AN EARLY COMPONENT OF
DELAYED-TYPE HYPERSENSITIVITY MEDIATED
BY T CELLS AND MAST CELLS*

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Delayed-type hypersensitivity (DTH) reactions are mediated by Ly-1+ T cells (1, 2), and the elicitation of DTH requires T cell-dependent release of the vasoactive amine serotonin by local mast cells (3–5). This causes gaps to form between vascular endothelial cells allowing circulating leukocytes to emigrate into the extravascular tissue spaces in response to chemoattractant lymphokines that are released by antigen-activated T cells. The fact that DTH reactions depend on T cell activation of local mast cells led us to search for factors that might mediate T cell activation of mast cells. Such a factor has been described recently (6, 7). It is a T cell-derived antigen-binding factor that transfers an antigen-specific immediate hypersensitivity-like reaction (6). The T cell factor has in vivo activity analogous to IgE antibody, but can be distinguished from IgE by a number of immunochemical and biological properties (6, 7). Importantly, the T cell factor, like IgE, is mast cell dependent, since neither transfer the ability to elicit skin reactions in mast cell-deficient W/Wv or Sl/Slg mice.

Thus, the T cell factor is a suitable candidate for participation in the mechanism by which T cells activate mast cells in DTH. The early onset of skin swelling (0.5–2 h) after antigen challenge in recipients of the T cell factor led us to investigate whether previously unrecognized early events were present in DTH reactions. We have found with careful time-course experiments in actively sensitized mice, or in animals passively sensitized by transfer of immune T cells, that DTH reactions have an early component that can be easily detected and quantitated. This early component of DTH, like responses in recipients of the T cell factor, has a 2-h peak after antigen challenge and does not occur in mast cell-deficient mice, and therefore probably is a reflection of in vivo functioning of the T cell factor in DTH responses.

Materials and Methods

Mice. Male CBA/J, BALB/c, B6D2F1, C57BL6/J, DBA/2, and two types of mast cell-deficient mice: [(WBB6F1) (WB-W/+ X C57BL/6- W/+)-W/Wv, and their normal litter-

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Abbreviations used in this paper: DNFB, dinitrofluorobenzene; DTH, delayed-type hypersensitivity; OX, oxazolone; PCL, picryl chloride.
mates (WB6F1: +/+; and WC6F1 (WC-SI+/ X C57BL/6 - SI+/+) - SI/SI, and their normal littermates (WC6F1: +/+), were obtained from The Jackson Laboratory, Bar Harbor, ME and were rested at least 1 wk in an air-filtered enclosure before use.

Reagents. Picryl chloride (PCL) (Chemotronix, Swannona, NC) recrystallized three times from methanol/H2O before use, and oxazolone (OX) (Gallard-Schlesinger, Carle Place, NY) recrystallized three times from ethanol, were protected from light in a desiccator during storage at room temperature. Dinitrofluorobenzene (DNFB) was obtained from Sigma Chemical Co., St. Louis, MO).

Tumor Cells. L5178Y lymphosarcoma cells, growing as an ascitic tumor in the peritoneal cavity of the syngeneic DBA/2 mice, were maintained by weekly intraperitoneal passage. The cells were a gift of Dr. N. Ruddle, Yale University.

Active Immunization and Adoptive Transfer. Unless stated otherwise, male mice (6-8 wk old) were contact-sensitized using three established protocols. Contact sensitization was performed by topical application of either 0.15 ml of 5% PCL (8) or 3% OX (8) in absolute ethanol and acetone (3:1), to the shaved abdomen and four feet of mice, or by application of 0.02 ml 0.5% DNFB in acetone and olive oil (1:4) to the shaved abdomen on two consecutive days (9). 4 d after completion of immunization, mice were either challenged by topical application of reactive sensitizer to the ears, or spleen and peripheral lymph node cells were harvested and 5 × 10⁷ cells were transferred by intravenous injection via the retro-orbital plexus of naive syngeneic recipients. C57BL/6J mice were immunized by subcutaneous injection of 10⁶ allogeneic L5178Y tumor cells in 0.2 ml saline at two separate sites on the chest. 7 d later the mice were challenged by injection of L5178Y cells in the hind feet (10).

