Dendritic Cell Modulation by Mast Cells Controls the Th1/Th2 Balance in Responding T Cells[1]

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The cytokines secreted by pathogen-activated human dendritic cells (DC) are strongly regulated in vitro by histamine, a major component of mast cell granules, ultimately modulating the capacity of the DC to polarize naive T cells. Because DC and mast cells are located in close proximity in peripheral compartments, we hypothesized that mast cell products would influence the maturation of DC and hence the Th balance of an immune response in vivo. In this study, we show that specific mast cell degranulation stimuli, given s.c. in mice with Ag and adjuvant, produce effector T cells that proliferate to Ag but secrete dramatically reduced levels of IFN-γ and increased amounts of IL-4 compared with control T cells primed in the absence of a mast cell stimulus. Immunization with Ag and adjuvant in the presence of a degranulation stimulus also resulted in the accumulation of DC in the draining lymph nodes that had reduced capacity to induce Ag-specific Th1 cells, in comparison with DC from mice lacking a degranulation stimulus. Therefore, by acting upon DC at sites of inflammation, mast cells play a critical role in determining the polarity of Ag-specific T cell responses in vivo. *The Journal of Immunology,* **2006, 177: 3577–3581.**

Both immature dendritic cells (DC)[3] and mast cells are strategically located at portals of pathogen entry and are therefore well-positioned to respond to infectious agents. DC serve as a bridge between the innate and acquired responses to pathogens (1). In the periphery, immature DC continually sample the microenvironment for potential Ags and receive instructional stimuli from bioactive substances (e.g., cytokines, chemokines, and a variety of small molecule mediators) in the surrounding medium. Immature DC express TLRs which recognize microbial structures (2, 3) and TLR ligation by pathogens stimulates the DC to mature and migrate to draining lymph nodes where they present pathogen-derived Ags to naive T cells. Notably, the instructional signals generated by neighboring cells at the site of infection modulate the capacity of DC to control the Th1/Th2 balance of Ag-specific effector cells (4, 5). DC control T cell polarization by using a number of cytokines and membrane molecules some of which have been well-characterized. For example, IL-12 is instrumental in promoting Th1 responses, and type I IFNs may play a role as well. IL-10 has been implicated in Th2 polarization, but factors controlling Th2 development are not well-characterized. Also, costimulatory molecules expressed by mature DC affect the Th balance, with OX40L, Jagged, high B7.2/B7.1 ratios and low peptide-MHC complex favoring Th2 responses (4, 5).

One instructional signal that affects the maturation of DC is histamine, a major component of cytoplasmic granules in mast cells. The release of histamine is normally triggered by multivalent ligands (e.g., allergens) that cross-link specific IgE Abs bound to FcεRI molecules on mast cell surfaces, ultimately leading to the fusion of granules with the plasma membrane (6). In addition to histamine, mast cell granules also contain proteases, proteoglycans, and in mice but not in humans, large amounts of serotonin. Degranulation is followed by the de novo synthesis and secretion of lipid-derived mediators (e.g., leukotrienes and prostaglandins) and, at later time points, cytokines such as IL-4 and GM-CSF. We and others (7–13) have previously shown that human DC express receptors for histamine, and that histamine profoundly alters the cytokines secreted by maturing DC. Importantly, histamine downregulates IL-12 and increases IL-10 production by DC, resulting in a decrease in expression of the Th1 cytokine, IFN-γ, and an increase in the Th2 cytokine, IL-4, by T cells primed in vitro by these DC. Th1 cells promote cell-mediated immunity, while Th2 cells induce humoral responses and, in unfavorable situations, allergy. The polarization of Th cells by DC is therefore crucial to the outcome of Ag-specific acquired immune responses.

In the current study, we asked whether mast cell degranulation in vivo affects the Ag-presenting and Th-polarizing functions of neighboring DC. We found that activation of mast cells at a site of immunization in mice markedly suppresses the development of Ag-specific Th1 responses in the draining lymph nodes, thus directly implicating mast cells in the control of acquired immune responses.

