

IgE Cross-Linking or Lipopolysaccharide Treatment Induces Recruitment of Th2 Cells to the Lung in the Absence of Specific Antigen¹

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We previously showed that Th1 cells can increase recruitment of Th2 cells to the lungs even in the absence of the Th2-specific Ag. The fact that Th2 recruitment is independent from the Th2 cell Ag suggested that Th1 cells may support Th2 cell recruitment using their Ag-nonspecific proinflammatory functions. To investigate the potential for inflammatory stimuli that are distinct from Ag-specific signals to affect the recruitment of T cells, we tested whether cross-linking of IgE or treatment with LPS modulated influx of Th2 cells into the airways in the presence or absence of inhaled Ag. When naive mice that had been treated with OVA-specific Th2 cells and passively sensitized with anti-DNP IgE were challenged by intranasal administration of either DNP-haptenated OVA or DNP-BSA, increased numbers of Th2 cells were recruited to the lung compared with mice challenged intranasally with OVA alone. Intranasal administration of LPS also increased recruitment of Th2 cells to the airways. These two distinct inflammatory stimuli increased the numbers of recruited Th2 cells equally with or without concurrent challenge using the cognate Th2 Ag. This Ag-independent recruitment of Th2 cells to the lung was not associated with localization of these cells to the regional lymph nodes and was independent of Th2 cell activation. Interestingly, P- or E-selectin contributed to Th2 cell recruitment to the lung. These data suggest that Th2 cells of the adaptive immune response are similar to cells of the innate immune response in their lack of requirement for protein Ag to initiate cell recruitment. They demonstrate further that recruitment can occur independently of Ag-dependent activation. *The Journal of Immunology*, 2002, 169: 5468–5476.

Naive Th cells circulate through the lymph and blood until they encounter their cognate Ag presented in the secondary lymphoid organs, and then they become activated to proliferate and express their effector functions. One to three days after activation, responding T cells leave the lymph nodes (LNs)⁴ and are increasingly recruited to effector sites where challenge has occurred (1, 2). Recruitment of immune cells into the tissues is an important regulatory checkpoint because regulated recruitment permits rapid amplification of local immune responses. The effect of Ag-induced activation on the migration potential of a T cell (3), the effect of T cell-specific Ag localized in a tissue on recruitment of T cells to that tissue (4), and the contribution of inflammatory signals delivered within a tissue on the recruitment potential of that tissue (5) are only beginning to be understood.

Using inherited memory in the form of pattern recognition receptors, tissue-resident cells of the innate immune system can rec-

ognize pathogens and regulate the induction of an immune response both locally and in secondary lymphoid tissues as part of their subsequent role as APCs (6–8). Once they have identified a pathogen and responded locally, these sentinels recruit nonresident effector cells to the site of insult. They accomplish this by using soluble mediators such as cytokines and chemokines that act on the local vascular endothelium to enhance its adhesive qualities and to recruit cells directly. Although the cognate Ag plays a central role in activating many functions of T cells, the specific stimulatory activities of Ag in the context of MHC have not been separated from the nonspecific inflammatory signals that local Ag may induce that then may play a role in T cell recruitment to the tissue. Antigenic peptide and MHC can affect the adherence of T cells to an ICAM-1-containing lipid bilayer in vitro (9); however, the effect of specific Ag on recruitment of T cells in vivo is poorly understood.

Leukocyte recruitment is regulated by three main families of molecules: selectins, integrins, and chemokines (10). Expression of the members of each of these families is differentially regulated by Th1 cells and Th2 cells as well as by regulatory T cells (11–15). The migration patterns of Th1 and Th2 cells are also distinct in vivo, both in recirculation (16) and during inflammatory responses (17). In several experimental model systems, Th1 cells are recruited into effector sites in response to Ag more readily than are Th2 cells. Th1 cells but not Th2 cells have been shown to migrate into inflamed skin, and the migration of Th1 cells was blocked by systemic treatment with Abs to P- and E-selectin (17). Adoptively transferred Th1 cells and Th2 cells also migrate differently into the inflamed peritoneum (3), pancreatic islets (18), and the gastric mucosa, where the data suggest that differential recruitment may genuinely play a role in defining the local milieu. Differential recruitment may even exclude Th2 cells that are produced in the draining LN from the effector site (19).

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⁴ Abbreviations used in this paper: LN, lymph node; i.n., intranasal; Tg, transgene; BAL, bronchoalveolar lavage; PAS, Periodate Acid Schiff's.

Studies by Xie et al. (3) demonstrated that the recruitment of Th1 cells to the peritoneal cavity is more efficient than that of Th2 cells, even when recruitment is enhanced by administration of adjuvant or by injection of the chemokine IFN-inducible protein 10 in the absence of specific Ag. This important finding suggested that Th1 cells can be recruited to sites of inflammation in an Ag-independent manner. Xie et al. (3) suggested that because Th1 cells were more readily recruited to the peritoneal cavity, Th2 cells may be particularly adapted for migration to sites such as the lungs that are known to host Th2-type inflammation. Arguing against this broad interpretation, Randolph et al. (20) showed that adoptively transferred Th1 cells are also recruited to the lungs more efficiently than are Th2 cells. Although these studies suggest that Th2 cells are intrinsically less competent for recruitment to peripheral tissues, it is well established that Th2 cells predominate in affected tissues, under conditions of parasitic infection (for example, see Ref. 21). Therefore, questions remain regarding the nature of the stimuli that elicit Th2 cell recruitment.