Anti-T Cell Antibody and Complement Treatment of Transferred Lymphoid Cells. Separate aliquots of cells (5 × 10⁷/ml) were incubated at 4°C for 45 min, or in a 1:1,000 dilution of a monoclonal anti-Thy-1 antibody (New England Nuclear, Boston, MA), and after washing, both aliquots were incubated subsequently at 37°C for 45 min in a 1:20 dilution of spleen cell-absorbed rabbit serum as a source of complement. After washing the cells were resuspended in saline, and equal numbers of cells were transferred intravenously into separate groups of syngeneic recipient mice.

Measurement of DTH Responses. Contact-sensitized mice were challenged by topical application to both sides of both ears of 1 drop (27-gauge needle) of either 0.8% PCL in olive oil, 0.8% OX in olive oil, or 0.2% DNFB in acetone and olive oil (1:4). Mice immunized with L5178Y cells were challenged by injecting 50 μl saline containing 4 × 10⁸ L5178Y cells into both hind footpads. Duplicate measurements of ear thickness or single measurements of footpad thickness were made bilaterally with an engineer’s micrometer (Digimic; Brown & Sharpe Co., North Kingston, RI) before and at various times after challenge. The increment of ear or footpad thickness was expressed as the mean ± SE in units of 10⁻² cm (8). In each experiment, the ears or footpads of a separate group of nonimmunized controls were challenged and measured similarly to immunized mice. The background swelling responses in these controls were subtracted from the swelling responses of experimental animals.

Statistics. Student’s t test was performed for statistical analysis of the data. Differences between groups with P < 0.05 were taken as significant.

Results

Biphasic Pattern of Ear- or Footpad-swelling Reactions after Challenge of Actively Immunized Mice. CBA mice were immunized by contact sensitization with PCL and 4 d later their ears were challenged by application of PCL. Ear-swelling responses were detected within 1 h, and this early response was maximal by 2 h (Fig. 1). The ear-swelling response declined completely by 4 h, and then rose again at 18 h, peaked at 24 h, and thereafter declined, but was still present at 48 h (Fig. 1). Occasionally the decline at 4 h was not complete. Challenge with the appropriate antigen resulted in similar biphasic patterns of ear-swelling reactions in DNFB-sensitized BALB/c mice (Fig. 2), OX-sensitized BDF1 (Fig. 3) or CBA mice (Fig. 5); and footpad-swelling reactions in L5178Y-immunized C57BL/6 mice (Fig. 4). The early and delayed components of
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Fig. 1. Time course of the ear-swelling response in mice contact sensitized with PCL. CBA/J mice were immunized by contact painting with PCL, and their ears were challenged by topical application of PCL 4 d later. Ear thickness was measured with an engineer's micrometer before challenge and at various times thereafter and the increase in ear thickness was calculated. The background increase in ear thickness of similarly challenged nonimmune controls was subtracted from responses of immunized animals at each time point. The data from six separate experiments were pooled and the number of mice used to compute the mean ± SE at each time point is given in parenthesis.

DTH responses were antigen-specific, as PCL immunized animals did not react at 2 or 24 h to challenge with OX, and conversely, OX-immunized animals did not react to PCL challenge at either time (Fig. 5).
Fig. 3. Time course of the ear-swelling response in BDF₁ mice contact-sensitized with oxazolone (OX). Mice were immunized by contact painting with OX and their ears were challenged by topical application of OX 4 d later. The increase in ear thickness was measured at various times thereafter and the response of nonimmune and similarly challenged controls was subtracted. Groups of six mice were used.

Fig. 4. Time course of the footpad DTH response in C57BL/6 mice immunized with DBA/2 lymphoma cells. Mice were immunized by subcutaneous injection of L5178Y lymphosarcoma cells into the chest. 7 d later their feet were challenged by subcutaneous injection of L5178Y cells and the increase in footpad thickness was measured at various times thereafter and the response of nonimmune and similarly challenged controls was subtracted. Groups of five mice were used.
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Fig. 5. Specificity of early and late components of DTH. Groups of CBA/J mice were immunized with either PCL or OX. 4 d later both ears of separate groups of each type were challenged with either PCL or OX. 2 and 24 h later the increase in ear thickness was measured and the response of appropriate nonimmune controls that were challenged either with PCL or OX were subtracted from responses of immune mice. The number of animals per group is given in parentheses.

| Immunization | Challenge | No of Mice | 2H (units x 10^-3 cm ± SE) | 24H (units x 10^-3 cm ± SE) |
|--------------|-----------|------------|---------------------------|---------------------------|
| PCL          | PCL       | (9)        |                           |                           |
| PCL          | OX        | (9)        |                           |                           |
| OX           | OX        | (6)        |                           |                           |
| OX           | PCL       | (6)        |                           |                           |

Fig. 6. Onset after immunization of early (2 h) and late (24 h) components of DTH. Groups of five CBA mice were immunized by contact painting with PCL, and at various times after immunization their ears were challenged by topical application of PCL. 2 and 24 h after challenge the increase in ear thickness was measured and the response of nonimmune and similarly challenged controls was subtracted.

