are formed but cannot function in the presence of large amounts of antigen. Since DTH to SRBC can be transferred adoptively with spleen cells (1), the immunological status of the activated T cells produced in response to high and low doses of antigen could be tested directly. Two experiments were performed: In the first, spleen cells were transferred to normal recipients from donors which had been sensitized or blocked with doses of $10^5$ and $10^6$ SRBC, respectively; in the second experiment, spleen cells were transferred from highly sensitive donors into anergic recipients which had themselves been immunized 4 days previously with the larger ($10^6$) dose of SRBC.

Fig. 2 A shows what levels of DTH to expect in recipients of a given number of spleen cells from sensitized donors. The recipients of $2 \times 10^8$ spleen cells (one spleen equivalent) commonly react with about half the level of DTH displayed by the donors (Fig. 2 A). But whereas one spleen-equivalent ($2 \times 10^8$) of cells from reactive donors conferred the expected levels of DTH upon normal recipients, twice that number of spleen cells from donors rendered anergic by an intravenous injection of $10^6$ SRBC did not make normal recipients hypersensitive (Fig. 2 B); and animals in the same anergic state failed to react after receiving cells from highly sensitive donors (Fig. 2 C). The latter finding confirms the observations of Dwyer and Kantor (9) who showed that DTH cannot be conferred upon sensitized animals in which hypersensitivity had been suppressed by a desensitizing dose of soluble antigen. But, in apparent contradiction of Dwyer and Kantor, the cells of spontaneously nonreactive donors (as opposed to the desensitized donors of their experiments) failed to transfer reactivity adoptively. A single large dose of SRBC, lying within the range of doses that gives an optimal antibody response (10), thus blocked the expression of both actively and adoptively acquired DTH.

Radovich and Talmage (11) had raised the possibility, in connection with the phenomenon of antigenic competition, that blocking of the second immune response is due to a humoral product of the first. The fact that footpad sensitization could not be achieved once SRBC had been injected intravenously (2) suggested that the block might be located at the level of T-cell induction. The question was not a trivial one because Diener and Feldmann (12) had concluded that the control of antibody production is exercised through B cells rather than T cells. To investigate this question, serum was obtained on day 4 from animals which had been immunized intravenously with a large ($10^6$) blocking dose of SRBC. This day was chosen for bleeding because it marks the end of the lymphoproliferative response to SRBC in regionally stimulated nodes (1) and
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Fig. 2. (A) Levels of DTH to SRBC in donors (D) which were immunized in one hind footpad after priming with BCG, and in adoptively sensitized recipients (©) given varying numbers of spleen cells from the donor panel. The control animals (on the right) received spleen cells from donors given BCG only. (B) Transfer of DTH with spleen cells (2 × 10⁸) from hypersensitive donors (black columns) which had been immunized with 10⁹ SRBC intravenously. The recipients showed the expected level of DTH (white column). Recipients of 4 × 10⁸ spleen cells from anergic donors which were immunized intravenously with 10⁹ SRBC were not sensitive. Means of 5 ± SE. (C) Anergic recipients (blocked by 10⁹ SRBC intravenously) were not rendered hypersensitive with 2 × 10⁸ spleen cells from the same panel of donors used in A (IR), but another group of normal recipients (NR) were. Controls (C) were untreated. Means of 5 ± SE.

because DTH declines rapidly from its peak on this day (2). Both of these findings suggested that blocking activity, if it existed in the serum, would be most readily detected in serum taken at this time.

Immune serum, drawn on day 4 of the immune response, hereafter referred to as S₄, was tested for blocking activity in four groups of mice which had been immunized by a footpad injection of 10⁸ SRBC, a highly sensitizing dose when given by this route (2). Mice of two groups were treated intravenously with 0.5 ml of S₄, mice of one group being injected at the time of sensitization, the others 12 h earlier. A third group of sensitized controls received serum from normal donors, also given at the time of sensitization, and a fourth group served as sensitized but untreated controls. When tests for DTH were performed 4 days later unsensitized controls were also included. The observations recorded in Fig. 3 shows that the induction of DTH was partially suppressed when S₄ was given immediately before the sensitizing dose of SRBC and more so in animals treated 12 h earlier.