Materials and Methods

Mice

Female BALB/c mice, 6–12 wk old, were obtained from the Frederick Cancer Research and Development Center (Frederick, MD). DO11.10 $\alpha\beta$ TCR-transgenic BALB/c mice (14) were maintained at Bioqual. The DO11.10-transgenic TCR recognizes chicken OVA peptide 323–339 (ISQAVHAAHAEINEAGR, pOVA) bound to I-A^d, and is specifically labeled by the clonotype-specific mAb KJ126. All procedures involving mice were approved by the National Cancer Institute (NCI) Animal Care and Use Committee.

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[3] Abbreviations used in this paper: DC, dendritic cell; ODN, oligodeoxynucleotide; TNP, trinitrophenyl.

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Passive cutaneous anaphylaxis

Mice were injected i.v. with 100 μl of a 0.2% Evans blue solution in PBS (Sigma-Aldrich). Ten minutes later, 20 μl of compound 48/80 (40 μg) or PBS were injected in the left and right hind footpad, respectively. After ~10 min, development of blue color and swelling in the footpads were examined.

Adoptive transfer and immunization

$CD4^+$ T cells from DO11.10 mice were purified from lymph node cells by negative selection using BioMag anti-mouse IgG beads (Qiagen) to remove B cells, and a rat anti-mouse CD8 Ab (clone 2.43; a gift from Dr. A. Singer, NCI, Bethesda, MD) plus BioMag anti-rat IgG beads to remove $CD8^+$ T cells. Purity of all preparations was >97% as determined by FACS staining with anti-CD4. On day 0, 2.5×10^6 $CD4^+KJ126^+$ cells were injected i.v. into BALB/c mice. One day later, mice were challenged in both hind footpads (20 μl/footpad) with combinations of: LPS-free OVA (a gift from Dr. B. Kelsall, National Institute of Allergy and Infectious Diseases (NIAID, Bethesda, MD); 100 μg/footpad); LPS (*Escherichia coli*, serotype 055:B5, Sigma-Aldrich; 0.05 μg/footpad); CpG oligodeoxynucleotide (ODN) 1d (15), 0.5 nM/footpad; and compound 48/80 (Sigma-Aldrich; 40 μg/footpad). Three mice per group were used in each experiment. On day 5, mice were sacrificed by CO_2 asphyxiation and popliteal lymph node cells were harvested and pooled within the same treatment group. In some experiments, mice were sensitized with anti-trinitrophenyl (TNP)-IgE (16) either in the footpad (2 μg IgE/footpad) or i.p. (~40 μg of IgE/mouse). Three days later, they received transgenic T cells, and 1 day after that they were injected in the footpads with OVA, LPS, and 2 μg/footpad of DNP-BSA (Molecular Probes). In some experiments, 50 mg/kg each of pyrilamine and cimetidine (Sigma-Aldrich), antagonists specific for histamine receptor HR1 and HR2, respectively, were administered i.p. 2 h before footpad challenge.

T cell polarization assays

Single-cell suspensions of lymph nodes from immunized mice (10^6 cells/ml in complete RPMI medium (RPMI 1640 plus 10% FCS, 55 μM 2-ME, glutamine, antibiotics, nonessential amino acids, and sodium pyruvate)) were stimulated with 10 ng/ml PMA and 1 μg/ml ionomycin for a total of 5 h. Brefeldin A (10 μg/ml; Epicentre Technologies) was added during the last 4 h of stimulation. Cells were then surface stained with KJ126 mAb and internally stained for IL-4 and IFN-γ. Parallel cultures were established in which single-cell suspensions from lymph nodes were stimulated with pOVA for 3 days, then supernatants were collected and assayed for cytokine content with DuoSet ELISA Development kits (R&D Systems). Levels of IL-4 were assessed in supernatants that contained 20 μg/ml of a neutralizing rat anti-mouse IL-4R mAb (clone mIL4R-M1; BD Pharmingen). For proliferation assays, single-cell suspensions of lymph nodes from immunized mice (10^6 cells/ml in complete RPMI medium) were stimulated in 96-well culture plates (200 μl/well) for 2 days with the indicated amounts of pOVA, then pulsed with $[^3H]$thymidine as described (7).