Onset after Immunization of Early and Late Components of DTH. CBA mice were immunized with PCL at various times before ear challenge. The 2-h ear-swelling responses were detected as early as 1 d after immunization (Fig. 6). Mice immunized from 1–5 d before challenge were able to mount this early reaction, and the magnitude of this response seemed not to depend on the duration of the immunization. However, 9 d after immunization, the effect was no longer observed. Unlike this early response, the classical 24-h component of DTH reactions was only seen in animals immunized
at least 3 d before challenge. This late component was maximal at 4 d after immunization, and, like the early component, was absent by 9 d after immunization (Fig. 6).

Transfer of Early and Late Components of DTH by Lymphoid Cells. 5 × 10⁷ spleen and lymph node cells from CBA mice immunized 4 d previously were injected intravenously into naive CBA mice. 24 h later the ears of the recipient mice were challenged. Ear reactions in these recipients paralleled those of actively sensitized animals, with an early component at 2 h, a decline of ear-swelling response at 4 h, and a late component at 24 h (Fig. 7a). Transfer of either 5 × 10⁷ spleen cells alone (Fig. 7b) or of 5 × 10⁷ lymph node cells alone (Fig. 7c) also resulted in sensitization for elicitation of approximately the same biphasic responses in the recipients as resulted from transfer of the cell mixture. The 2-h response in the L5178Y lymphoma cell-induced footpad DTH reaction of C57BL/6 mice (Fig. 4) was also transferred to naive recipients with immune lymphoid cells (data not shown).

The minimal time interval between injection of immune lymphoid cells and skin challenge to produce the 2-h response was 12 h (Fig. 8). CBA mice that were injected intravenously with immune lymphoid cells and were then challenged immediately, responded with an early response that began 6 h after challenge, and was maximal at 12 h (Fig. 9). In this case the early response declined completely by 18 h and thus the onset of the late component of DTH was somewhat delayed until 21 h (Fig. 9), compared with that of actively sensitized mice (Fig. 1). Thus the detailed time course of DTH was completely different in recipients that were challenged immediately after cell transfer. Although the classical 24-h component of DTH was present in these mice, responses were absent at 18 h (Fig. 9). Therefore the exact time course of DTH responses, as occurs in actively sensitized animals (Fig. 1), was faithfully reproduced in immune cell recipients that were challenged 24 h after transfer, and did not occur when recipients were challenged immediately, which produced an atypical time course (Fig. 9).

T Cell Dependence of Early and Late Components of DTH. To investigate whether the early response was dependent on T cells, as is the late component of DTH responses (1, 2), the adoptively transferred cells were treated with monoclonal anti-Thy-1 antibodies and complement. Treatment of the cells with complement alone did not affect their ability to transfer both early and late responses (Fig. 10). In contrast, both the early and late components of DTH were abolished by treatment of the transferred cells with anti-T cell antibodies and complement, showing dependence of both components of DTH reactions on T cells (Fig. 10).

Mast Cell Dependence of Early and Late Components of DTH. Actively sensitized mast cell-deficient W/W' mice were not able to mount an ear-swelling response at 2 h or at 24 h, in contrast to their normal +/+ counterparts (Fig. 11). Both the 2- and 24-h ear-swelling responses in actively sensitized mast cell deficient SI/SI^d mice were also greatly reduced compared to 2- and 24-h reactions in their actively sensitized +/+ controls (Fig. 11). These results indicate that both the early and late components of DTH are dependent on mast cells.

Discussion

We have shown, in four unrelated systems, that DTH reactions have an expected delayed time course, but that this is preceded by an early response that is maximal 2
h after challenge. Although many studies (including our own [11]) have overlooked this early component of DTH, a biphasic pattern is evident in the work of others (12–14), although the impact of these findings was not discussed in these publications. We found that this early response, like the classical 24-h component of DTH, could be transferred to naive recipients with spleen and lymph node T cells from immunized donors. Morley et al. (15) noted early phase increases in vascular permeability in tuberculin reactions of guinea pigs, and were unable to transfer this aspect of classical
DTH reactions with immune serum. Thus an early vasoactive component of DTH, that like the classical later component is T cell dependent, may be an inherent aspect of these reactions.