Since antibody opsonizes red cells, it had to be considered possible that a
FIG. 3. The effect of an intravenous injection of 4 day immune serum (0.5 ml) on the induction of DTH by a sensitizing dose of SRBC (10⁶) given in one hind footpad. Untreated controls (group A) and recipients of normal mouse serum (group B) developed DTH in the usual way. Animals given immune serum immediately before (group C) or 12 h before sensitization (group D) were partially suppressed. Unsensitized controls (group E) did not react. Means of 5 ± SE.

major portion of the inoculum used in the foregoing experiment had been destroyed in nonimmunological organs as the liver. Although diversion, neutralization, or destruction of antigen by specific antibody seemed unlikely explanations for the inhibitory effect that immune serum had on the induction of DTH, it was nonetheless important to establish whether antibody as such can affect the antigenicity of SRBC. If antigenicity were reduced by opsonization a larger dose of opsonized SRBC would be required to achieve maximum DTH and the dose-response curve of Fig. 1 would be shifted to the right. SRBC were therefore treated with normal or immune serum (54) by adding packed SRBC to whole serum in a ratio of 1:10. The cells were mixed and allowed to stand for 15 min at room temperature. After centrifugation the supernatant serum was decanted and the opsonized red cells were washed three times with normal saline at 4°C. Tests for opsonization were then performed by injecting samples of each cell suspension (5 × 10⁷ in 0.1 ml) into the peritoneal cavity. Cells were recovered 1 and 3 h later by introducing 2.5 ml of heparinized saline through the abdominal wall of three mice from each group, at each time point. The total numbers of extracellular SRBC in the washings were counted directly in a hemocytometer, and are recorded in Table I. In addition, samples of the cells washed from the peritoneal cavity were deposited on slides with a cytocentrifuge (Fig. 4).

As indicated in Table I, and illustrated in Fig. 4, red cells were effectively
opsonized by exposure to S4, phagocytosis of S4-treated SRBC being virtually complete within 1 h of injection into the peritoneal cavity. The opsonized SRBC were apparently no more or less immunogenic, however, for the numbers needed to produce maximum DTH were the same for normal and opsonized cells (Fig. 5 A). Even if a larger proportion of the opsonized SRBC had been diverted to highly phagocytic organs, such as the liver, the process of T-cell activation in the spleen, where the response to SRBC is centered (2), was obviously unaffected. This is not surprising in light of Wason's observation (13) showing that antibody-treated SRBC reach the spleen with added efficiency.

Although opsonization did not seem to alter the antigenicity of SRBC, the possibility remained that antibody might be capable of inhibiting DTH in other ways. This possibility was tested with the supernatant serum remaining after opsonization. It was first absorbed with an equal volume of packed SRBC to reduce its antibody titer still further. After incubation for 30 min at 37°C the hemagglutinating titer of the absorbed (AS4) and unabsorbed (US4) sera were 1:32 and 1:1024, respectively. Despite this loss of antibody, the absorbed serum remained highly active in inhibiting the induction of DTH. Mice given 0.2 ml of AS4 intravenously 1 h before immunization failed to develop more than a minor degree of DTH in response to any dose of SRBC (Fig. 5 B).

