Recruitment of Neutrophils during IgE-dependent Cutaneous Late Phase Reactions in the Mouse Is Mast Cell–dependent

Partial Inhibition of the Reaction with Antiserum against Tumor Necrosis Factor-Alpha

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Abstract

Much of the clinically important pathology associated with IgE-dependent disorders is thought to reflect the actions of the blood-borne leukocytes recruited during these responses. To evaluate the extent to which mast cells are responsible for the leukocyte infiltration associated with IgE-dependent cutaneous reactions, we attempted to elicit these responses in normal mice, genetically mast cell–deficient W/W^v mice, and in W/W^v mice selectively repaired of their mast cell deficiency by the intradermal injection of cultured mast cells derived from the congenic normal (+/+) mice. We found that the tissue swelling associated with IgE-dependent passive cutaneous anaphylaxis reactions developed rapidly and diminished markedly from 2 to 4 h after antigen challenge, but remained detectable for at least 24 h after elicitation of the responses. Infiltration of leukocytes (predominantly neutrophils) also occurred at these sites, but reached maximal levels 6–12 h after antigen challenge, persisted at high levels for 24 h, and largely waned by 48 h. Virtually all of the tissue swelling and leukocyte infiltration associated with IgE-dependent cutaneous reactions was mast cell dependent. Intradermal injection of 40 U of recombinant murine TNF-α (rmTNF-α) elicited neutrophil infiltration similar in magnitude and kinetics to that observed after IgE-dependent mast cell degranulation. A rabbit anti-rmTNF-α (R anti-rmTNF-α) antiserum, which was able to inhibit 84% of the neutrophil infiltration observed after i.d. injection of rmTNF-α, inhibited IgE-, and mast cell–dependent leukocyte infiltration by 47±7% in three separate experiments. These findings indicate that TNF-α contributes to mast cell–dependent recruitment of leukocytes during IgE-dependent cutaneous late phase reactions, but suggest that other mast cell–associated mediators probably also contribute to this response. (*J. Clin. Invest.* 1991. 87:446–453.) Key words: inflammation • mast cell–deficient mice • local mast cell reconstitution • anaphylactic degranulation • passive cutaneous anaphylaxis

Introduction

In many allergic patients, intradermal challenge with specific antigen or anti-IgE induces an immediate wheal and flare reaction which is followed, 4–8 h later, by a period of persistent swelling and leukocyte infiltration termed the late phase cutaneous reaction (1–4). Late phase reactions (LPR)[1] were initially described in the skin (1, 2). However, it is now clear that late consequences of IgE-dependent reactions, notably including infiltration of the reaction sites with blood-borne leukocytes, also occur in the respiratory tract and other anatomical locations (3, 4). Indeed, it has been argued cogently that many of the clinically significant consequences of IgE-dependent reactions, in both the skin and the respiratory system, reflect the actions of the leukocytes recruited to these sites during the LPR rather than the direct effects of the mediators released at early intervals after antigen provocation (3, 4).

Several lines of evidence support the hypothesis that the leukocyte infiltration associated with LPRs occurs as a result of mast cell degranulation. In both man and experimental animals, agents that induce cutaneous mast cell degranulation by either IgE-dependent or certain other mechanisms can also promote infiltration of the reaction sites with leukocytes (1–9). In the rat, Tannenbaum et al. (10) demonstrated that the intradermal injection of cytoplasmic granules purified from rat peritoneal mast cells induced leukocyte infiltration with kinetics similar to those of IgE-dependent cutaneous LPRs. Additional studies by Kaliner and his associates indicated that among several rat mast cell granule–associated factors which could induce leukocyte infiltration, the most potent was a protein of ~ 1,400 D molecular weight (3, 11, 12). However, a review of the mediators that can be elaborated by activated mast cells reveals many that might contribute to leukocyte infiltration in LPRs, including lipid mediators such as LTB_4, LTC_4, LTD_4, PGD_2, and PAF (platelet activating factor), as well as several peptide or proteinacious chemotactic factors (13–15). The latter agents range in size from tetrapeptide "eosinophil chemotactic factors of anaphylaxis" to very high molecular weight "neutrophil chemotactic factors" (13–15).

Even more candidate mast cell–associated mediators of leukocyte infiltration recently have been identified, including cy-

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1. *Abbreviations used in this paper:* DNP, dinitrophenol; $Fc_\epsilon RI$ and $Fc_\epsilon RII$, receptors that bind the Fc region of IgE antibodies with high ($Fc_\epsilon RI$) or low ($Fc_\epsilon RII$) affinity; IL-1α, interleukin 1α; IL-3, interleukin 3; LPR, late phase reaction; LTB_4, LTC_4, LTD_4, leukotrienes B_4, C_4, and D_4; MIP-1α, -1β, macrophage inflammatory proteins 1α and 1β; NRS, normal rabbit serum; PAF, platelet activating factor; PCA, passive cutaneous anaphylaxis; PGD_2, prostaglandin D_2; R anti-rmTNF-α, rabbit antiserum against recombinant murine TNF-α; TCA3, T cell activation antigen 3; TNF-α, tumor necrosis factor-α/cachetin.

tokines similar or identical to tumor necrosis factor-alpha (TNF-α; 16–21), interleukin-1α (22), and four members of the MIP-1 gene family of small secreted peptides, T cell activation antigen 3 (TCA3), JE, and the macrophage inflammatory proteins 1α and 1β (MIP-1α and MIP-1β, reference 22). Four of these cytokines (TNF-α, IL-1α, MIP-1α, and MIP-1β) have been demonstrated to have the ability to promote leukocyte infiltration (23–28), whereas mouse TCA3 and JE are regarded as likely to have such activity based on their structural homology to other members of the MIP-1 gene family (29).

Given this evidence, the case that mast cells are required for the leukocyte infiltration observed in IgE-dependent LPRs would appear to be compelling. Yet several considerations prompted us to test this hypothesis directly. For example, IgE or its aggregates can interact with several cell types in addition to the mast cell, either via $Fc_\epsilon RI$ (basophils) or $Fc_\epsilon RII$ (monocytes, platelets, B cells, and perhaps eosinophils, references 30–32). Many of these cell types can produce mediators that might contribute to the initiation and/or perpetuation of LPRs. In addition, the fact that mast cell degranulation occurs before the development of the LPR does not necessarily prove that the first event is required for the second. Mast cell activation also occurs at early intervals during the development of contact hypersensitivity responses, but the magnitude of the leukocyte infiltration that develops in cutaneous contact sensitivity reactions in genetically mast cell–deficient mice equals or, in some cases, exceeds that observed in the congenic normal animals (reviewed in 33–35). Moreover, Dolovich et al. (36) reported that the early wheal responses induced by intracutaneous injection of codeine were rarely followed by clinically detectable late reactions, indicating that human cutaneous mast cell activation *per se* may not in all cases be sufficient for production of a LPR. Finally, three groups have reported that fatal systemic anaphylaxis can be elicited in appropriately sensitized genetically mast cell–deficient mice that are virtually devoid of mast cells (37–39). Passive transfer studies indicate that the antibodies responsible for these reactions are of the IgE class (40). Thus, the occurrence of mast cell degranulation and leukocyte infiltration at the same site does not prove the two events are causally related, nor does the fact that a reaction is IgE-dependent prove that the critical source of mediators in the reaction is the mast cell.