Our laboratory has previously investigated early events in the challenge phase of Th cell-dependent eosinophilic airway inflammation. Using a short protocol in which *in vitro*-differentiated Ag-specific transgenic Th cells were adoptively transferred into naive mice and challenged via the airway 1 day later, we simulated the initial phase of T cell recruitment in isolation from the full endogenous response. The unique characteristics of this system allowed us to investigate the regulation of the threshold for T cell recruitment to the lung *in vivo*. Using this model, we previously observed that adoptively transferred Th2 cells retained their differentiated Th2 phenotype for many days after transfer (22), but when administered in the absence of Th1 cells, they did not enter the lungs in response to airway Ag challenge. Under similar conditions, Th1 cells were efficiently recruited. Importantly, when Th1 and Th2 cells were transferred together, airway challenge led to successful recruitment of both subsets (23). These observations indicated that Ag challenge alone was not sufficient for recruitment of Th2 cells to the airways, but that recruitment of Th1 cells can condition the environment to enable Th2 cell migration. We hypothesized that the recruitment of Th1 cells might influence Th2 cell accumulation in the tissues due to the inflammation they induce independent of their Ag specificity. Interestingly, as shown in the accompanying report (56), when Th2 cell recruitment was elicited by cotransfer of Th1 cells with an antigenic specificity different from that of the Th2 cells, the Th2 cells were recruited even in the absence of Th2-specific Ag. Surprisingly, the recruited Th2 cells showed aspects of an activated phenotype even in the absence of the Th2 Ag. This suggested that recruitment might lead directly to T cell activation. In this report, to investigate the factors influencing Th2 migration into the lungs and to distinguish the effects on recruitment of local inflammation from the effects of Th2 cell activation, we tested the impact of two different types of innate cell stimuli on T cell recruitment both in the presence and absence of the specific Th2 Ag. We showed that mast cell stimulation by cross-linking IgE receptors can induce Th2 cell migration into the lung. Intranasal (*i.n.*) administration of LPS also induces Th2 cell migration into the airways. Both of these innate cell stimuli were able to affect Th2 cell recruitment in a T cell Ag- and activation-independent manner.

Materials and Methods

Mice

Female BALB/c mice (Harlan Sprague Dawley, Indianapolis, IN) were used between 6 and 8 wk of age. DO11.10 mice (24) that are transgenic for a TCR that recognizes chicken OVA_{323–339} in I-A^d were the generous gift of K. Murphy (Washington University, St. Louis, MO). Thy1.1⁺ DO11.10

mice were generated as previously described (23) and splenocytes were differentiated into Th1 and Th2 cells as described below. The resulting Thy1.1⁺ cells were transferred into Thy1.2⁺ BALB/c mice. OT-II.2 mice (25) are transgenic for a TCR that recognizes the same OVA peptide in I-A^b. Because the transgene (Tg) is inserted into the Y chromosome, only males are Tg positive. OT-II.2 mice were a generous gift of Paul Allen (Washington University). Cells from these mice were transferred into 6- to 8-wk-old male C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) and used in Fig. 6. P- and E-selectin double-deficient mice (mixed C57BL/6 and Sv129 background; The Jackson Laboratory) (26) were maintained by homozygous mating. All mice were kept in microisolator cages in the specific pathogen-free Division of Comparative Medicine facility at Washington University Medical Center. They were fed and watered *ad libitum*. All experiments were approved by the Washington University Institutional Committee for the Humane Use of Laboratory Animals.

Preparation of differentiated Th cells

Splenocytes from the DO11.10 or OT-II.2 TCR Tg⁺ mice were made into single cell suspensions with a disposable mesh (BD Falcon, Bedford, MA), cultured at $2.5\text{--}5 \times 10^6/\text{ml}$ with $0.3 \mu\text{M}$ peptide, and split as needed with 40 U/ml IL-2 (BD PharMingen, San Diego, CA). T cells were cultured in 10% FBS (HyClone Laboratories, Logan, UT) in IMDM with $2 \times 10^{-5} \text{ M}$ 2-ME, 1 mM sodium pyruvate, $1 \times$ nonessential amino acids, $10 \mu\text{g/ml}$ penicillin, $10 \mu\text{g/ml}$ streptomycin, and 2 mM L-glutamine (all from Life Technologies, Grand Island, NY). Th2 cells were prepared by culturing in 40 ng/ml recombinant murine IL-4 (R&D Systems, Minneapolis, MN), $1/8$ dilution of anti-IFN- γ (clone TOSH; kindly provided by E. Unanue, Washington University) hybridoma supernatant, and Th1 cells were made by adding 10 ng/ml IL-12 (BD PharMingen) and $1/8$ dilution of anti IL-4 (11B11) hybridoma supernatant. By intracellular staining, 30–60% of the differentiated Th1 cells were potential IFN- γ producers and 20–30% of the Th2 cells were IL-4 single producers. Cells were used for adoptive transfer 7 days after the last stimulation. At this time, these cells did not make detectable IL-4 or IFN- γ by intracellular staining and flow cytometry or by ELISA (data not shown).

Adoptive transfer and Ag challenge

Differentiated, resting DO11.10 Th cells were adoptively transferred *i.v.* via the retro-orbital plexus. The next day, 1% (w/v) Ag in PBS was administered *i.n.* or by aerosol with similar results. In the case of *i.n.* exposure, $40 \mu\text{l}$ of 1% solution was administered twice 6 h apart to mice anesthetized with Metaflane (methoxyflurane; Schering-Plough Animal Health, Union, NJ). Aerosol Ag was administered as described previously (20). Where indicated, $25 \mu\text{g}$ of *Salmonella typhosa* LPS (Sigma-Aldrich, St. Louis, MO) was administered *i.n.* in $20 \mu\text{l}$ of PBS on the day after T cell transfer and immediately before aerosol Ag.