The unexpected findings that opsonized SRBC retained their immunogenicity while serum freed of most of its hemagglutinating activity still blocked the development of DTH implied that T-cell function was being obstructed by a product of the antigen-antibody reaction, but whether at the stage of induction or expression had yet to be determined. The effects of immune serum on afferent and efferent arms of the cellular response were therefore compared in the two separate experiments recorded in Fig. 6. The immune serum used for this comparison was 4-day heat-inactivated serum, half of which had been absorbed

### Table I

**Total and Mean Numbers of SRBC in the Extracellular Phase 1 and 3 h after Intraperitoneal Injection of Red Cells Treated with Normal or 4-Day Immune Serum**

| Mouse | Unopsonized 1 h | Opsonized 1 h | Unopsonized 3 h | Opsonized 3 h |
|-------|-----------------|---------------|-----------------|---------------|
| 1     | 5.1 X 10⁷       | 2.5 X 10⁶     | 3.0 x 10⁶       | 5 x 10⁴       |
| 2     | 4.8 X 10⁷       | 2.0 X 10⁶     | 2.2 x 10⁶       | 1 x 10⁵       |
| 3     | 6.3 X 10⁷       | 1.2 X 10⁶     | 6.5 x 10⁶       | 4 x 10⁴       |
| Mean  | 5.4 X 10⁷       | 1.9 X 10⁶     | 3.9 x 10⁶       | 3.3 x 10⁴     |

% ingested

- * Inoculum: 0.1 ml containing 5 x 10⁷ SRBC.
- † Washout: 2.5 ml Hank's BSS containing 5 intravenous heparin/ml.
- ‡ Assuming no loss of SRBC by lymphatic drainage from the peritoneal cavity.
with an equal volume of packed SRBC. The agglutinin titers for the AS4 and US4 sera were 1:16 and 1:1,024, respectively.

The effects of AS4 and US4 on the induction of DTH were tested by injecting 0.5 ml intravenously 1 day before giving the sensitizing injections of SRBC (10⁶) into one hind footpad. A group of controls received an equal volume of normal mouse serum. The hypersensitivity reactions elicited in the opposite footpad 4 days later showed that AS4 was significantly more suppressive than US4 (Fig. 6 A). The same two preparations were then tested for their capacity to interfere with the expression of an established state of hypersensitivity in mice which had been immunized intravenously with 10⁶ SRBC. On day 4, when DTH was at its peak (1, 2), AS4 or US4 were injected intravenously in doses of 0.2 ml. Controls were treated as before with serum from unimmunized mice. 1 h later mice of all groups, including unsensitized controls, were tested for hypersensitivity with an eliciting footpad injection of 10⁶ SRBC. The reactions read 24 h later were depressed in treated mice, especially in those given AS4 (Fig. 6 B).

In that intravenously immunized mice had failed to develop DTH and had shown no sign of a lymphoproliferative response in the popliteal lymph node when subsequently inoculated in the footpad with a sensitizing dose of SRBC (2), it was confidently expected that AS4 would have a similar inhibitory effect on cell proliferation and PFC production in popliteal lymph nodes. It emerged, however, that an intravenous injection of AS4 had almost no effect on either of these features of the immune response in the popliteal lymph nodes (Fig. 7 A and D), yet caused the expected depression of DTH (Fig. 7 B). This dissociation of effects was further emphasized by the fact that the hemagglutinating titers on day 5 were actually increased in the pooled serum of mice treated with AS4 (Fig. 7 C). This increase was not due to transferred antibody because a separate study had shown that hemagglutinating activity was already below detectable levels within 1 day of injecting the same volume of unabsorbed serum into normal mice. It will be noted, too, that PFC production was actually increased in the spleens of an additional group of mice which had received AS4 before immunization with 10⁶ SRBC in both hind footpads (Fig. 7 D). This accords with the findings of Wason (13) who found that PFC formation by the spleen is actually increased in mice given specific IgM antibody before immunization.