We therefore decided to evaluate directly the role of the mast cell in the development of the leukocyte infiltration associated with IgE-dependent cutaneous LPRs, by comparing the expression of these reactions in normal mice, genetically mast cell–deficient W/W^v mice, and W/W^v mice that had been selectively and locally repaired of their cutaneous mast cell deficiency by the injection of IL-3-dependent mast cells derived in vitro from the bone marrow cells of the congenic normal (+/+) mice.

Methods

Mice. Genetically mast cell–deficient mice and the congenic normal (+/+) mice (WB/ReJ-W+ × C57BL/6J-W^v/+) F_1-(W/W^v, +/+), designated here WBB6F_1-(W/W^v, +/+), were purchased from the Jackson Laboratory (Bar Harbor, ME). The skin of adult WBB6F_1-W/W^v mice contains < 1% the number of mast cells present in the skin of the congenic normal (+/+) mice (41, 42). C57BL/6 mice were purchased from the Charles River Laboratories. All mice were used at 2–7 mo of age unless stated otherwise.

Elicitation of IgE-dependent cutaneous reactions. Unless otherwise specified, each mouse was primed to express an IgE-dependent passive cutaneous anaphylaxis (PCA) reaction in the left ear and a control reaction in the contralateral (right) ear. Mice were lightly anesthetized with ether and received an i.d. injection to the left ear of 20 μl of a 1:10,000 dilution of ascites containing a monoclonal mouse anti-DNP IgE (43) in HMEM (Gibco Laboratories, Grand Island, NY) containing 0.47 g/l Pipes buffer instead of $NaHCO_3$ (HMEM/Pipes). The amount of IgE injected per site was ~ 20 ng, determined in a rat PCA assay by comparison with purified monoclonal anti-DNP IgE derived from the same hybridoma (44). The right ear received an i.d. injection of 20 μl of the diluent alone. The next day, the mice received an i.v. injection of 100 μg of $DNP_{30\text{-}40}$-human serum albumin (HSA) (Sigma Chemical Co., St. Louis, MO) in saline, as previously described (44). In some experiments, the $DNP_{30\text{-}40}$-HSA solution also contained 1% Evans blue (Sigma Chemical Co., St. Louis, MO) to permit visualization of sites of increased vascular permeability.

Measurement of tissue swelling, mast cell degranulation, and neutrophil infiltration. Ear swelling was determined by measurement of ear thickness with a micrometer before and at various intervals after the challenge; swelling was expressed as the increment (Δ) of thickness (postinjection value − preinjection baseline value) in units of 10^{-4} inch (45).

In all experiments, tissue from sites of PCA reactions (left ears) and control sites (right ears) were obtained after sacrifice by cervical dislocation and were processed for 1-μm Epon-embedded, Giemsa-stained sections (33, 46). Coded sections were examined by one observer (Z.-S. Wang) who was unaware of the identity of individual sections. The number of mast cells per square millimeter of dermis was determined as previously described (7, 9), and the mast cells were classified morphologically as extensively degranulated (≥ 50% of the cytoplasmic granules exhibiting fusion, staining alterations, and/or extrusion from the cell), slightly to moderately degranulated (10–50% of the granules exhibiting fusion or discharge), or normal (7, 9). We also quantified the number of granulocytes present at the reaction sites, and expressed the results as the number of granulocytes per square millimeter of dermis, as previously described (7, 9). In all of the PCA reactions analyzed in this study, > 90% of the infiltrating leukocytes were neutrophils.

Mast cell reconstitution of genetically mast cell–deficient W/W^v mice. WBB6F_1-W/W^v mice were repaired of their mast cell deficiency selectively and locally by the injection of growth factor–dependent, bone marrow–derived cultured mast cells into one ear (7, 9, 44, 47). We reported that IL-3-dependent, bone marrow–derived, WBB6F_1-+/+ mast cells maintained in suspension culture represent immature mast cells that express certain phenotypic similarities to mucosal mast cells, but that upon injection into the skin (7, 9, 44, 47) of WBB6F_1-W/W^v mice, these mast cell populations gradually (over a 10-wk period) acquire multiple phenotypic characteristics of the mature connective tissue–type mast cells present in the dermis of normal mice. Moreover, injection of cultured mast cells into one ear of W/W^v mice repairs the mast cell deficiency in that ear alone. The mice remain mast cell–deficient at other cutaneous sites and in other organs, and remain anemic as well (7, 9, 44). Briefly, bone marrow cells from WBB6F_1-+/+ mice were grown in vitro for 3–5 wk in concanavalin A–stimulated mouse spleen cell–conditioned medium until mast cells represented > 95% of the total cells as determined by neutral red staining. Mast cells (0.5×10^6) in 20 μl of DMEM (Gibco) were injected into the left ears and 20 μl of medium alone into the right ears. 10 wk after injection of mast cells, IgE anti-DNP antibodies were injected into both ears and antigen challenge was performed as described above. In all experiments, we confirmed histologically that local reconstitution of dermal mast cell populations had occurred and determined that the W/W^v mice locally reconstituted with mast cells remained anemic.

Assessment of the effects of rabbit antiserum raised against TNF on tissue swelling or neutrophil accumulation in response to rmTNF-α or IgE-dependent reactions. In preliminary experiments, various doses of recombinant murine TNF-α (rmTNF-α, Genzyme, Boston, MA) in 20 μl of HMEM/Pipes were injected into the left ears of C57BL/6 mice

and similar dilutions of normal rabbit serum (NRS) were injected into the right ears as a control. In some experiments, the left ears of mice were injected with rmTNF-α and various doses of a polyclonal rabbit antiserum raised against rmTNF-α (R anti-rmTNF-α, reference 21), whereas the right ears received rmTNF-α and similar dilutions of normal rabbit serum. The preparation and characterization of the R anti-rmTNF-α antiserum has been described in detail (21). Briefly, rmTNF (Genentech, Inc., South San Francisco, CA) was reduced and alkylated with 50 mM dithiothreitol and 100 mM iodoacetate, respectively. New Zealand white rabbits were primed by intramuscular injection of 250 μg of protein in Freund's complete adjuvant and boosted with 50 μg in Freund's incomplete adjuvant. When titered against rmTNF-α in an L929 cell cytotoxicity assay, the R anti-rmTNF-α antiserum had an activity of $> 8 \times 10^5$ neutralizing U/ml (21).

Finally, the effect of R anti-rmTNF-α was examined in IgE-dependent reactions. Mice were sensitized by injection of monoclonal IgE anti-DNP antibodies into *both* ears. The next day, immediately before systemic challenge with $DNP_{30\text{-}40}$-HSA, all mice received an i.d. injection of R anti-rmTNF-α (1/100 dilution) into the left ear and the same dilution of normal rabbit serum into the right ear. Tissue swelling and neutrophil accumulation were assessed as described above.