Immediate type hypersensitivity skin test

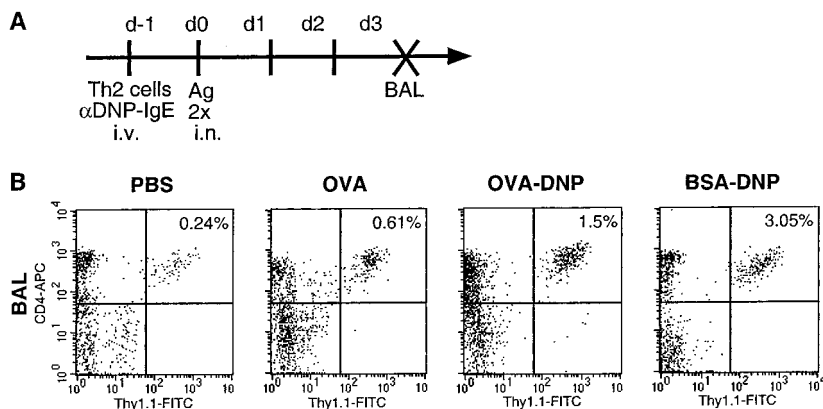
The dose of IgE required to produce a detectable immediate type hypersensitivity response was determined by injecting various amounts of monoclonal anti-DNP IgE (clone SPE-7; Sigma-Aldrich) *i.v.* (modified from Ref. 27). The abdomens of the mice were shaved. The next day, $50 \mu\text{l}$ of 0.5% Evan's blue dye (Sigma-Aldrich) in PBS was given *i.v.*, and then OVA-DNP₁₂ or BSA-DNP₂₂ was injected intraepidermally to form a blister. When injected, Ag causes increased vascular permeability, and Evan's blue leaks out of the vasculature and stains the blister blue. At 30 min after challenge, both $0.1 \mu\text{g}$ and $1.0 \mu\text{g}$ of IgE were sufficient to allow a blue wheal to form at the site of Ag injection.

AgS used for airway challenges

OVA (grade V) and *Salmonella typhosa* LPS were purchased from Sigma-Aldrich. OVA-DNP was initially prepared according to the method of Eisen et al. (28) as follows. OVA-DNP (Fig. 1) was made by mixing 1 g of OVA and 1 g of KCO₃ in 20 ml of water together with 2 g of dinitrochlorobenzene (either liquid or crystalline) in 30 ml of water. The reaction was mixed for 4 h or more at room temperature in the dark. The color changed from yellow to orange as the conjugate formed. The product was dialyzed exhaustively against PBS.

The number of DNP groups (*n*) per OVA molecule was estimated as follows: $(\text{DNP})(\text{moles/liter}) = \text{OD}_{355}/14,900$ (ϵ of DNP), $(\text{OVA})(\text{moles/liter}) = A_{595}/45,000$ (molecular mass of OVA), $\text{OVA-DNP}_n = (\text{DNP})/(\text{OVA})$. *n* was always found to be 10 for both OVA-DNP and BSA-DNP₂₂. For some experiments, OVA-DNP and BSA-DNP₂₂ were purchased from Biosearch Technologies (Novato, CA).

FIGURE 1. Cross-linking IgE receptors with multivalent haptens recruit Th2 cells to the airway. **A**, 10^7 in vitro-differentiated Thy1.1⁺ DO11.10 Th2 cells and 1.0 μ g of anti-DNP IgE in 100 μ l of PBS were transferred i.v. into naive BALB/c mice (Thy1.2). One day later, the indicated Ag (1.0% in 40 μ l of PBS) was administered i.n. twice $\sim$ 6 h apart. Seventy-two hours after the first challenge, BAL cells were collected and analyzed by flow cytometry. **B**, Representative FACS data showing the recruitment to the airways of adoptively transferred Th2 cells (CD4⁺ Thy1.1⁺ lymphocytes). Percents shown represent the fraction of total cells in the BAL that have the forward and side scatter properties of lymphocytes and that are CD4⁺ Thy1.1⁺. Similar results were obtained in four different experiments. Each dot plot represents $\sim$ 10,000 cells.



Analysis of airway and lung inflammation

Three days after challenge, mice were sacrificed by lethal injection using 50 μ l of a mixture of ketamine (100 mg/ml) and xylazine (20 mg/ml), and bisection of the descending vena cava and their airways were assessed for inflammation. The trachea was cannulated and airway inflammatory cells were obtained by bronchoalveolar lavage (BAL) with four 0.8-ml aliquots of ice-cold 2% FBS in PBS. In some experiments, the left upper lobe was tied off after lavage and kept at 4°C in T cell medium until it was dissociated through a 40- μ m nylon mesh (BD Falcon). This fraction was designated "lung cells." The numbers of live cells counted in this fraction were proportional to the total number of cells in the BAL. RBCs were lysed using 8.3 g/L ammonium chloride, 10 mM Tris base. Nucleated cells were counted in a hemacytometer. Eosinophil counts were determined in cytopsin preparations of the BAL cells using Wright's stain. Other BAL cells were identified by Ab staining and FACS analysis. The transferred Th2 cells were followed using the clonotypic Ab for the DO11.10 TCR, KJ1-26-tricolor (29), or anti-V β 8.1, 8.2-tricolor (both purchased from Caltag, Burlingame, CA, and used at 1/25 dilution). Other Abs used were anti-Thy1.1-biotin, anti-CD25-PE, anti-CD4-cyc, anti-CD4-APC, anti-TCR V α 2 and V β 5.1/2, and streptavidin-APC at 1/50 (BD Pharmingen). The analysis was done using a FACSCalibur (BD Immunocytometry Systems, San Jose, CA) and Cell Quest (version 3.3; BD Immunocytometry Systems). Staining for carbohydrate-modified mucins was performed by the Barnes-Jewish Hospital Morphology Core Facility (St. Louis, MO) using periodic acid Schiff's (PAS) and Mucicarmine on formaldehyde-fixed and paraffin-embedded sections.