Since AS4 did not interfere with the lymphoproliferative response or with the resulting appearance of PFC in regionally stimulated nodes, yet almost abolished the state of DTH that is a normal accompaniment of these responses, it was reasonable to conclude that AS4 must act by blocking effector cells. This interpretation of the foregoing experiment assumes that helper cells are unaffected by AS4 while the mediators of DTH are blocked. If so, it should be possible to demonstrate direct inhibition of effector cells in adoptively sensitized animals. To establish this point, advantage was taken of the fact that
Fig. 4. Cytocentrifuge preparations of peritoneal washings made 1 h after intraperitoneal injection of SRBC treated with (a) normal or (b) 4 day specific immune serum. Leishman stain × 350.
spleen cells from cyclophosphamide-treated donors confer much higher levels of hypersensitivity than do cells obtained from conventionally immunized donors. In the experiment recorded in Fig. 8, one spleen equivalent of cells was transferred intravenously to four groups of recipients: A control group which received 0.2 ml of normal serum intravenously just before cell transfer and three test groups which were injected intravenously with AS4 (0.2 ml) at an interval of 24, 12, or 1 h before cell transfer. The sensitized cells were almost completely blocked in animals given AS4 1 h before cell transfer, but blocking was progressively less in animals treated 12 and 24 h earlier. Apparently, blocking of DTH was due to immediate and direct inhibition of effector cells by a constituent of AS4 and not by something elaborated in recipients. This stands in contrast to the more slowly evolving inhibition of DTH that results from injecting excess antigen intravenously (2).

DISCUSSION

It has been apparent since methods became available for studying the cellular kinetics of the immune response that recruitment and proliferation of precursor cells is normally limited to a brief period of escalation before a regulatory

\[ \text{Fig. 5. (A) The dose-response relationship was identical for SRBC treated with normal (○-○) or specific antibody (●-●). Both gave maximum DTH at an intravenous dose of } 10^6 \text{ (cf. Fig. 1). The antibody-treated cells were agglutinated, but were readily dispersed after washing. They remained opsonized, however, as shown in Fig. 4. (B) After absorption with an equal volume of SRBC, the hemagglutinin titer of 4-day immune serum fell from 1:1,024 to 1:16. The absorbed serum (0.2 ml) almost completely blocked the induction of DTH at all antigen doses (○-○), whereas normal serum (also absorbed with SRBC) gave a normal dose-response with maximum DTH at } 10^6 \text{ SRBC (●-●). Means of } 5 \pm SE. \]

\[ \text{Lagrange, P. H., G. B. Mackaness, and T. E. Miller. 1974. Potentiation of T-cell response by selective suppression of antibody formation with cyclophosphamide. Manuscript submitted for publication.} \]
mechanism comes into play. If cell proliferation is a valid criterion, it seems that the immune response is abruptly interrupted on the 4th day in lymph nodes responding to SRBC (2). The steep rise and subsequent decay of DTH from its peak on day 4 in intravenously immunized mice also indicates that an inhibitory mechanism is activated at this time, and that T cells may be the target of the inhibitor. Indeed, the mediators of DTH are so susceptible to inhibition that there may be no peripheral evidence of DTH to indicate that T cells have been activated at all in intravenously immunized mice (2 and Fig. 1). Since this peculiar sensitivity of intravenously immunized animals to T-cell inhibition was abolished by splenectomy, it seemed that antibody must be implicated in the inhibitory mechanism. But whether inhibition was due to antibody, as such, and the site of action of the inhibitor, were important questions that remained unanswered by the preceding study (2). They have been examined here and the results leave little doubt that the inhibitor is not antibody alone but a product of its interaction with antigen, and that activated T cells are the prime target.

The first indication came with the finding that T-cell activity, as measured by DTH, increased with increasing intravenous doses of SRBC until the threshold for antibody
Fig. 7. (A) Rates of \[^{3}H\]thymidine incorporation into DNA by popliteal lymph nodes during the peak response to a footpad inoculation of 10^6 SRBC, given intravenously to mice treated 24 h before sensitization with 0.2 ml of normal mouse serum (●—●) or AS4 (○—○). Means of 5 ± SE. (B) 24-h footpad reactions elicited on day 4 and read on day 5 in the control (black) and AS4-treated (hatched) mice described in A. Means of 5 ± SE. (C) Antibody titers (log2) in pooled, 5-day serum from mice belonging to the groups described in A. (D) Direct PFC on successive days in spleens (—) or lymph nodes (——) of mice injected intravenously with 0.2 ml of normal serum (●) or AS4 (○) given 24 h before bilateral footpad inoculations of 10^6 SRBC. Means of 5 ± SE.