Assessment of endotoxin content of reagents injected into mice. According to the limulus amebocyte lysate chromogenic assay (LAL Assay, Whitaker Bioproducts Inc., Walkersville, MD), the endotoxin content of our HMEM, IgE-containing ascites, R anti-rmTNF-α, NRS, or rmTNF-α preparations, when tested at the dilutions actually injected into the mice, ranged from 0.23 to 0.50 EU/ml (all values < 0.05 ng/ml). Based on these values and the volumes of the reagents injected, the amounts of endotoxin which were administered in these preparations were ~ 1,000-fold below those required to elicit significant leukocyte infiltration when injected into the skin of mice (48).

Statistical analysis. The results of ear swelling assays or differences in mast cell or granulocyte counts in different groups of mice were analyzed for statistical significance ($P < 0.05$) by Student's t test (two-tailed); values for left and right ears in individual mice were analyzed by the paired Student's t test (two tailed).

All results are expressed as the mean±SEM.

Results

Kinetics of tissue swelling and neutrophil infiltration during IgE-dependent PCA reactions. As shown in Fig. 1, swelling developed rapidly at sites of PCA reactions but not at contralateral control sites. Swelling at PCA sites diminished markedly between 2 and 4 h after i.v. antigen challenge then waned more slowly. By 24 h after challenge, the swelling associated with PCA reactions was greatly reduced, although still significant ($P < 0.02$) when compared to contralateral control sites.

PCA and control sites were assessed histologically at 6, 12, 24, or 48 h after i.v. antigen challenge. The majority of mast cells at PCA sites exhibited extensive or moderate degranulation at the first interval examined, and evidence of mast cell degranulation at these sites persisted for at least 48 h (Fig. 2 *A*). By contrast, < 3% of mast cells at contralateral control sites exhibited extensive degranulation at any interval tested (data not shown). As shown in Fig. 2 *B*, neutrophil infiltration at PCA sites reached near maximal levels by 6 h after antigen challenge, remained stable until 24 h after antigen challenge, but declined by 48 h. A sparse neutrophil infiltration was seen in the contralateral control sites (12–29 neutrophils/mm^2 of dermis), which probably reflected the mild trauma of injection of medium at those sites 24 h before antigen challenge.

Cutaneous swelling and neutrophil infiltration during IgE-dependent PCA reactions are mast cell dependent. We next compared the expression of IgE-dependent cutaneous reactions in genetically mast cell-deficient $WBB6F_1$-W/W^v mice and the congenic normal +/+ mice. Fig. 3 shows that swelling responses occurred only in the IgE-injected left ears of +/+ mice; little or no swelling was noted in the control (right) ears of +/+ mice nor in the IgE-injected or contralateral control ears of the W/W^v mice. Histological examination at 6 and 12 h after elicitation of PCA reactions demonstrated mast cell degranulation at sites of IgE injection in the ears of +/+ mice; few or no mast cells (0.2±0.1/mm^2; < 0.5% the number in +/+ mice, Fig. 4 *A*) were present in the ears of the W/W^v mice.

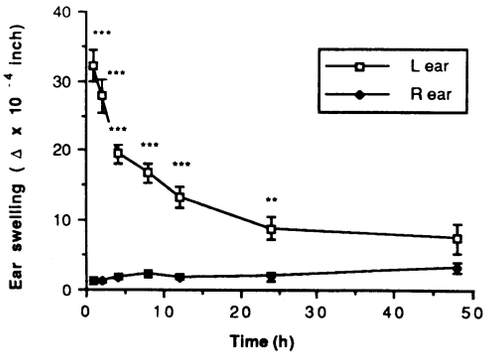

Figure 1. Ear swelling responses during IgE-dependent reactions. Ear swelling responses (Δ = postchallenge thickness − prechallenge baseline value) in C57BL/6 mice that had been injected in the left ear with ~ 20 ng of IgE anti-DNP antibodies (in 20 μl HMEM/Pipes) and in the right (control) ear with HMEM/Pipes (20 μl). 1 d later, the mice were challenged with $DNP_{30\text{-}40}$-HSA i.v. (100 μg in 0.1 ml of 0.9% NaCl). Data represent mean±SEM (n = 5 to 20/point). Significant differences between values for L (IgE-injected) and R (vehicle-injected) ears are shown as $^{**}P < 0.02$, $^{***}P < 0.001$.

In the +/+ mice, neutrophil infiltration into IgE-injected left ears exceeded that in contralateral control ears at both 6 h (116±8 vs. 35±13 neutrophils/mm^2 of dermis, respectively, L vs. R, $P < 0.01$) and 12 h (L vs. R, 81±11 vs. 28±8 neutrophils/mm^2 of dermis, respectively, $P < 0.05$) after antigen challenge (Fig. 4 *B*). By contrast, the numbers of neutrophils in the IgE-injected ears of W/W^v mice did not differ significantly from those in the contralateral control ears or those in the control ears of the +/+ mice.

These results suggested that mast cells were required for both the tissue swelling response and the neutrophil infiltration associated with IgE-dependent PCA reactions. To confirm this point, we examined PCA reactions in W/W^v mice that had undergone selective, local reconstitution of the left ear with congenic +/+ mouse bone marrow–derived mast cells. 10 wk after mast cell reconstitution of the left ears, both left and right ears were injected with IgE anti-DNP and the mice were challenged i.v. with $DNP_{30\text{-}40}$-HSA the next day. A swelling response with kinetics similar to those seen in normal mice developed in the IgE-injected mast cell-reconstituted left ears but not in the IgE-injected mast cell–deficient right ears (Fig. 5). Moreover, significant neutrophil accumulation occurred within 6 h of antigen challenge in the mast cell–reconstituted left ears, whereas the mast cell–deficient right ears exhibited little or no reaction (126±43 vs. 10±2 neutrophils/mm^2 of dermis, respectively, L vs. R, $P < 0.01$, Fig. 6). These results confirm that mast cells are necessary for both the tissue swelling and the neutrophil infiltration associated with IgE-dependent cutaneous reactions in the mouse.