Results

IgE cross-linking stimulates recruitment of Th2 cells

IgE that is bound by the Fc ϵ Ig receptors on mast cells and basophils leads to cross-linking of these receptors after challenge with multivalent Ag and induces activation of the mast cell, one facet of a typical Th2-type inflammatory response (30). To simulate the potential effect of Fc ϵ R cross-linking-induced mast cell activation on recruitment of Th2 cells, IgE anti-DNP Abs and DO11.10 OVA-specific Th2 cells were passively transferred into naive mice and the mice were challenged with DNP-haptenated protein Ags. The dose of IgE used was determined by titrating IgE and testing epicutaneously for an immediate type hypersensitivity response. We used a dose of IgE sufficient to activate mast cells after cross-linking with 1% Ag. We used the allotypic marker Thy1.1 to track the transferred T cells from Thy1.1⁺ DO11.10 mice in Thy1.2⁺ BALB/c recipients. A total of 10^7 Thy1.1⁺ DO11.10 Th2 cells and 1.0 μ g IgE anti-DNP Abs were transferred i.v. into naive BALB/c recipient mice whose T cells express Thy1.2 (Fig. 1A). On the next day, the mice were challenged by i.n. administration of 1% OVA or OVA-DNP₁₂ solution. Negative controls received PBS alone. On the third day after challenge, BAL cells were analyzed for the percentage of CD4⁺ Thy1.1⁺ transferred Th2 cells by flow cytometry and the number of Th2 cells calculated as the product of this percentage multiplied by the

total number of nucleated cells in the airway. Challenge with OVA induced a small increase in CD4⁺ lymphocytes that were Thy1.1⁺ Th2 cells (Fig. 1) (23). In contrast, challenge with OVA-DNP, which both cross-links the IgE and stimulates the Th2 cells, as well as with BSA-DNP₂₂, which triggers the mast cells without activating the transgenic T cells, resulted in a greater than sixfold increase in the percent BAL Th2 cells compared with PBS-challenged mice. The percentage of Th2 cells in the BAL is even larger than that seen in the OVA-DNP challenge, due to the lack of eosinophils in the BSA-DNP₂₂ BAL (Fig. 2B). Although a change in the percent of BAL cells that are Th2 cells is observed in Figs. 1B and 2A, this difference is more striking when measured as the change in the actual number of BAL Th2 cells seen in Fig. 2. Cross-linking IgE using either OVA-DNP or BSA-DNP₂₂ induced a dramatic increase in the total number of recruited Th2 cells compared with OVA alone (Fig. 2). The increase in recruitment of Th2 cells was greater at 1 μ g (0.72% transferred cells/total cells or 2×10^5) than at 0.1 μ g (0.46%, 1.1×10^5) of IgE, suggesting that Th2 cell recruitment is proportional to the dose of IgE used for sensitization. In this experiment, mice that received i.n. PBS without Ag

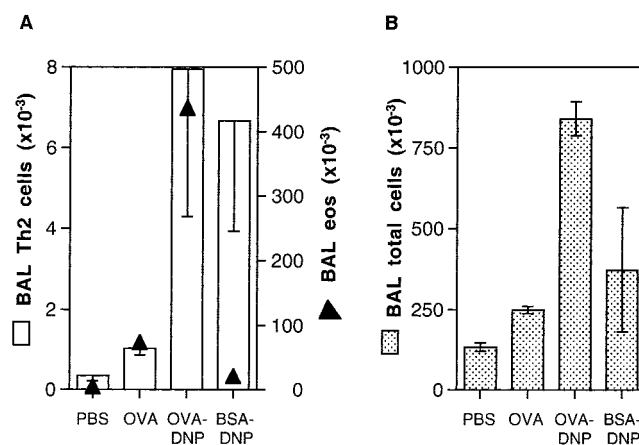


FIGURE 2. T cell Ag-independent recruitment of Th2 cells to the airways by cross-linking IgE receptors with multivalent haptens. A total of 10^7 in vitro-differentiated Thy1.1⁺ DO11.10 Th2 cells were transferred i.v. into naive BALB/c mice (Thy1.2) together with 1.0 μ g of anti-DNP IgE. The next day, the indicated Ag (40 μ l of a 1% solution in PBS) was administered i.n. twice, $\sim$ 6 h apart. Seventy-two hours after the first challenge BAL cells were collected and analyzed by FACS for the presence of the transferred Thy1.1⁺ CD4⁺ Th2 cells and by Wright's stain of cytopsin preparations for eosinophils. **A**, The numbers of Th2 cells and eosinophils in the BAL. **B**, The total numbers of cells in the BAL. Similar data were obtained in four separate experiments. Data represent mean $\pm$ SEM ($n = 5$).

had BAL with 0.12% Th2 cells (total 1.1×10^4 recruited T cells). These data suggest that IgE-dependent stimulation, presumably via high-affinity FcεRs on the surfaces of mast cells and basophils, is capable of stimulating production of inflammatory mediators that increase either the migration potential of the Th2 cells or the recruitment potential of the lung microenvironment.