Fig. 8. Effect of AS4 on DTH in mice sensitized adoptively to SRBC. Donors (D) were immunized intravenously with 10^5 SRBC 2 days after an intraperitoneal injection of cyclophosphamide (200 mg/kg). Spleen cells (10^8) were harvested 5 days later. Recipients were treated intravenously with AS4 immediately (T0), 12 h (T-1/2), or 24 h (T-1) before cell transfer. Sensitized controls received normal serum (NS). Unsensitized controls (C) received spleen cells from normal donors. Means of 5 ± SE.
production was reached (Fig. 1). When the dose of antigen was raised above this level hemagglutinins appeared in the serum to increasing titer and the accompanying level of DTH fell proportionally, disappearing completely in animals stimulated with $10^8$ SRBC or more. Since the dose of antigen required to produce complete blocking of DTH was no more than needed to produce a maximum antibody response (10), it was clear that helper cell activity can proceed at a normal rate in the spleens of intravenously immunized animals without producing DTH as a peripheral sign that T cells have been activated. This raised the question of whether the mediators of DTH are produced at all in heavily immunized animals; but it could not be answered by cell transfer studies because spleen cells from sensitive donors failed to confer hypersensitivity on anergic recipients, and spleen cells from anergic donors were inactive when transferred to normal (antigen-free) recipients. This implied that sensitized cells, if produced, were irreversibly blocked in the anergic animal. But blocking was apparently not due to antigen acting directly on specifically sensitized lymphocytes because a large dose of SRBC ($10^9$) had failed to suppress an existing state of DTH when given intravenously to animals sensitized by footpad inoculation (2). This left three possibilities: (a) that specifically sensitized lymphocytes are never formed, (b) are not released, or (c) are neutralized in situ when high doses of antigen are given intravenously. Since the development of DTH was not suppressed by a massive intravenous dose of SRBC in splenectomized mice it was logical to inquire whether reactive cells become trapped in the spleen. The argument against trapping as an explanation for the anergic state has been discussed already (2), and seems conclusive.

The two remaining possibilities presuppose the existence of an inhibitor which prevents the further induction of precursor cells and suppresses effector cells after they have been formed. Early serum from heavily immunized animals did, in fact, suppress the development of DTH in response to an optimal sensitizing dose of SRBC (Fig. 3), thereby suggesting that the block is situated early in the sequence of events leading to the production of activated T cells. As blocking was more marked when immune serum was given before rather than at the time of sensitization, a passive role for antibody seemed unlikely because antibody-mediated effects tend to diminish with time. The short half-life of immunoglobulins in mice (14, 15) dictates this tendency. Moreover, and in confirmation of Dennert (16), the antigenicity of SRBC was unimpaired by opsonic antibody (Fig. 5 A), but the serum itself was even more suppressive after partial absorption (Fig. 5 B). This made it virtually certain that antibody alone was not the inhibitor of activated T cells.

When absorbed immune serum was injected into mice with an established state of DTH it caused immediate and almost complete suppression of reactivity (Fig. 6). This created an enigma, for it was now not clear whether animals fail to develop DTH in response to a large primary dose of SRBC because activated T cells are not produced or because they are blocked by a humoral factor as fast as they are formed. In an effort to answer this question, the kinetics of the cellular response to SRBC were studied in the popliteal lymph nodes of animals treated with blocking serum. An intravenous injection of AS4
caused the expected block to the development of DTH, but had virtually no effect on the lymphoproliferative response in nodes or on the number of PFC produced therein (Fig. 7). Apparently, specifically sensitized lymphocytes could be inhibited without affecting helper cell activity; unless, of course, the mediators of DTH were not produced by serum-treated animals, whereas the helper cells needed for a full-blown PFC response were formed in normal numbers (Fig. 7).