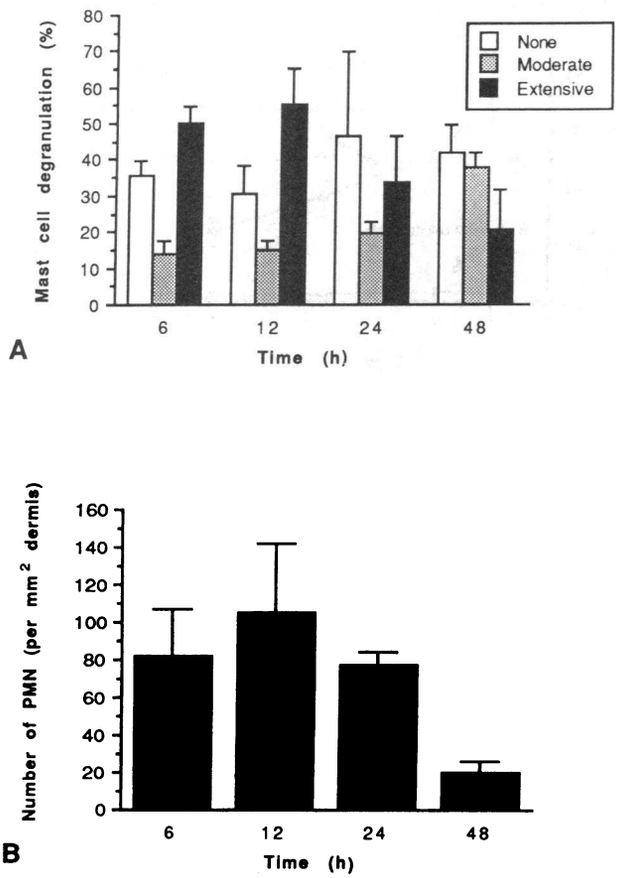

Figure 2. (*A*) Mast cell degranulation during IgE-dependent reactions. The number of mast cells per square millimeter of dermis was determined in C57BL/6 mice which were injected in the left ear with IgE anti-DNP antibodies and in the right (control) ear with vehicle and then challenged the next day with $DNP_{30\text{-}40}$-HSA i.v. At various intervals after challenge, the mice were killed, specimens of the ears were processed into 1-μm Epon-embedded Giemsa-stained sections, and the mast cells were counted and classified (at ×1,000) as extensively degranulated, slightly to moderately degranulated, or normal (see Methods). (*B*) Neutrophil infiltration during IgE-dependent reactions. Neutrophil accumulation, expressed as PMN per square millimeter of dermis in the IgE-injected (left) ear of C57BL/6 mice, was determined at various times after i.v. injection of $DNP_{30\text{-}40}$-HSA. Data represent mean±SEM (n = 4–5/point).

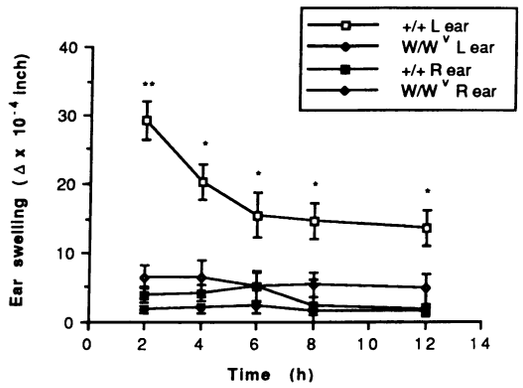

Figure 3. Ear swelling responses in mast cell-deficient $WBB6F_1$-W/W^v and congenic normal (+/+) mice during IgE-dependent reactions. $WBB6F_1$-W/W^v or congenic normal (+/+) mice were injected in the left ear with IgE anti-DNP antibodies and in the right (control) ear with vehicle. The next day, the mice were challenged with $DNP_{30\text{-}40}$-HSA i.v. Data represent mean±SEM (n = 5/point). Significant differences between L (IgE-injected) ears of +/+ mice and the R (control) ears of +/+ mice or between the L and R ears of W/W^v mice are shown as $^*P < 0.05$, $^{**}P < 0.02$.

The cutaneous neutrophil accumulation elicited by rmTNF-α is inhibited by a rabbit antiserum to rmTNF-α. We first showed that the intradermal injection of 40 or 400 U, but not 4 U, of rmTNF-α induced significant neutrophil accumulation within 6 h of administration when compared to medium alone injected into the contralateral (control) ears (Fig. 7). We then tested the ability of our R anti-rmTNF-α antiserum to inhibit the cutaneous reaction elicited by intradermal injection of 40 U of rmTNF-α, a dose which elicited a level of neutrophil infiltration similar to that observed in the PCA reactions of normal or mast cell-reconstituted W/W^v mice. The simultaneous injection into the left ear of rmTNF-α and the antiserum (at 1:100 dilution) resulted in significantly less neutrophil accumulation at 6 h than that observed in the contralateral right ears which were injected with 40 U of rmTNF-α and a 1:100 dilution of NRS (18±7 vs. 115±15 neutrophils/mm² of dermis, respectively, $P < 0.001$).

Antiserum to rmTNF-α partially inhibits the neutrophil infiltration associated with an IgE-dependent cutaneous reaction. We next tested the effect of the R anti-rmTNF-α antiserum on the mast cell–dependent neutrophil infiltration associated with IgE-dependent PCA reactions. In these experiments, both ears of C57BL/6 mice were sensitized with IgE anti-DNP antibodies. Before systemic challenge with $DNP_{30\text{-}40}$-HSA, the left ears were injected with a 1:100 dilution of R anti-rmTNF-α, whereas the right ears received a 1:100 dilution of NRS. In the experiment shown in Fig. 8, significantly less neutrophil accumulation was seen 6 h after initiation of PCA reactions in the left (R anti-rmTNF-α-treated) ears compared to the values for the reactions in the contralateral right (NRS-treated) ears (60±19 vs. 151±37 neutrophils/mm² dermis, respectively, $P < 0.05$). Similar results were seen in two other experiments; the reduction in neutrophil infiltration observed in R anti-rmTNF-α- as opposed to NRS-treated PCA sites in the three experiments was 47±7% (mean±SEM).

Discussion

We found that virtually all of the leukocyte infiltration associated with IgE-dependent late phase reactions in mouse skin was mast cell dependent. In accord with our previous work (44), i.v. administration of specific antigen also rapidly induced the mast cell–dependent development of tissue swelling at sites injected 1 d previously with monoclonal anti-DNP IgE antibodies. Thus, antigen-induced tissue swelling did not occur either at cutaneous sites containing mast cells but not injected with IgE, or at IgE-injected sites in genetically mast cell–deficient W/W^v mice. Although IgE-induced tissue swelling diminished rapidly from 2 to 4 h after antigen challenge, IgE-injected sites remained significantly swollen compared to contralateral control sites for at least 24 h after initiation of the response.

Histological analysis indicated that leukocyte infiltration developed rapidly at sites of IgE-induced mast cell degranulation in normal mice. Some neutrophils were present as early as 2 h after antigen challenge (44, and unpublished data). How-