T cell Ag is not required for local recruitment of Th2 cells

As we have shown previously (23), when Th2 cells are adoptively transferred to naive mice, Ag challenge results in insufficient Th2 recruitment to induce eosinophilic airway inflammation. Increasing the number of repetitions of Ag challenge leads to increased recruitment of Th2 cells (our unpublished data and Ref. 31). Having shown that an innate stimulus could recruit DO11.10 Th2 cells to the airways in the presence of OVA, we tested whether T cell-specific Ag was required for this recruitment. Using an Ag, BSA-DNP₂₂, which can cross-link anti-DNP IgE but which is not an Ag for the OVA-specific T cells, we could examine the role of specificity in Ag-mediated recruitment. Intranasal administration of BSA-DNP₂₂ to mice that had been passively sensitized with anti-DNP IgE increased recruitment to the airway of transferred OVA-specific Th2 cells in numbers similar to those recruited after challenge with OVA-DNP (Fig. 2). This indicates that the T cell-specific Ag is not required for recruitment of Th2 cells to the airway. Importantly, although BSA-DNP₂₂ elicited recruitment of OVA-specific Th2 cells, it did not elicit recruitment of eosinophils, whereas the Th2-activating Ag OVA-DNP did. These data indicate that mast cell inflammatory stimuli induced the recruitment of Th2 cells to the airway, but did not activate these cells to perform their full spectrum of effector functions. Additionally, this experiment showed no evidence of direct recruitment of eosinophils by mast cells. On the contrary, in this system, unless the Th2 cells have been activated by their cognate Ag, eosinophil recruitment does not occur. The total number of cells in the BAL of mice challenged with BSA-DNP₂₂ (Fig. 2B) increased only modestly over background, and the increase in total cells in the airways of OVA-DNP-challenged mice can be accounted for entirely by the increase in eosinophils in the infiltrate. These data suggest that stimulation of mast cells through the IgE receptor FcεRI does not lead to additional monocyte or macrophage infiltration. Furthermore, the effect of IgE on the recruitment of Th2 cells in these experiments is not dependent on the presence of T cell Ag.

Recruitment of Th2 cells to the airway is not inherently associated with T cell activation

In most systems it is difficult to separate T cell recruitment from T cell activation because the presence of T cells in a tissue is generally a consequence of effector processes for which both activation and recruitment are required. Consequently, the role of Ag in the recruitment phase of the Th2 cell response remains unknown. Here, challenge with BSA-DNP₂₂ resulted in recruitment of OVA-specific Th2 cells without eosinophils, suggesting that the Th2 cells that had been recruited to the lungs did not express an activated phenotype. These findings highlight questions regarding how closely linked Th cell activation and Th cell recruitment are. For example, Th cells might become "recruitable" due to experiences in the LN during activation. To test whether the cells recruited to the lung were also present in the local LN and to determine whether the transferred CD4⁺ cells were activated, we extended our analysis to evaluate expression of IL-2Rα (CD25), which is transiently up-regulated on activated T cells. High expression of CD25 is observed on transferred cells that have migrated to the lungs and the regional LNs of mice treated with airway OVA or OVA-DNP (Fig. 3). When passively sensitized mice were challenged with OVA-DNP, transferred Th2 cells positive for the DO11.10 clonotype KJ1-26 were recruited to both the airway and the paratracheal LNs. Few KJ1-26⁺ cells were detected in nondraining LNs (data not shown). Because the proportion of CD25⁺ transgenic cells is lower in the lung compared with the LNs, it would appear that activation is not a prerequisite for lung migration. These findings are underscored by the results of airway challenge with BSA-DNP₂₂. Here, in the absence of the T cell Ag, although there was substantial recruitment of the transgenic cells to the airway, the Th2 cells do not accumulate in the regional LN. This is consistent with previous studies from our lab and others indicating that Ag-experienced cells are not recruited to the LNs in the absence of Ag (32). In contrast, although BSA-DNP₂₂ led to the recruitment of large numbers of Th2 cells to the airway, this Ag-independent T cell recruitment was not associated with activation of the recruited cells, as evidenced by the absence of CD25^{high} Th2 cells in the lung in this setting (Fig. 3). Thus, in contrast to recruitment of Th2 cells by Th1 cells (56), local cross-linking of IgE leads to recruitment to the lung of Th2 cells that are not activated.

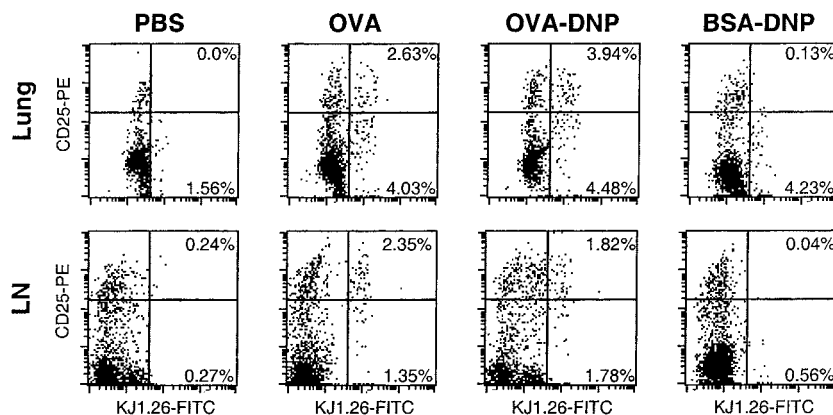


FIGURE 3. T cell Ag is required for LN accumulation and activation of Th2 cells recruited in response to IgE cross-linking. Naive BALB/c mice were passively sensitized with anti-DNP IgE and Thy1.1⁺ DO11.10 Th2 cells as in Fig. 2. Three days after airway Ag challenge, the lung and the paratracheal LNs were collected. The tissues were dissociated as described in *Materials and Methods*, and the activation state of the transgenic Th2 cells was analyzed by FACS using the DO11.10 clonotype Ab KJ1-26 (KJ-FITC) and anti-IL-2Rα (CD25). Profiles shown are gated on CD4⁺ cells with the forward and side scatter characteristics of lymphocytes. Percents shown represent the fraction of CD4⁺ cells in the BAL that are KJ1⁺ and CD25⁺ or CD25⁻. Each dot plot represents 10,000 lymphocyte events collected. Similar data were obtained in three additional experiments.