The idea that helper cells and the mediators of DTH belong to different cell populations, only one of which is produced in response to a strong antigenic stimulus, is inconsistent with the observations of Kettman (17) who has adduced strong circumstantial evidence that the same thymus-derived cells perform both functions. Nevertheless, blocking serum did inhibit the adoptive transfer of DTH (Fig. 8) without interfering with antibody formation (Fig. 7). This means only that the mediators of DTH are susceptible to blocking, and does not deny them an identity with helper cells. It is entirely possible for T cells to function as helper cells in one location, yet be prevented from behaving as the mediators of DTH within the vascular compartment.

Total suppression of DTH and maximum PFC production in the spleen are achieved with comparable doses of SRBC (about 2–4 × 10⁸) (2, 10, 18). If the T cells which serve as helper cells are also the mediators of DTH one function must be lost in performing the other. Without entering too deeply into the nature of helper cell function it is possible to examine this question and comment briefly on the possible relationship between helper cells and the mediators of DTH. The former appear to function by surrendering up a macromolecular product that serves as a specific inducer of B cells (19–21). Feldmann (20) believes that the B-cell inducer is a complex of antigen and the T-cell receptor, and that it needs macrophages in order to fulfill its role. Having performed this initial act of providing a specific inducer of B cells, the responding T cells are presumably free to leave the scene, enter the blood, and perform a second function as mediators of DTH. It is clear, however, that they may fail to do so once antibody production has set in, a circumstance that clearly depends upon an adequate dose of antigen. Perhaps the divestment of receptors is sometimes so extreme that T cells are stripped of all receptors, leaving them unable to function either as helper cells or as mediators of DTH. Sprent and Miller (22) have described a phenomenon which could be interpreted in this way.

A more certain consequence of having been induced by antigen is for T cells to transform and divide (23). It is by no means clear at what stage in this sequence of events that responding T cells become capable of mediating DTH reactions, or what relationship the mediators of hypersensitivity bear to the memory cells that are created at the same time. Coe and Salvin (24) have shown, however, that guinea pigs which develop DTH in response to a minute dose of antigen may have no detectable antibodies in circulation but are primed to give a secondary antibody response. The appearance of antibodies in response to the second antigenic stimulus is accompanied by loss of DTH.
This points again to the reality of a T-cell blocking mechanism that depends not on antigen or antibody, but on a product of the two. This dual requirement explains the apparent discrepancy between the present findings and those of Axelrad (25) who observed that an existing state of DTH can be suppressed by a subcutaneous or intraperitoneal injection of antigen alone. Axelrad used Freund’s complete adjuvant and waited 9 days before injecting the desensitizing dose of antigen. At this time circulating antibodies were present to high titer. In the present studies, a modest dose of SRBC was inoculated into the footpad, and antibodies were low or absent on day 4 when desensitization was attempted. If blocking is due to interaction between antigen and antibody, antigen injected at this time would not encounter the antibodies needed for the formation of blocking factors and should not cause desensitization. This view is consistent with the findings (2), and gains support from Axelrad’s own observation that the desensitizing effects of antigen and antibody were synergistic.