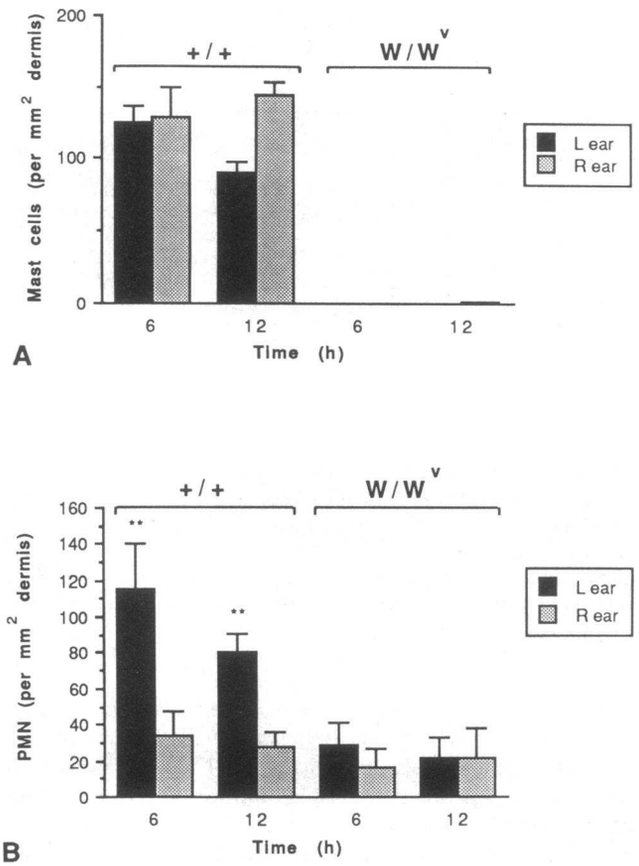

Figure 4. (*A*) Mast cell numbers in mast cell–deficient WBB6F$_1$-*W/W^v* and congenic normal (+/+) mice during IgE-dependent reactions. The numbers of mast cells per square millimeter of dermis were determined in the ears of *W/W^v* or +/+ mice injected in the left ear with IgE anti-DNP antibodies and the right ear with vehicle and then challenged the next day with DNP$_{30\text{-}40}$-HSA i.v. Data represent mean±SEM (n = 5/point). (*B*) Neutrophil infiltration in mast cell–deficient WBB6F$_1$-*W/W^v* and congenic normal (+/+) mice during IgE-dependent reactions. Neutrophil accumulation in the left (IgE-injected) ears or right (vehicle-injected) ears was determined as in Fig. 2 *B*. Data represent mean±SEM (n = 5/point). Significant differences between values for L (IgE-injected) ears of +/+ mice and the R (vehicle-injected) ears of +/+ mice or the L (IgE-injected) and R (vehicle-injected) ears of *W/W^v* mice are shown as $^{**}P < 0.02$.

ever, the numbers of neutrophils reached the highest levels by 6–12 h, diminished slightly by 24 h, and had waned significantly by 48 h. At all intervals analyzed, neutrophils represented > 90% of infiltrating leukocytes; the rest were primarily mononuclear cells with occasional eosinophils.

Two lines of evidence indicated that the leukocyte infiltration that followed IgE-dependent PCA reactions was mast cell dependent. First, the minimal levels of neutrophil infiltration observed at IgE-injected sites of antigen challenged mast cell–deficient *W/W^v* mice were not statistically distinguishable from those observed either in the contralateral control (medium-injected) ears of these mice or in the control sites in antigen-injected congenic normal (+/+) mice (Fig. 4 *B*). In addition to supporting the hypothesis that mast cells are required for expression of IgE-dependent recruitment of leukocytes to the skin, these findings are consistent with our previous work indicating that the ability of dilute preparations of IgE-containing ascites to elicit cutaneous inflammation reflects primarily the preparation's content of biologically active IgE (44). Thus, heating to 56°C for 1 h, which destroys the ability of IgE to bind to Fc$_\epsilon$RI, completely abrogated the ability of IgE-containing preparations to prime skin for the expression of a PCA reaction (44).

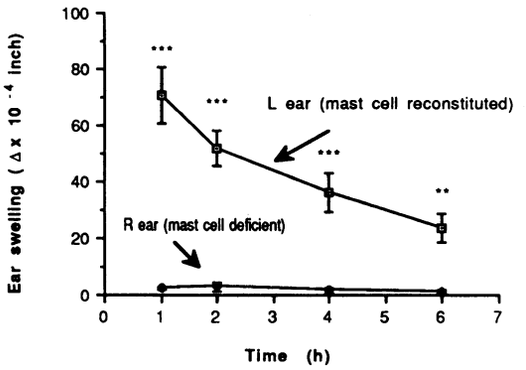

Figure 5. Ear swelling responses during IgE-dependent reactions in WBB6F$_1$-*W/W^v* mice locally reconstituted with mast cells in one ear. Ear swelling responses were determined in both mast cell–reconstituted (left) and mast cell-deficient (right) ears of *W/W^v* mice that had been injected > 10 wk before the experiment with IL-3-dependent mast cells generated in vitro from the bone marrow of congenic +/+ mice (see Methods). Both ears were injected with IgE anti-DNP antibodies and, 1 d later, the mice were challenged with DNP$_{30\text{-}40}$-HSA i.v. Data represent mean±SEM (n = 4/point). Significant differences between values for L and R ears are shown as $^{**}P < 0.02$, $^{***}P < 0.001$.

Second, local selective repair of the mast cell deficiency of *W/W^v* mice by injection, 10 wk before the experiment, of bone marrow–derived cultured mast cells of congenic +/+ mouse origin permitted such sites to express both the tissue swelling response and the neutrophil infiltration associated with IgE-dependent reactions (Fig. 5 and 6). By contrast, neither the tissue swelling nor the leukocyte infiltration observed in the IgE-injected contralateral mast cell–deficient ears of the same mice

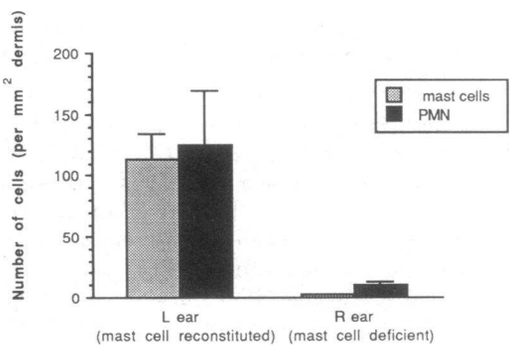

Figure 6. Total mast cell number and neutrophil infiltration during IgE-dependent reactions in WBB6F$_1$-*W/W^v* mice locally reconstituted with mast cells in one ear. The total numbers of mast cells and neutrophils were measured in the left (mast cell–reconstituted) and right (mast cell–deficient) ears of *W/W^v* mice that > 10 weeks earlier underwent local reconstitution of the left ear with bone marrow–derived WBB6F$_1$-+/+ mast cells (see Methods). Data are from the same mice shown in Fig. 5, in which IgE had been injected into both ears 1 d before antigen challenge (mean±SEM).

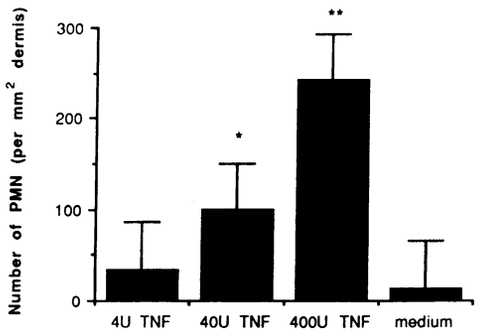

Figure 7. Neutrophil infiltration induced by cutaneous injection of rmTNF-α. Neutrophil accumulation was determined in the left ear of C57BL/6 mice injected with various doses of rmTNF-α in HMEM/Pipes (20 μl) and in the contralateral (right) ear injected with HMEM/Pipes alone (20 μl). Mice were killed 6 h after injection. Data represent mean±SEM (n = 8–24/point). Significant differences between L (rmTNF-α injected) and R (medium injected) ears are shown as: $^*P < 0.05$, $^{**}P < 0.001$.

differed significantly from those seen in non-IgE injected control sites in W/W^v mice. Thus, the inability of W/W^v mouse skin to express either the immediate swelling associated with IgE-dependent PCA reactions or the later infiltration of neutrophils at these sites reflected the mast cell deficiency of this tissue, not some other aspect of the *W* locus/*c-kit* (49) mutations of these mice.