We also found that there was an important difference in the exact time course of DTH in T cell recipients. This depended on the interval between transfer and challenge. Recipients that were challenged 1 d after transfer had the same response as actively sensitized mice, while transfer with immediate challenge resulted in an
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Fig. 9. Cell transfer of PCL sensitivity with immediate challenge. Time-course of the DTH response in passively immunized mice that were challenged immediately after cell transfer. Groups of CBA/J mice were injected intravenously with 5 X 10^7 mixed spleen and lymph node cells from PCL-sensitized CBA/J donors. Immediately thereafter the ears of these mice were challenged by topical application of PCL. The increase in ear thickness at various times thereafter was measured and the response of nontreated and similarly challenged controls was subtracted. The data from four separate experiments were pooled and the number of mice were used to compute the mean ± SE at each time point is given in parenthesis.

| Response | Treatment | Ear Swelling Response (units x 10^-3 cm ± SE) |
|----------|-----------|---------------------------------------------|
| 2 h      | Complement Alone | ![Graph](image) |
| 2 h      | Anti-Thy I + Complement | ![Graph](image) |
| 24 h     | Complement Alone | ![Graph](image) |
| 24 h     | Anti-Thy I + Complement | ![Graph](image) |

Fig. 10. T cell dependence of the early (2 h) and late (24 h) components of DTH. Mixed spleen and lymph node cells from PCL-sensitized CBA/J mice were treated with either anti-thy 1 antibodies and rabbit complement, or with rabbit complement only. Then 5 X 10^7 cells of each type were injected intravenously into groups of three naïve CBA/J mice. 24 h later the ears of these mice were challenged by topical application of PCL. At 2 and 24 h after challenge the increase in ear thickness was measured and the response of nontreated and similarly challenged controls was subtracted.

Atypical time course, with an early response at 6–12 h, and a somewhat later delayed response. Thus transfer with immediate challenge, as is commonly performed, results in DTH responses that have a completely different time course compared to responses
Fig. 11. Mast cell dependence of the early (2 h) and late (24 h) components of DTH. Groups of five mast cell-deficient W/Wv mice and their normal +/+ littermates, and groups of five mast cell-deficient SI/SI\(d\) mice and their normal +/+ littermates, were immunized by contact painting with PCL. 4 d later their ears were challenged by topical application of PCL. At 2 and 24 h after challenge the increase in ear thickness was measured and the response of four groups of nonimmune controls (W/Wv and +/+; SI/SI\(d\) and +/+\) was subtracted from responses of appropriate sensitized groups.

It is likely that the early component of DTH is due to the ability of immune T cells to release skin-sensitizing, antigen-specific factors. When T cells that are capable of transferring the early component of DTH are cultured in vitro, they release an antigen-binding factor that is capable of transferring such an early response (6, 7). Moreover, both early reactions that depend on transfer of the T cell factor and early reactions that depend on transfer of T cells are maximal 2 h after challenge, and both are mast cell dependent (reference 7, and Fig. 11 herein). This indicates that immediate reactions due to the T cell factor and early reactions due to the T cells are essentially the same. Moreover, CBA mice that were immunized with PCL 1-5 d but not 9 d, before challenge were able to mount the early reaction (Fig. 6), which parallels the ability of lymphoid cells taken from these animals to produce the T cell factor in vitro (data not shown). The only contradiction to the formulation that early reactions due to active sensitization, or due to passive sensitization with T cells or the T cell factor, are the same is that the decline of the response in recipients of the T cell factor at 4 h after challenge is less pronounced (6, 7), compared with responses at 4 h in actively sensitized mice (Figs. 1-4), or recipients of cell transfers that are challenged 1 d later (Fig. 7). This difference might be explained by assuming that regulatory (suppressor) cells are induced with the factor-producing cells, which is not the case in recipients of the factor. Another explanation may be that much more factor is transferred in the experiments involving transfer with the antigen-binding T cell factor than is produced by the sensitized T cells in vivo.

The fact that the early ear-swelling responses were already present within 1 d after immunization (Fig. 6) is somewhat surprising, since in vivo induction of T cells to
mediate reactions usually takes more time. In addition, the transferred cells seem to need at least 12 h before challenge to optimally produce the mast cell-activating factor (Figs. 8 and 9), which leaves the T cells of actively sensitized animals even less time (~12–18 h) to become sensitized and begin to elaborate the factor. However, activation of T cells (16), or eosinophil reactions that may depend on T cells (17), very soon after sensitization, have been described in other systems.