Similar reasoning may be used to explain why spleen cells of spontaneously anergic mice failed to transfer sensitivity to recipients (Fig. 2), whereas Dwyer and Kantor (9) succeeded in transferring DTH with peripheral lymph node cells of guinea pigs which had been actively desensitized by injections of antigen. Here the difference lies not in the absence of proper conditions for the creation of blocking factors, but in the accessibilities of the cells chosen for transfer to the blocking factors that accumulate in serum. There is, in fact, precedent for believing that functionally active cells may be found in one location but not in another (26). Blocking factors which can interfere with the mediators of DTH in spleen and blood but not in peripheral lymphoid tissue are likely to be macromolecules that are mainly confined to the blood. There is, of course, substantial evidence to suggest that antigen-antibody complexes can interfere with the function of lymphocytes. Diener and Feldmann (12) showed that immune complexes, formed in antibody excess, act rapidly to suppress an antibody response in vitro. As tolerance is difficult to induce in vitro, except to T-cell-independent antigens (27), it seems that this example of inhibition by immune complexes involves suppression of B cells. There is little doubt, however, that immune complexes can also interfere with the performance of T cells. The Hellströms (28) have made observations which suggest that lymphocyte-mediated destruction of target cells can be blocked by immune complexes in vitro, but it has not been established whether effector cell or target cell is affected in this system. The present studies provide inferential evidence that the blocking factors that inhibit the mediators of DTH act directly on the effector cells. A mechanism whereby this effect of immune complexes, if such they are, inhibit the mediators of DTH may be deduced from the findings of Yoshida and Andersson (29) who have shown that activated T cells can bind antigen-IgG complexes. The present studies hint at the possibility that they may also find complexes formed with IgM and become
functionally suppressed as a consequence. The present studies have not pro-
vided any evidence, however, concerning the identity of the blocking factors
that appear in the serum of anergic mice.

SUMMARY

An explanation was sought for the fact that delayed-type hypersensitivity
(DTH) does not normally occur in response to T-cell-dependent antigens
unless an adjuvant is used. But when sheep red blood cells (SRBC) were
administered intravenously DTH did appear, provided that the dose of antigen
was less than that required to give a maximum antibody response. Animals in
which T-cell activity had been blocked by a large dose of antigen could not be
sensitized adoptively, and their spleen cells failed to transfer DTH to normal
recipients. The serum of blocked animals partially inhibited the induction of
DTH, and after absorption with SRBC its blocking activity increased sub-
stantially. Moreover, absorbed serum inhibited DTH in previously sensitized
animals, but it did not inhibit the proliferative response to SRBC in peripheral
lymph nodes or reduce the number of plaque-forming cells produced therein.
On the contrary, the hemagglutinating titer was actually increased by block-
ing serum even though DTH was totally suppressed. It is concluded that a
product of the interaction between antigens and antibody blocks the activated T
cells which mediate DTH without interfering with helper cells.