As pointed out in the Introduction, the influx of leukocytes into sites of mast cell degranulation might reflect the actions of several mast cell–derived mediators, including products of arachidonic acid oxidation, chemotactic peptides or proteins, and/or several different cytokines. Upon activation via the $Fc_\epsilon RI$, IL-3-dependent or -independent cloned mouse mast cells have been shown to develop increased levels of mRNA for at least six cytokines with demonstrated or potential ability to elicit leukocyte infiltration: TNF-α, IL-1α, MIP-1α, MIP-1β, TCA3, and JE (21, 22). IgE-dependent activation of cloned mouse mast cells also results in the release of bioactivity for TNF-α and IL-1α (21, 22). However, studies of cytokine production by mature, physiologically relevant mouse mast cell populations have been rather limited. Plaut et al. (50) reported that mouse peritoneal mast cells can release IL-3 upon activation via the $Fc_\epsilon RI$, and we have shown that the same population of mast cells represents a source of preformed TNF-α that can be released rapidly upon IgE-dependent stimulation (21). Using an organ culture system, Klein et al. (51) demonstrated that IgE-dependent activation of human foreskin mast cells resulted in the expression of a TNF-α-inducible leukocyte adherence molecule (ELAM-1, reference 52) on adjacent vascular endothelial cells. Although the cellular source of the TNF-α responsible for the augmented ELAM-1 expression observed in this model system was not specifically identified, the findings support the possibility that mast cell–derived TNF-α may contribute to the leukocyte infiltration observed in IgE-dependent LPRs.

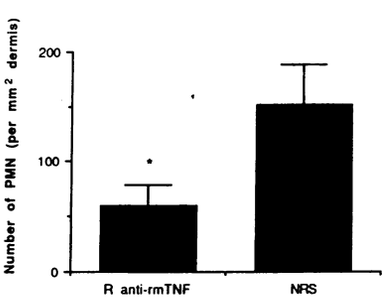

Figure 8. Inhibition of the neutrophil infiltration associated with IgE-dependent reactions by R anti-rmTNF-α antiserum. Both ears of C57BL/6 mice were injected with IgE anti-DNP antibodies. The next day the left ears were injected with a 1:100 dilution of R anti-rmTNF antiserum and the right ears with a 1:100 dilution of NRS. The mice were immediately challenged with $DNP_{30\text{-}40}$-HSA i.v. and sacrificed 6 h later for measurement of neutrophil infiltration. Data represent mean±SEM (n = 8). Significant differences between the L and R ears are shown as $^*P < 0.05$.

In the present study, we demonstrated that the intradermal injection of 40 U of rmTNF-α induced an interstitial infiltration of neutrophils similar in magnitude and kinetics to that observed after IgE-dependent mast cell degranulation. We then showed that a rabbit antibody raised against rmTNF-α, which is able to inhibit completely the cytotoxicity of both rmTNF-α and connective tissue–type mast cells against L929 cells in vitro (21), was able to inhibit by ~ 84% the neutrophil infiltration induced by intradermal injection of rmTNF-α into mouse skin. Finally, we demonstrated that when compared to similar dilutions of NRS, rabbit anti-rmTNF-α was able to diminish significantly (by 47±7%) the neutrophil infiltration elicited in association with IgE-dependent cutaneous mast cell degranulation.

Our findings thus support the hypothesis that TNF-α contributes to the mast cell–dependent leukocyte infiltration associated with cutaneous LPRs. This result is not surprising, given the many studies demonstrating that TNF-α can promote leukocyte infiltration by effects on both vascular endothelial cells and leukocytes (reviewed in 23, 24, 53), and our recent demonstration that mouse connective tissue–type mast cells can represent a source of both preformed and IgE-inducible TNF-α (19, 21). However, it should be noted that the anti-rmTNF-α antiserum was more effective at diminishing the leukocyte infiltration elicited by rmTNF-α (decreased by 84%) than that observed after mast cell degranulation (decreased by 47±7% in three separate experiments). One possible explanation for this difference is that the intradermally injected rmTNF-α was more accessible to the R anti-rmTNF-α than was endogenous TNF-α of mast cell origin. In mouse mast cells generated in vitro, a significant fraction of the TNF-α bioactivity is associated with the cells' cytoplasmic granules (19). If the same is true for dermal mast cells, the physical association of TNF-α with other components of the mast cell granule may have limited access to the cytokine by R anti-rmTNF-α antibodies. Moreover, the rmTNF-α was given as a single injection, whereas mast cells activated via IgE in vitro can release TNF-α over a prolonged period after stimulation (21).

On the other hand, given the large number of products of mast cell origin that might contribute to leukocyte infiltration, we feel that it is unlikely that mast cell–derived TNF-α would represent the sole mediator of leukocyte infiltration in the LPR in mice. We think that it is more reasonable to propose that the leukocyte infiltration which follows IgE-dependent mast cell activation is orchestrated by several products of the mast cell, perhaps in concert with mediators from other cells that are recruited to these reactions. It should also be remembered that while mouse and human mast cells share broad similarities, they also express certain differences in mediator content (reviewed in 13, 14, 54, 55). It is entirely possible that patterns of

cytokine production in mouse and human mast cells will also be found to differ (56). Therefore, even though the mast cell–reconstituted, mast cell–deficient mouse model is very useful for identifying the roles of mast cells in biological responses in the mouse, the conclusions of such studies cannot be extrapolated uncritically to human systems.

One final point deserves brief consideration. The mast cell generally is thought to contribute to inflammation by releasing proinflammatory mediators acutely in response to immunologically specific or nonspecific stimuli (reviewed in 13–15, 20, 35, 54, 55). However, it has been proposed that mast cells may also release very small quantities of mediators, either intermittently or over long intervals, by vesicular transport or other mechanisms (reviewed in reference 54). It is possible that such low level release of cytokines or other mast cell–associated mediators might alter the properties of the vasculature or other elements of the tissues in which mast cells reside (56). If this indeed were the case, reconstitution of mast cell populations to the tissues of mast cell–deficient mice might influence the development of inflammatory responses both because the reconstituted tissues could express the acute effects of mast cell activation and also because of more chronic, "priming" effects of mast cells on the tissue microenvironment. Unfortunately, several technical considerations may make it difficult to quantify or even to establish the existence of such mast cell–dependent "priming" effects in vivo. For example, there are no satisfactory techniques to ablate mast cells selectively from mast cell–containing tissues, and it may be difficult to distinguish chronic "priming" effects of mast cells on the tissue microenvironment from the acute effects of low levels of mast cell activation produced by the experimental manipulations of mast cell–containing tissues. As a result, at this time the existence and nature of mast cell–dependent chronic effects on vascularized tissues remain the subject of speculation.