The finding that the 2-h response can be transferred by immune T cells, and also by cell-free products, but that the late reactions are only transferred by cells, suggests that these components of DTH reactions depend on different T cell subsets, or similar T cells at different stages of activation. This is further substantiated by the finding that early responses are present in animals immunized 1–5 d before challenge, whereas the late component of DTH could only be detected after 3 d (Fig. 6).

These findings have led us to formulate the hypothesis that immunization of mice for DTH stimulates a certain population of T cells to produce a factor in vivo, that can lead to mast cell sensitization. Upon antigen challenge these mast cells probably are activated to release the vasoactive amine serotonin. This results in an early reaction that is caused by an increased permeability of the local vasculature. Pharmacologic studies have confirmed that early ear-swelling reactions are dependent on release of serotonin. Electron microscopy studies demonstrate mast cell activation, and alterations of the local vasculature with the formation of gaps between endothelial cells and increased permeability, that are probably due to serotonin.

T cells that are capable of producing the factor that leads to mast cell activation probably need not be at the site of challenge, since cells producing the factor are found in the spleen and lymph nodes; moreover, activity of the factor has also been found in serum of immunized animals (H. Van Loveren and P. W. Askenase, unpublished observations). According to our hypothesis, alterations in the local vasculature allow other antigen-specific T cells (although not necessarily different ones) to penetrate into the extravascular spaces, and these cells are then activated by antigen to produce lymphokines that recruit circulating bone marrow-derived leukocytes, such as monocytes and neutrophils, to comprise the majority of cells infiltrating the local DTH site. Our findings raise the possibility that a similar collaboration between T cell factor-dependent and T cell lymphokine-dependent mechanisms may underly the attraction of leukocytes at various examples of cellular immunity where IgE antibodies do not seem to play an apparent role, such as: hypersensitivity granulomas, autoallergic diseases (18), immune responses to tumors (19) and parasites (20), and chronic graft rejection (21).

Our findings may also imply that the traditional classification of immune tissue responses into “immediate” and “delayed” hypersensitivity reactions is no longer adequate, a view that is supported by the finding of a late phase of IgE- and IgG-mediated anaphylactic reactions (22–27). Thus, both classical immediate and delayed hypersensitivity are in fact biphasic, and these early and late components of both reactions are mast cell dependent.

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Summary

In four different systems it was shown that murine delayed-type hypersensitivity (DTH) responses at 18-48 h were preceded by early 2-h responses. CBA mice immunized with picryl chloride, BDF1 mice immunized with oxazolone, BALB/c mice immunized with dinitrofluorobenzene, and C57BL/6 mice immunized with L5178Y lymphoma cells, and challenged with the appropriate specific antigen, all gave rise to expected 18-48 h delayed-in-time hypersensitivity reactions, but all of these responses were preceded by early hypersensitivity reactions that peaked at 2 h. These early 2-h reactions are transferable with T cells or with a T cell-derived, antigen-binding factor and are antigen-specific. The early and late components of DTH reactions are mast cell dependent since neither are elicited in mast cell deficient W/Wv or Sl/SIa mice. The T cell activity mediating the early component of DTH is demonstrable as early as 24 h after immunization, while the classical late component of DTH is not demonstrable until days 3-4.

The difference in onset after immunization of the early and late components of DTH, and the different kinetics of these components in recipients of cell transfers that were challenged immediately or 24 h after transfer, led to the hypothesis that immunization for DTH leads to rapid induction in lymphoid organs of a certain population of T cells to produce an antigen-binding factor. This factor sensitizes peripheral tissues, probably mast cells, and local challenge with appropriate antigen leads to mast cell activation and release of the vasoactive amine serotonin, resulting in increased permeability of the local vasculature. This allows other circulating antigen-specific T cells, which are induced later after immunization, to enter the tissues and interact with antigen, resulting in production of chemotactic lymphokines that recruit accessory leukocytes such as monocytes and polymorphs to enter the tissues via gaps between endothelial cells. These inflammatory cells, that are recruited to the site via two different T cell activities, constitute the characteristic infiltrate of DTH responses. Identification of an early 2-h component of DTH that is T cell- and mast cell-dependent provides evidence that the tissue-sensitizing, antigen-binding, T cell factor probably functions in vivo in the early phases of DTH responses.

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