In vitro stimulation of DO11.10 T cells

Draining popliteal lymph nodes were harvested 24 h after footpad challenge and DC-containing single-cell suspensions were obtained after a 30-min incubation at 37°C in serum-free medium containing collagenase (Liberase; Roche Diagnostics). DC were removed from some samples using CD11c MicroBeads (Miltenyi Biotec). Unfractionated and DC-depleted lymph node cells (4×10^5 cells/well) were cultured with purified $CD4^+$ T cells from DO11.10 mice (4×10^4 cells/well) in complete RPMI medium (200 μl/well) for 3 days, then restimulated for 5 h with PMA and ionomycin in the presence of brefeldin A. Cells were then surface stained with KJ126 mAb and internally stained for IL-4 and IFN-γ.

Flow cytometry

CyChrome-conjugated streptavidin and all Abs used for surface and intracellular staining were obtained from BD Pharmingen. Surface staining was performed in the presence of saturating levels of the anti-FcγR mAb 2.4G2. Permeabilization of cells for intracellular staining was performed using a commercial kit from BD Pharmingen, as described (7). A total of 3×10^5 events were collected per sample. Dead cells were excluded by gating on forward and side light scatter.

Statistical analyses

Data were analyzed by using a paired Student's *t* test. Values of p of 0.05 or less were considered significant.

Results

Mast cell degranulation affects T cell polarization but not proliferation, in vivo

It has been shown that in vitro treatment of maturing human monocyte-derived DC with histamine, a major component of mast cell granules, alters their cytokine secretion patterns and Th cell-polarizing capacities; in particular, IL-12 secretion is blocked and T cell responses are switched from a Th1 to a Th2 type (7). Therefore, we hypothesized that in vivo, degranulation of mast cells at a site of immunization could affect the Th1/Th2 balance of Ag-specific T cell responses in the draining lymph nodes. To test this hypothesis, $CD4^+$ T cells from DO11.10 TCR-transgenic mice were adoptively transferred into BALB/c mice and 1 day later, OVA, adjuvant, and a mast cell stimulus were injected into the hind footpads. We expected that the adjuvant would trigger the maturation and migration of peripheral DC to the draining (popliteal) lymph nodes, where they would specifically prime transgenic T cells. In the experiment shown in Fig. 1, degranulation of tissue mast cells was induced at the site of Ag challenge by coinjecting compound 48/80, a known mast cell secretagogue, with OVA and LPS, a TLR4 activator, as adjuvant. Injection of compound 48/80 alone induced extravasation of Evans blue dye and swelling in the footpads, indicative of mast cell activation (Fig. 1*A*) and caused a small increase in the total number of popliteal