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References

1. Dolovich, J., F. E. Hargreave, R. Chalmers, K. J. Shier, J. Gauldie, and J. Bienenstock. 1973. Late cutaneous allergic responses in isolated IgE-dependent reaction. *J. Allergy Clin. Immunol.* 52:38–46.

2. Solley, G. O., G. Gleich, R. Jordan, and A. Schroeter. 1976. The late phase of the immediate wheal and flare skin reaction. Its dependence on IgE antibodies. *J. Clin. Invest.* 58:408–420.

3. Lemanske, R. F., and M. Kaliner. 1988. Late-phase allergic reactions. *In* Allergy: Principles and Practice. Vol. 1. E. Middleton, Jr., C. E. Reed, E. F. Ellis, N. F. Adkinson, Jr., and J. W. Yunginger, editors. C. V. Mosby Co., St. Louis, MO. 224–246.

4. Schleimer, R. P., C. C. Fox, R. M. Naclerio, M. Plaut, J. A. Warner, A. Kagey-Sobotka, and L. M. Lichtenstein. 1985. Role of human basophils and mast cells in the pathogenesis of allergic diseases. *J. Allergy Clin. Immunol.* 76:369–374.

5. Charlesworth, E. N., A. F. Hood, N. A. Soter, A. Kagey-Sobotka, P. S. Norman, and L. M. Lichtenstein. 1989. Cutaneous late-phase response to allergen. Mediator release and inflammatory cell infiltration. *J. Clin. Invest.* 83:1519–1526.

6. Lemanske, R. F., and M. Kaliner. 1981–1982. Mast cell-dependent late phase reactions. *Clin. Immunol. Rev.* 1:547–580.

7. Wershil, B. K., T. Murakami, and S. J. Galli. 1988. Mast cell-dependent amplification of an immunologically nonspecific inflammatory response. Mast cells are required for the full expression of cutaneous acute inflammation induced by phorbol 12-myristate 13-acetate. *J. Immunol.* 140:2356–2360.

8. Matsuda, H., K. Kawakita, Y. Kiso, T. Nakano, and Y. Kitamura. 1989. Substance P induces granulocyte infiltration through degranulation of mast cells. *J. Immunol.* 142:927–931.

9. Yano, H., B. K. Wershil, N. Arizono, and S. J. Galli. 1989. Substance P-induced augmentation of cutaneous vascular permeability and granulocyte infiltration in mice is mast cell-dependent. *J. Clin. Invest.* 84:1276–1286.

10. Tannenbaum, S., H. Oertel, W. Henderson, and M. Kaliner. 1980. The biologic activity of mast cell granules. I. Elicitation of inflammatory responses in rat skin. *J. Immunol.* 125:325–335.

11. Kaliner, M., and R. Lemanske. 1984. Inflammatory responses to mast cell granules. *Fed. Proc.* 43:2846–2851.

12. Oertel, H., and M. Kaliner. 1981. The biologic activity of mast cell granules. III. Purification of inflammatory factors of anaphylaxis (IF-A) responsible for causing late-phase reactions. *J. Immunol.* 127:1398–1402.

13. Schwartz, L. B., and K. F. Austen. 1984. Structure and function of the chemical mediators of mast cells. *Prog. Allergy.* 34:271–321.

14. Galli, S. J., and L. M. Lichtenstein. 1988. The biology of mast cells and basophils. *In* Allergy: Principles and Practice. Vol. 1. E. Middleton, Jr., C. E. Reed, E. F. Ellis, N. F. Adkinson, Jr., and J. W. Yunginger, editors. C. V. Mosby Co., St. Louis, MO. 106–134.

15. Holgate, S. T., C. Robinson, and M. K. Church. 1988. Mediators of immediate hypersensitivity. *In* Allergy: Principles and Practice. Vol. 1. E. Middleton, Jr., C. E. Reed, E. F. Ellis, N. F. Adkinson, Jr., and J. W. Yunginger. editors. C. V. Mosby Co., St. Louis, MO. 135–178.

16. Ghiara, P., D. Boraschi, L. Villa, G. Scapigliatti, C. Taddei, and A. Tagliabue. 1985. In vitro generated mast cells express natural cytotoxicity against tumour cells. *Immunology.* 55:317–324.

17. Okuno, T., Y. Takagaki, D. H. Pluzuik, and J. Y. Djeu. 1986. Natural cytotoxic (NC) cell activity in basophilic cells: release of NC-specific cytotoxic factor by IgE receptor triggering. *J. Immunol.* 136:4652–4658.

18. Jadus, M. R., G. Schmunk, J. Y. Djeu, and R. Parkman. 1986. Morphology and lytic mechanisms of interleukin 3-dependent natural cytotoxic cells: tumor necrosis factor as a possible mediator. *J. Immunol.* 137:2774–2783.

19. Young, J. D.-E., C.-C. Liu, G. Butler, Z. A. Cohn, and S. J. Galli. 1987. Identification, purification, and characterization of a mast cell-associated cytolytic factor related to tumor necrosis factor. *Proc. Natl. Acad. Sci. USA.* 84:9175–9179.

20. Galli, S. J., B. K. Wershil, J. R. Gordon, and T. R. Martin. 1989. Mast cells: immunologically specific effectors and potential sources of multiple cytokines during IgE-dependent responses. *CIBA Found. Symp.* 147:53–73.

21. Gordon, J. R., and S. J. Galli. 1990. Mast cells as a source of both preformed and immunologically-inducible TNF-α/cachectin. *Nature (Lond.).* 346:274–276.

22. Burd, P. R., H. W. Rodgers, J. R. Gordon, C. A. Martin, S. Jayaraman, S. D. Wilson, A. M. Dvorak, S. J. Galli, and M. E. Dorf. 1989. Interleukin 3-dependent and -independent mast cells stimulated with IgE and antigen express multiple cytokines. *J. Exp. Med.* 170:245–247.

23. Ming, W. J., L. Bersani, and A. Mantovani. 1987. Tumor necrosis factor is chemotactic for monocytes and polymorphonuclear leukocytes. *J. Immunol.* 138:1469–1474.

24. Sayers, T. J., T. A. Wiltrout, C. A. Bull, A. C. Denn III, A. M. Pilaro, and B. Lokesh. 1988. Effect of cytokines on polymorphonuclear neutrophil infiltration in the mouse. *J. Immunol.* 141:1670–1677.

25. Mantovani, A., and E. Dejana. 1989. Cytokines as communication signals between leukocytes and endothelial cells. *Immunol. Today.* 10:370–375.

26. Dinarello, C. A. 1988. Biology of interleukin 1. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 2:108–115.

27. Wolpe, S. D., G. Davatelis, B. Sherry, B. Beutler, D. G. Hesse, H. T. Nguyen, L. L. Moldawer, C. F. Nathan, S. F. Lowry, and A. Cerami. 1988. Macrophages secrete a novel heparin-binding protein with inflammatory and neutrophil chemokinetic properties. *J. Exp. Med.* 167:570–581.

28. Wolpe, S. D., and A. Cerami. 1989. Macrophage inflammatory proteins 1 and 2: members of a novel superfamily of cytokines. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 3:2565–2573.