Mucus production is not induced by Ag-nonspecific T cell recruitment

The Th2-specific cytokines IL-9 and IL-13 are excellent promoters of airway mucus secretion. Sensitized mice carrying targeted null mutations of the genes encoding either these molecules or their receptors have decreased mucus production in the lungs after Ag challenge (33, 34). Conversely, transgenic mice producing these cytokines under the lung-specific rat Clara cell 10-kDa protein promoter have severe goblet cell hyperplasia and increased production of mucins (35, 36). These data suggest that up-regulation of airway epithelial mucus production is a process driven by Th2 cells. Enhanced mucus production, therefore, can be used as a marker of the action of activated Th2 cells. This is consistent with our observation here that there is a lack of increased epithelial mucus production after administration of BSA-DNP₂₂, which induces recruitment of Th2 cells but does not activate them (Fig. 4). Although Th2 cells accumulate in the lungs of passively sensitized mice in response to airway challenge with BSA-DNP₂₂, they localize there without expression of their effector functions. There were no eosinophils and little epithelial mucus production in the BSA-DNP₂₂-challenged lungs. This indicated that recruitment of Th2 cells into the lung and activation of these Th cells are separable events.

Intranasal LPS enhances recruitment of Th2 cells and produces a large infiltrate

Our initial experiments using IgE cross-linking showed that this kind of Th2-associated inflammation could elicit Ag-independent recruitment of Th2 cells to the airways. To test the generalized role of inflammation for recruitment of Th2 cells and to explore the range of inflammatory signals that could elicit Th2 cell recruitment, we tested whether LPS could modulate Th2 cell recruitment. LPS induces activation of macrophages, dendritic cells, and endothelial cells by binding the LPS receptor (CD14) and signaling through the Toll-like receptor 4 and MD2, which activates NF- κ B. This activated NF- κ B, together with other signals, can lead to IL-12 production. Because IL-12 drives the differentiation of Th1 cells and the secretion of IFN- γ by NK cells, this kind of stimulus leads to inflammation with features quite distinct from the inflammation that occurs after mast cell activation by cross-linking surface IgE.

To test the hypothesis that signals common to many types of inflammatory responses are the primary determinant of Th2 cell recruitment to the lung, we instilled *Salmonella typhosa* LPS i.n. into recipients of adoptively transferred DO11.10 Th2 cells. Recruitment of Th2 cells was observed in both the lungs and the airways of recipient mice receiving LPS (Figs. 5, A and B). The LPS-induced inflammation was associated with recruitment of Th2 cells and other cell types yielding a substantial increase in the total BAL cell numbers (Fig. 5C). These cells included macrophages and monocytes as well as lymphocytes and eosinophils.

An important finding in these studies is that inflammatory stimuli as different as LPS and IgE cross-linking are both potent at recruiting Th2 cells to the lungs. That such diverse types of inflammation are capable of permitting Th2 cell recruitment suggests that the molecules responsible for this effect are not unique to either IgE- or LPS-responsive cells. Using repeated Ag exposure as a stimulus for Th2 cell recruitment, Lloyd et al. (37) showed that the eosinophil attractant, eotaxin, and the macrophage-derived chemokine were involved in Th2 cell recruitment. This suggests that multiple molecular mechanisms may contribute to recruitment of Th2-type inflammation to the lung.

Endothelial selectins contribute to the recruitment of Th2 cells to the lung

Several elegant studies that have investigated the usage of P- and E-selectins by Th1 and Th2 cells have indicated that Th1 cells use selectins for recruitment to sites of inflammation, but have suggested that Th2 cells do not. Th2 cells neither bind P- or E-selectin Ig fusion proteins (3, 38) nor express high levels of the fucosylating enzyme $\alpha(1,3)$ -fucosyltransferase VII (39) necessary to generate functional P-selectin glycoprotein ligand (40), although they do express the P-selectin glycoprotein ligand protein backbone, CD34 (38). In contrast, it is clear that recruitment of Th1 cells into the skin and the peritoneal cavity is dependent on the endothelial selectins. Systemic administration of blocking Abs to P- and E-selectin blocks Th1 cell recruitment (17, 41, 42). We have extended these studies to investigate the role of P- and E-selectin in the recruitment of Th2 cells to the lungs and airways. OVA-specific Th2 cells were fully differentiated in vitro from male OT-II.2 mice (H-2^b) (25) and were transferred i.v. into P- and E-selectin

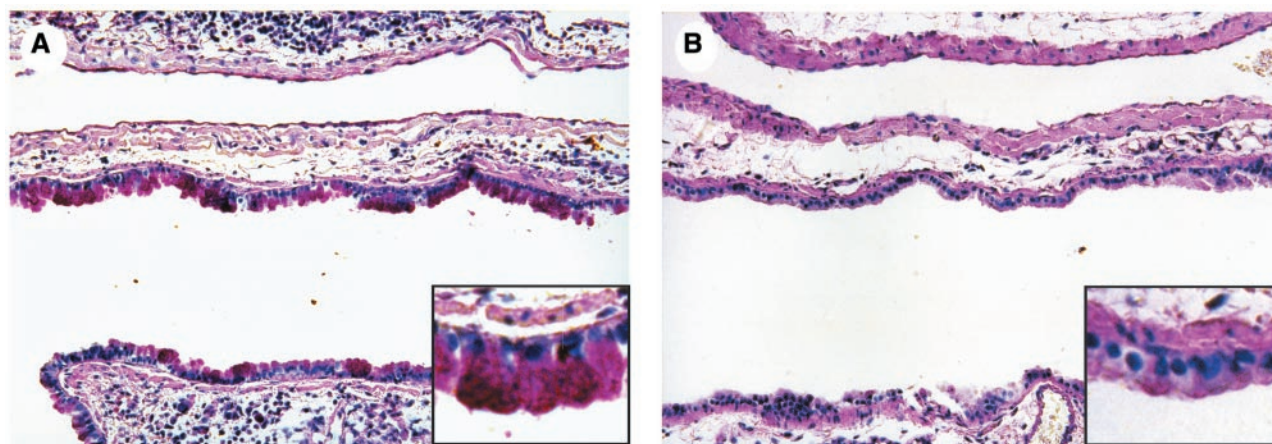


FIGURE 4. Ag-independent recruitment of Th2 cells does not stimulate production of airway epithelial mucus. Formalin-fixed and paraffin-embedded lung sections 8 μ m thick from the experiment in Fig. 2 were analyzed using PAS stain. Paraffin-embedded, PAS-stained lung sections of sensitized mice challenged 72 h before tissue harvesting with OVA-DNP (A) or BSA-DNP₂₂ (B). The magenta staining of the swollen epithelial goblet cells represents carbohydrate-modified mucins. Original magnification, $\times 100$ (inset, digitally enlarged to show detail). These micrographs are representative of two experiments.