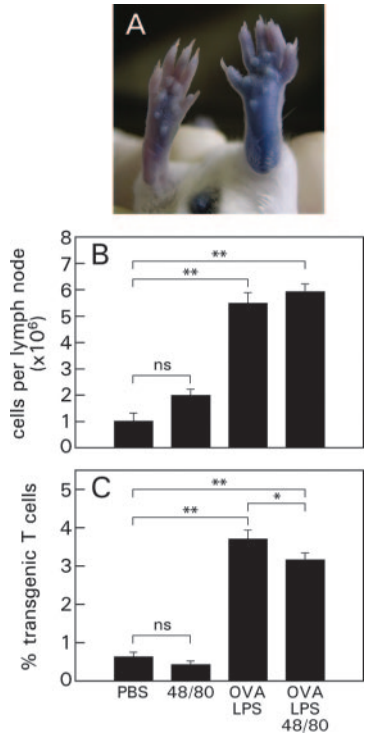

FIGURE 1. Administration of a mast cell secretagogue at the immunization site has minimal effect on the expansion of Ag-specific T cells. *A*, BALB/c mice were injected i.v. with Evans blue and 10 min later 20 μl of PBS or compound 48/80 were injected in the right and left hind footpads, respectively. Vascular leakage and swelling developed in the left footpad within 10 min. *B* and *C*, BALB/c mice adoptively transferred with DO11.10 TCR-transgenic T cells were challenged in the hind footpads with the indicated stimuli. Four days later, the total number of cells (*A*) and the percent of transgenic $CD4^+KJ126^+$ T cells (*B*) in the draining popliteal lymph nodes were determined. Means and SE of data from 3 to 12 separate experiments are shown. In each experiment, three mice per group were used, and lymph node cells within the same treatment group were pooled. **, $p < 0.0001$; *, $p < 0.05$; ns, nonsignificant.

lymph node cells (Fig. 1*B*). Although this increase was not statistically significant, the trend was consistent with results from a previous report (17). Injection of OVA with LPS resulted in a substantial increase in lymph node cellularity, which was not affected by coinjection of compound 48/80 (Fig. 1*B*). Transgenic T cells were not selectively expanded by compound 48/80 (Fig. 1*C*), but were selectively expanded by Ag (OVA) and LPS, as expected. Coadministration of 48/80 had only a minor effect on this expansion (14% inhibition). Thus, degranulation of mast cells does not impair the Ag-presenting capacity of the DC.

We next assessed the Th1/Th2 polarity of transgenic T cells from the draining lymph nodes. Immunization with OVA using LPS as adjuvant, resulted in the generation of significant numbers of transgenic T cells that expressed IFN-γ but very few that expressed IL-4, consistent with a Th1 type of response (Fig. 2, *left panel*). Strikingly, immunization in the presence of compound 48/80 resulted in a >2-fold loss in IFN-γ^+ cells and >3-fold increase in IL-4^+ cells (Fig. 2, *right panel*), indicative of Th2 polarization. We confirmed this polarization by stimulating lymph node cells with titrated amounts of pOVA, and measuring the levels of IFN-γ and IL-4 secreted into culture supernatants. As expected, lymph node cells from mice immunized with OVA and LPS released high levels of IFN-γ (Fig. 3*A*) and little IL-4 (Fig. 3*B*) in response to Ag, but inclusion of 48/80 in the immunization mix resulted in a loss in IFN-γ secretion, and an increase in IL-4. These differences in cytokine expression were not due to differential priming of T cells by DC, because T cells proliferated identically to secondary Ag challenge, independent of mast cell degranulation at the site of immunization (Fig. 3*C*).

We next performed experiments in which degranulation of mast cells was induced by FcεRI cross-linking, rather than by compound 48/80. Mice were sensitized with an anti-TNP IgE mAb, and 4 days later the footpads were injected with OVA and adjuvant together with DNP-BSA to cross-link the anti-TNP IgE bound to FcεRI on neighboring mast cells. In the mouse, only mast cells and basophils (not DC) express FcεRI (18). Specific mast cell degranulation following DNP-BSA treatment was confirmed by Evans blue extravasation (not shown). The total number of popliteal lymph node cells was similar in mice that had or had not been presensitized with IgE and a minor, but significant, reduction (~14%) in the expansion of transgenic T cells was observed in the sensitized mice (Table I). In addition, mast cell degranulation induced by IgE cross-linking had no effect upon the capacity of popliteal T cells to proliferate in response to a secondary in vitro challenge with Ag (Fig. 3*C*). Notably, IgE cross-linking at the immunization site caused a >2-fold decrease in IFN-γ^+-transgenic T cells; this decrease was accompanied by a modest increase in the percentage of IL-4^+ cells (Fig. 4*A*). Similar results were obtained when a TLR9 ligand, CpG ODN, was used as an adjuvant (Fig. 4*B*). We conclude that mast cell degranulation at the site of immunization, whether induced by compound 48/80 or FcεRI cross-linking, blocks the generation of Ag-specific Th1 effector cells in the draining lymph nodes and enhances, to a different degree depending on the mast cell stimulus, the development of Th2 effector cells.