29. Burd, P. R., B. J. Rollins, S. D. Wilson, P. R. Billings, C. D. Stiles, and M. E. Dorf. 1988. Comparison of fibroblast and T-cell activation genes. *Cell Immunol.* 115:481–483.

30. Metzger, H., J.-P. Kinet, H. Blank, L. Miller, and C. Ra. 1989. The receptor with high affinity for IgE. *CIBA Found. Symp.* 147:93–113.

31. Capron, A., M. Capron, C. Grangette, and J. P. Dessaint. 1989. IgE and inflammatory cells. *CIBA Found. Symp.* 147:153–170.

32. Kikutani, H., A. Yokato, N. Uchibayashi, K. Yukana, T. Tanaka, K. Sugiyama, E. E. L. Barsumian, M. Suemrua, and T. Kishimoto. 1989. Structure and function of Fc$_\epsilon$ receptor II (Fc$_\epsilon$RII/CD23): a point of contact between the effector phase of allergy and B cell differentiation. *CIBA Found. Symp.* 147:23–35.

33. Galli, S. J., and I. Hammel. 1984. Unequivocal delayed hypersensitivity in mast cell-deficient and beige mice. *Science (Wash. DC).* 226:710–713.

34. Mekori, Y. A., J. C. C. Chang, B. K. Wershil, and S. J. Galli. 1987. Studies of the role of mast cells in contact sensitivity responses: passive transfer of the reaction into mast cell-deficient mice locally reconstituted with cultured mast cell; effect of reserpine on transfer of the reaction with DNP-specific cloned T cells. *Cell Immunol.* 109:39–52.

35. Wershil, B. K., Y. A. Mekori, and S. J. Galli. 1989. The contribution of mast cells to immunological responses with IgE- and/or T cell-mediated components. *In* Mast Cell and Basophil Differentiation and Function in Health and Disease. S. J. Galli and K. F. Austen, editors. Raven Press, Ltd., New York. 229–246.

36. Dolovich, J., J. Denberg, Y. N. Kwee, T. Belda, J. Blajchman, and F. E. Hargreave. 1983. Does non-immunologic mast cell mediator release/activation elicit a late cutaneous response? *Ann. Allergy.* 50:241–244.

37. Jacoby, W., P. V. Cammarata, S. Findlay, and S. H. Pincus. 1984. Anaphylaxis in mast cell-deficient mice. *J. Invest. Dermatol.* 83:302–304.

38. Ha, T. Y., N. D. Reed, and P. K. Crowle. 1986. Immune response potential of mast cell-deficient *W/W^v* mice. *Int. Arch. Allergy Appl. Immunol.* 80:85–94.

39. Martin, T. R., J. M. Drazen, and S. J. Galli. 1989. Active anaphylaxis is associated with tachycardia in normal but not mast cell (MC)-deficient mice. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 3:A790.

40. Ha, T.-Y., and N. D. Reed. 1987. Systemic anaphylaxis in mast cell-deficient mice of *W/W^v* and *Sl/Sl^d* genotypes. *Exp. Cell. Biol.* 55:63–68.

41. Kitamura, Y. S., S. Go, and S. Hatanaka. 1978. Decrease of mast cells in *W/W^v* mice and their increase by bone marrow transplantation. *Blood.* 52:447–452.

42. Galli, S. J., and Y. Kitamura. 1987. Animal model of human disease. Genetically mast cell-deficient *W/W^v* and *Sl/Sl^d* mice: their value for the analysis of the roles of mast cells in biological responses *in vivo*. *Am. J. Pathol.* 127:191–198.

43. Liu, F.-T., J. W. Bohn, E. L. Ferry, H. Yamamoto, C. A. Molinaro, L. A. Sherman, N. R. Klinman, and D. H. Katz. 1980. Monoclonal dinitrophenyl-specific murine IgE antibody: preparation, isolation, and characterization. *J. Immunol.* 124:2728–2737.

44. Wershil, B. K., Y. A. Mekori, T. Murakami, and S. J. Galli. 1987. ^{125}I-Fibrin deposition in IgE-dependent immediate hypersensitivity reactions in mouse skin: demonstration of the role of mast cells using genetically mast cell-deficient mice locally reconstituted with cultured mast cells. *J. Immunol.* 139:2605–2614.

45. Mekori, Y. A., and S. J. Galli. 1985. Undiminished immunologic tolerance to contact sensitivity in mast cell-deficient mice. *J. Immunol.* 135:879–885.

46. Dvorak, H. F., M. C. Mihm, Jr., A. M. Dvorak, B. A. Barnes, E. J. Manseau, and S. J. Galli. 1979. Rejection of first set skin allografts in man. The microvasculature is the critical target of the immune response. *J. Exp. Med.* 150:322–337.

47. Nakano, T., T. Sonada, C. Hayashi, A. Yamatodani, Y. Kanayama, T. Yamamura, H. Asai, Y. Yonezawa, Y. Kitamura, and S. J. Galli. 1985. Fate of bone marrow-derived cultured mast cells after intracutaneous, intraperitoneal and intravenous transfer into genetically mast cell-deficient *W/W^v* mice. Evidence that cultured mast cells can give rise to both connective tissue-type and mucosal mast cells. *J. Exp. Med.* 162:1025–1043.

48. Granstein, R. D., R. Margolis, S. B. Mizel, and D. N. Suader. 1986. *In vivo* inflammatory activity of epidermal cell-derived thymocyte activating factor and recombinant interleukin 1 in the mouse. *J. Clin. Invest.* 77:1020–1027.

49. Geissler, E. N., M. A. Ryan, and D. E. Housman. 1988. The dominant-white spotting (W) locus of the mouse encodes the c-kit proto-oncogene. *Cell.* 55:185–192.

50. Plaut, M., J. H. Pierce, C. J. Watson, J. Hanley-Hyde, R. P. Nordon, and W. E. Paul. 1989. Mast cell lines produce lymphokines in response to cross linkage of Fc$_\epsilon$RI or to calcium ionophore. *Nature (Lond.).* 339:64–67.

51. Klein, L. M., R. M. Lavker, W. L. Matis, and G. F. Murphy. 1989. Degranulation of human mast cells induces an endothelial antigen central to leukocyte adhesion. *Proc. Natl. Acad. Sci. USA.* 86:8972–8976.

52. Bevilacqua, M. P., S. Stengelin, M. A. Gimbrone, and B. Seed. 1989. Endotheiial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science (Wash. DC).* 243:1160–1165.

53. Pober, J. S. 1987. Effects of tumour necrosis factor and related cytokines on vascular endothelial cells. *CIBA Found. Symp.* 131:170–184.

54. Galli, S. J., A. M. Dvorak, and H. F. Dvorak. 1984. Basophils and mast cells: morphologic insights into their biology, secretory patterns, and function. *Prog. Allergy.* 34:1–141.

55. Galli, S. J. 1990. Biology of disease. New insights into "the riddle of the mast cells": microenvironmental regulation of mast cell development and phenotypic heterogeneity. *Lab. Invest.* 62:5–33.

56. Gordon, J. R., P. R. Burd, and S. J. Galli. 1990. Mast cells are a source of multifunctional cytokines. *Immunol. Today.* 11:458–464.