REFERENCES

1. Miller, T. E., G. B. Mackaness, and P. H. Lagrange. 1973. Immunopotentiation
with BCG. II. Modulation of the response to sheep red cells. J. Natl. Cancer
Inst. 51:1669.
2. 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. 139:000.
3. Asherson, G. L., and S. H. Stone. 1965. Selective and specific inhibition of 24 hour
skin reactions in the guinea-pig. I. Immune deviation: description of the phe-
nomenon and the effect of splenectomy. Immunology 9:205.
4. Kabat, E. A., and M. M. Mayer. 1961. Experimental Immunochemistry. Charles
C Thomas, Pub., Springfield, Ill.
5. Adler, F. L. 1965. Studies on mouse antibodies. I. The response to sheep red cells.
J. Immunol. 95:26.
6. Mackaness, G. B., D. J. Auclair, and P. H. Lagrange. 1973. Immunopotentiation
with BCG. I. The immune response to different strains and preparations. J.
Natl. Cancer Inst. 51:1655.
7. Mackaness, G. B. 1969. The influence of immunologically committed lymphoid
cells on macrophage activity in vivo. J. Exp. Med. 129:973.
8. Rowley, D. A. 1950. The effect of splenectomy on the formation of circulating
antibody in the adult male albino rat. J. Immunol. 64:289.
9. Dwyer, J. M., and F. S. Kantor. 1972. Regulation of delayed hypersensitivity.
Failure to transfer delayed hypersensitivity to desensitized guinea pigs. J.
Exp. Med. 137:32.
10. Adler, F. L. 1965. Studies on mouse antibodies. I. The response to sheep red cells. *J. Immunol.* 95:26.
11. Radovich, J., and D. W. Talmage. 1967. Antigenic competition: cellular or humoral. *Science (Wash. D. C.)* 161:512.
12. Diener, E., and M. Feldmann. 1972. Relationship between antigen and antibody-induced suppression of immunity. *Transplant. Rev.* 8:76.
13. Wason, W. M. 1973. Regulation of the immune response with antigen specific IgM antibody: a dual role. *J. Immunol.* 110:1245.
14. Britton, S., T. Wepsic, and G. Müller. 1968. Persistence of immunogenicity of two complex antigens retained in vivo. *Immunology.* 14:291.
15. Fahey, J. L., and S. Sell. 1965. The immunoglobulins of mice. V. The metabolic (catabolic) properties of five immunoglobulin classes. *J. Exp. Med.* 122:41.
16. Dennert, G. 1961. The mechanism of antibody-induced stimulation and inhibition of the immune response. *J. Immunol.* 106:951.
17. Kettman, J. 1972. Delayed hypersensitivity: Is the same population of thymus-derived cells responsible for cellular immunity reactions and the carrier effect? *Immunol. Commun.* 1:289.
18. Sell, S., A. B. Park, and A. A. Nordin. 1970. Immunoglobulin classes of antibody-forming cells in mice. I. Localized hemolysis-in-agar plaque-forming cells belonging to five immunoglobulin classes. *J. Immunol.* 104:483.
19. Feldmann, M., and A. Basten. 1972. Specific collaboration between T and B lymphocytes across a cell impermeable membrane in vitro. *Nat. New Biol.* 237:13.
20. Feldmann, M. 1972. Cell interactions in the immune response in vitro. V. Specific collaboration via complexes of antigen and thymus-derived cell immunoglobulin. *J. Exp. Med.* 136:737.
21. Yu, H., and J. Gordon. 1973. Helper function in antibody synthesis mediated by soluble factor(s). *Nat. New Biol.* 244:20.
22. Sprent, J., and J. F. A. P. Miller. 1973. Effect of recent antigen priming on adoptive immune responses. I. Specific unresponsiveness of cells from lymphoid organs of mice primed with heterologous erythrocytes. *J. Exp. Med.* 138:143.
23. Lamelin, J. P., B. Lisowska-Bernstein, A. Matter, J. E. Ryser, and P. Vassalli. 1972. Mouse thymus-independent and thymus-derived lymphoid cells. I. Immunofluorescent and functional studies. *J. Exp. Med.* 136:984.
24. Coe, J. E., and S. B. Salvin. 1964. The immune response in the presence of delayed hypersensitivity or circulating antibody. *J. Immunol.* 93:495.
25. Axelrad, M. A. 1968. Suppression of delayed hypersensitivity by antigen and antibody. *Immunology.* 18:159.
26. Schlossman, S. F., H. A. Levin, R. E. Rocklin, and J. R. David. 1971. The compartmentalization of antigen-reactive lymphocytes in desensitized guinea pigs. *J. Exp. Med.* 134:741.
27. Weigle, W. O. 1973. Immunological unresponsiveness. *Adv. Immunol.* 18:51.
28. Sjögren, H. O., I. Hellström, S. C. Bansai, and K. E. Hellström. 1971. Suggestive evidence that the "blocking antibodies" of tumor-bearing individuals may be antigen-antibody complexes. *Proc. Natl. Acad. Sci. U. S. A.* 68:1372.
29. Yoshida, T. O., and B. Andersson. 1972. Evidence for a receptor recognizing antigen complexed immunoglobulin on the surface of activated mouse thymus lymphocytes. *Scand. J. Immunol.* 1:401.