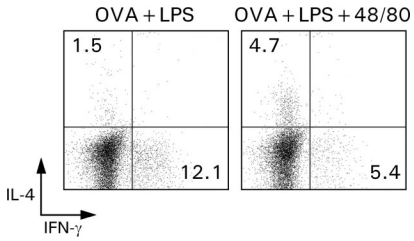

FIGURE 2. Induction of mast cell degranulation at the immunization site with compound 48/80 alters the balance of Th1/Th2 cells. BALB/c mice adoptively transferred with DO11.10-transgenic T cells were injected in the hind footpads with the indicated stimuli. Four days later, popliteal lymph node cells were harvested and stimulated with PMA and ionomycin in the presence of brefeldin A. Cells were surface stained with the KJ126 mAb, and then internally labeled with Abs against IFN-γ and IL-4. KJ126-positive transgenic cells were gated and analyzed. The numbers indicate the percent of transgenic T cells producing IL-4 (*upper left quadrant*) and IFN-γ (*lower right quadrant*). One representative experiment of three is shown.

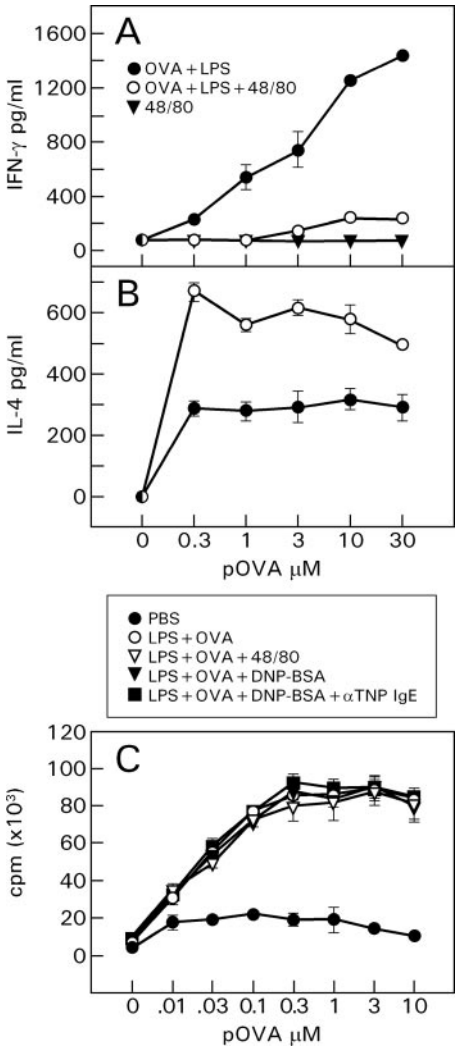

FIGURE 3. Mast cell activation at the site of immunization generates effector cells that secrete less IFN-γ and increased IL-4 upon secondary stimulation. DO11.10 reconstituted BALB/c mice were injected in the hind footpads with the indicated stimuli. Anti-TNP IgE was administered i.p. Four days later, popliteal lymph node cells were harvested and stimulated in vitro with titrated amounts of pOVA. IFN-γ (*A*) and IL-4 secretion (*B*) was assessed in supernatants after 3 days. No cytokines were detected in supernatants from lymph node cells of unimmunized mice. Proliferative responses to pOVA were measured by [^{3}H]thymidine incorporation (*C*). Data are representative of four separate experiments. Three mice per group were used, and lymph node cells from mice within the same treatment group were pooled.

Mast cell factors act on DCs to inhibit Th1 responses in vivo

To demonstrate that mast cell degranulation directly regulates the T cell-polarizing capacity of DC, BALB/c mice were challenged in

Table I. *FcεRI cross-linking in vivo does not hamper the expansion of transgenic T cells*[a]

	% Transgenic T Cells	Cells per Lymph Node ($\times 10^6$)
OVA + LPS + DNP-BSA (no anti-TNP IgE)	3.58 ± 0.12	5.06 ± 0.43
OVA + LPS + DNP-BSA (anti-TNP IgE)	3.09 ± 0.08	4.93 ± 0.30