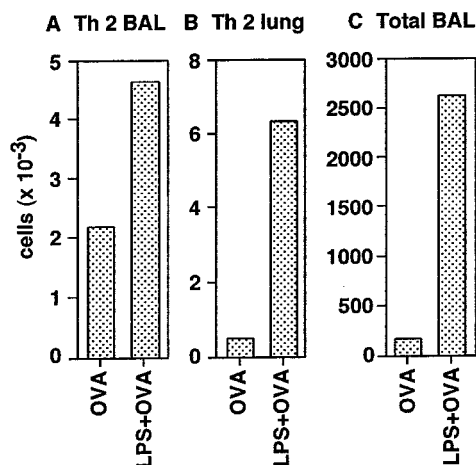


FIGURE 5. Instillation of LPS into the airway recruits Th2 cells and a large leukocyte infiltrate. A total of 10^7 DO11.10 Th2 cells were adoptively transferred i.v. into naive BALB/c mice. On the next day, OVA aerosol was administered two times for 30 min each, 6 h apart. Where indicated, 25 μ g of LPS was administered i.n. immediately before the first aerosol. BAL and lung cells were recovered 3 days after the challenge and were analyzed by flow cytometry. A, Numbers of CD4⁺ KJ1.26⁺ Th2 cells in the BAL. B, Numbers of Th2 cells in the lung. C, Numbers of total cells in the BAL. Similar results were obtained in three experiments.

double-deficient mice (26). Intranasal administration of LPS stimulated recruitment of Th2 cells to the BAL in P- and E-selectin-deficient mice (Fig. 6A). As seen in our other experiments, only small numbers of Th2 cells were recovered in the BAL of mice that received OVA alone. There were substantial numbers of eosinophils recovered by BAL from P- and E-selectin-deficient mice 72 h after i.n. challenge with OVA and LPS, suggesting that in mice, use of P-selectin by eosinophils is not absolutely required for these cells to pass through the lung vascular endothelium and ep-

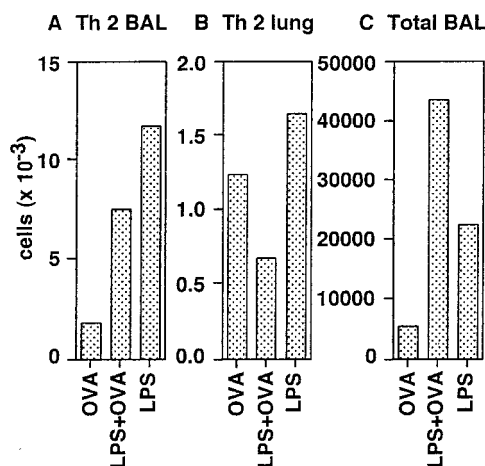


FIGURE 6. Recruitment of Th2 cells to the lung by LPS is diminished in P- and E-selectin-deficient mice. A total of 10^7 OT-II.2 Th2 cells ($\text{V}\alpha 2^+ \text{V}\beta 5^+$) on the C57BL/6 background were transferred into P- and E-selectin-deficient mice (C57BL/6 $\times$ 129/Sv), and OVA and LPS were administered the next day. BAL cells were collected and analyzed by FACS 3 days after the challenge. A, Numbers of CD4⁺ $\text{V}\alpha 2^+ \text{V}\beta 5^+$ Th2 cells in the BAL. B, Numbers of Th2 cells in the lung. C, Total numbers of cells in the BAL. Similar results were obtained in two experiments. In one experiment, LPS (25 μ g in 40 μ l) was administered i.n. immediately before the first exposure of the mice to aerosolized OVA. In the second experiment, OVA (1%) and LPS (25 μ g) were delivered together i.n. in a total volume of 40 μ l.

ithelium into the airway. Our data do not exclude a requirement for P- or E-selectin to support the normal rate of eosinophil migration from the circulation to the airway. Although recruitment of Th2 cells to the airway of P- and E-selectin double-deficient mice (as assessed by BAL) was increased in response to LPS plus OVA to levels similar to those seen in wild-type mice, the numbers of Th2 cells found in the lung parenchyma itself were reduced (Fig. 6B). In both wild-type and selectin-deficient mice, an increase in recruitment of Th2 cells to the airways was observed in response to LPS alone as well as in response to LPS plus the specific T cell Ag. However, in selectin-deficient mice, recruitment and accumulation of Th2 cells to the lung tissue were diminished when recruited by LPS alone (Fig. 6, A and B). These observations indicate that an endothelial selectin contributes to Th2 cell recruitment into the lungs.

Interestingly, the numbers of total cells in the BAL were dramatically increased in LPS-treated selectin-deficient animals (Fig. 6C). These numbers were even higher than those seen in wild-type mice. This is likely due to the fact that the double-deficient mice have much higher numbers of circulating leukocytes than do wild-type animals. This leukocytosis, first observed by Frenette et al. (26), could result in increased tissue infiltrates if the magnitude of these infiltrates is in proportion to the numbers of circulating cells. That P- or E-selectin participates in Ag-nonspecific recruitment was not predicted from previous experiments by Xie et al. (3), who showed that following Ag stimulation *in vivo*, only Ag-specific CD4⁺ cells in the lymph node acquire the ability to bind P-selectin Ig after stimulation with Ag. This indicated that in the lymph node, only Ag-specific cells express the selectin ligand. Their observations, however, do not exclude the possibility that Ag-nonspecific T cells migrate to the site of inflammation using this receptor without first proliferating in the LN (3).