[a] BALB/c mice sensitized or not with anti-TNP IgE, were reconstituted with DO11.10 TCR-transgenic T cells, and challenged in the footpads with the indicated stimuli. The percent of transgenic $CD4^+KJ126^+$ T cells and the total number of cells in the draining popliteal lymph nodes were determined four days after Ag challenge. Mean ± SE of data from seven experiments are shown. The small difference in the percentage of transgenic T cells between the two groups is significant ($p < 0.05$).

the footpads with OVA and adjuvant, and local mast cells were either activated or not by FcεRI cross-linking. The next day, cells from the draining lymph nodes were harvested and cultured with freshly isolated $CD4^+$ T cells from DO11.10-transgenic mice. Three days later, DO11.10 cells were analyzed for IFN-γ and IL-4 expression. As seen in Fig. 5*A*, lymph node cells from mice receiving a degranulation stimulus induced half the number of IFN-γ-producing DO11.10 cells as did lymph nodes from control animals. Moreover, selective removal of DC from the lymph node cells abolished their capacity to stimulate IFN-γ production (Fig. 5*B*). These data show that the differentiation of naive T cells into Th1 effector cells requires DC, and that DC in the draining lymph node have the capacity to regulate T cell polarization which is dependent upon prior mast cell activation at the site of immunization.

Discussion

Mast cells are traditionally viewed as effector cells in immediate-type hypersensitivity allergic reactions, but their role in the regulation of adaptive and innate immune responses has only recently been appreciated (19, 20). In this report, we demonstrate for the first time that in vivo, activated mast cells release mediators that alter the T cell-polarizing capacities of DC, thereby acting as regulators of acquired immune responses. Previously, Jawdat et al. (21) reported that, in the absence of a maturation stimulus, histamine released by mast cells in ear skin induced the migration of resident Langerhans cells (skin DC) to the draining lymph nodes, where they displayed a mature surface phenotype. However, the T cell-priming capacity of these DC was not investigated, and a recent report (22) suggests that DC are unable to generate effector T cells in the absence of a microbial stimulus in vivo. In our experiments, by contrast, mice were immunized s.c. with Ag and either *E. coli* LPS or CpG ODN to drive DC maturation, and test animals were given simultaneous mast cell degranulation stimuli; after several days to allow for T cell priming, the polarity of Ag-specific T cells from the draining lymph nodes was assessed. Consistently, mast cell degranulation resulted in a severe suppression of Th1 effector cell generation, as indicated by the abrogation of IFN-γ secretion, but had no effect upon the expansion or priming of Ag-specific T cells. We also observed an increase in the generation of IL-4-producing Th2 effector cells, although this effect was much more pronounced when degranulation was induced by compound 48/80 than by FcεRI cross-linking. One caveat of using compound 48/80 to induce mast cell degranulation is that it might also act directly on DC. Indeed, we observed that addition of compound 48/80 to LPS-stimulated cultures of splenic or bone marrow dendritic cells did cause a partial (maximum 40%) inhibition of IL-12 production (A. Mazzoni, unpublished observation). Thus, results using compound 48/80 as a degranulating agent should be considered supportive of experiments in which degranulation was induced by IgE cross-linking, but with the possibility that 48/80 may produce some effects that are independent of degranulation.

Because histamine alone regulated the polarizing capacity of human DC in vitro (7, 9), we performed preliminary experiments

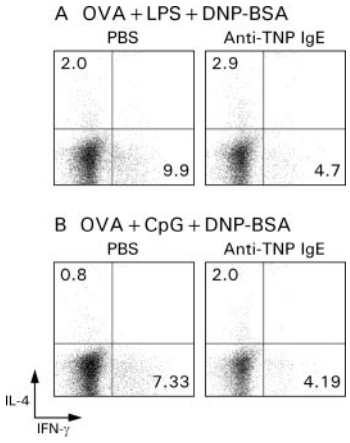

FIGURE 4. Degranulation of mast cells by FcεRI cross-linking at the site of immunization alters the Th1-Th2 ratio of effector cells. Mice were sensitized i.p. with anti-TNP IgE (*right upper* and *lower panels*) or PBS (*left upper* and *lower panels*), adoptively transferred with DO11.10 TCR-transgenic T cells, and challenged in the hind footpads with OVA, LPS, and DNP-BSA (*A*) or OVA, CpG, and DNP-BSA (*B*). After 4 days, draining lymph node cells were harvested and analyzed as described in Fig. 2. The numbers indicate the percent of transgenic T cells producing IL-4 (*upper left quadrant*) and IFN-γ (*lower right quadrant*) after gating on the KJ126-positive cells. Data are representative of three separate experiments.

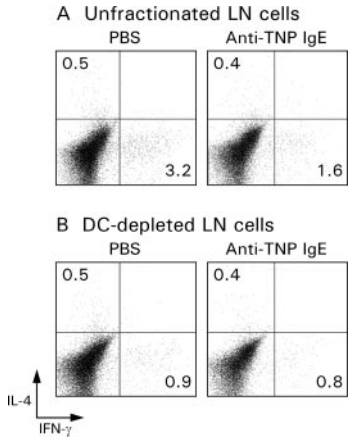

FIGURE 5. DC from mice immunized in the presence of a mast cell stimulus have a reduced ability to prime Th1 responses in vitro. Two days after i.p. administration of either anti-DNP IgE (*right panels*) or PBS (*left panels*), mice were challenged in the hind footpads with CpG, OVA, and DNP-BSA. One day later, unfractionated (*A*) and DC-depleted (*B*) cells from draining lymph nodes were mixed with freshly isolated DO11.10 cells and cultured for 3 days. Cells were internally labeled for IFN-γ and IL-4 and gated on KJ126-positive cells. The numbers indicate the percent of transgenic T cells producing IL-4 (*upper left quadrant*) and IFN-γ (*lower right quadrant*). One representative experiment of two is shown.

in an attempt to reverse the effects of mast cell degranulation on T cell polarization, using histamine receptor antagonists. We found, however, that high doses of the HR1 and HR2 histamine receptor antagonists, pyrilamine and cimetidine, administered i.p. before Ag challenge, failed to reverse the regulation of T cell polarity by mast cells (A. Mazzoni, unpublished observations). It is highly likely that in the s.c. microenvironment, DC are exposed to a variety of mediators released by activated mast cells in addition to histamine, including proteases, leukotrienes, prostaglandins, cytokines, and particularly serotonin, a potent mediator found in mouse, but not human, mast cell granules. Because any of these mediators could act in parallel with histamine to affect DC function, it is not surprising that histamine receptor antagonists failed to block the regulation of T cell polarization by degranulating mast cells.

The ability to integrate signals derived from invading pathogens with stimuli coming from neighboring cells is an essential feature of DC which allows them to regulate and fine-tune acquired immune responses against microbes (4, 5). DC interact with and receive signals from cells of the immune system such as mast cells, neutrophils, and NK cells (23–25); even cell types that do not traditionally perform immunological functions such as epithelial cells, keratinocytes, and stromal cells serve as important regulators of DC functions (26–29). Our results provide the first clear in vivo evidence that mast cells can affect the character of T cell responses in vivo. The s.c. model used in this study most closely parallels atopic dermatitis in the human, but may apply to other types of hypersensitivity as well. Interestingly, Piggott et al. (30) recently showed that the response of DC to pathogens depends upon the location of the DC, namely that pulmonary DC respond differently to LPS than do bone marrow-derived DC. This raises the possibility that mast cell degranulation might provoke a different type of reaction in the airways of asthmatic patients than, for example, in the skin of patients with atopic dermatitis. Also, at least one allergen, pollen, has the ability to act directly upon DC, and in the absence of mast cells, to block IL-12 production and promote Th2 responses in pathogen-activated DC (31). Taken together, the results of the current and previous studies indicate that in allergic individuals, a combination of environmental factors and endogenous mediators converge on DC to ultimately control the Th balance of immune responses. These findings suggest that DC may serve as a critical point of intervention for the therapeutic treatment of atopic disorders.

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Disclosures

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