Discussion

Many aspects of the way T lymphocytes respond to cognate Ag have been well studied; however, the way these cells respond to inflammatory signals is less well understood. Cells of the innate immune system, which recognize evolutionarily conserved microbial patterns and endogenous immune and inflammatory mediators, migrate to sites of inflammation and perform their effector functions independently of specific protein Ag. Mechanisms governing the inflammatory recruitment of immune cells to peripheral tissues using selectins, integrins, and chemokines and other chemoattractants are well established (43). Expression of adhesion molecules and chemoattractants by endothelial cells is up-regulated by inflammatory signals (reviewed in Ref. 44). Specific Ag has also been implicated in the recruitment of T cells by its ability to up-regulate P-selectin ligands on naive Ag-specific T cells in the LN (3) and by its ability to stop these cells from rolling on ICAM-1 *in vitro* (9). Until the development of T cell transfer models, however, it was difficult to separate T cell migration and effector functions, and as a result the differential effects of specific protein Ag and Ag-nonspecific inflammatory mediators on the recruitment phase of T cell responses *in vivo* remain poorly understood.

In previous studies we showed that endogenous Th1 cells precede Th2 cells into the airways in a model that includes both systemic priming and aerosol challenge with the protein Ag OVA (20). In another model designed to separate the recruitment process from sensitization events, we found that adoptively transferred OVA-specific Th2 cells were not effectively recruited to the lungs and airways of naive mice by challenge with aerosolized Ag alone. In contrast, adoptive transfer of Th2 cells together with Th1 cells

followed by airway Ag challenge resulted in dramatically increased Th2 cell recruitment (23). Our current data, however, demonstrate clearly that Th1 cells per se are not required for Th2 cell recruitment. Rather, a diverse collection of inflammatory stimuli can cooperate in the recruitment of Th2 cells. The role of non-Th1 cell factors is also supported by recent experiments in animals deficient for the Th1-promoting transcription factor T-bet (45). Finotto et al. (45) showed that SCID recipients of T-bet-deficient CD4⁺ T cells, which are unable to differentiate into Th1 cells, develop airway hyperreactivity and have IL-4 in the airways after aerosol Ag challenge, indicative of the development of the transferred cells into Th2 cells and their recruitment into the lungs in the absence of a Th1 response (Fig. 4).

We have recently shown that Th1 cells provide assistance for Th2 cell recruitment using a mechanism that is not dependent on Th2 cell-specific Ag (56). This is consistent with it being inflammatory signals downstream of Th1 cell activation that increase Th2 cell recruitment to the tissue. Our current study provides evidence that two additional stimulators of inflammation, Ag-specific IgE of the adaptive immune response and LPS, acting via the innate immune system can recruit Th2 cells to the lungs and airway in a T cell Ag-independent fashion. The Th2 cells that are recruited to the lung are not required to traffic through the local LN or to become activated to be recruited. Although Ag may affect the outcome of recruitment in ways not measured here, our experiments suggest a model for recruitment of Ag-experienced Th2 cells that, as in recruitment of innate immune cells, depends solely on the coordinated up-regulation of adhesion molecules and chemoattractants in target tissues to render the tissue receptive for immune cell entry.

We and others have investigated potential mediators and factors responsible for induction of Th2 cell recruitment in response to tissue inflammation. Although Th1 cells can stimulate inflammation, leading to increased Th2 recruitment, experiments using Abs to block the primary Th1-specific mediators IFN- γ , lymphotoxin, and IL-2 proved that the effects triggered by Th1 cells are not dependent on these mediators individually. Thus, major mediators that are products of Th1 cells uniquely do not appear to be the primary agents inducing recruitment. We anticipate that similar findings will hold for the inflammatory stimuli tested in the present study. Rather than cell type-specific mediators driving Th2 cell recruitment, we anticipate that mediators common to many inflammatory processes drive the Th2 cell response. For example, neutralization of the broad inflammatory mediator TNF did dampen Th2 cell recruitment induced by Th1 cells (23). We have also investigated representatives of each of the main categories of adhesion receptors—the integrins, the selectins, and the chemokine receptors. We have shown that blocking VCAM-1 reduces Th2 cell recruitment (23), that P- or E-selectin is involved in this recruitment (this manuscript), and that various chemokines are up-regulated in the presence of Th1 cells (56). Using a similar adoptive transfer model with repetitive aerosol Ag challenge as a stimulus, Mathew et al. (46) have underscored the potential role of chemokine receptors, showing that blockage of G-protein-coupled receptors on Th2 cells with pertussis toxin limits their recruitment. Furthermore, the work of Mathew et al. (47) has elegantly demonstrated that STAT6 signaling in the lung parenchyma is necessary for recruitment of wild-type Th2 cells. This work suggests that Th2 cells themselves can also facilitate their own recruitment via local cytokine production, as first suggested by Cohn et al. (31) in their studies of IL-4-deficient T cells.

If Ag-independent T cell recruitment to sites of tissue inflammation were to be obligatorily linked to T cell activation or if only activated cells could be recruited, then Th2 cell recruitment would

generally confer a Th2 character on the local inflammatory response by importing Th2 effector cells. If, in contrast, recruitment of Th2 cells could occur without cell activation and inflammation could lead to the influx of primarily resting Th2 cells, then the physiological impact of this recruitment would be expected to be less profound. Other circulating cell types may also be recruited in an Ag-independent manner that could also modify the outcome.

Prior studies using transgenic models of autoimmune diabetes have investigated this issue but suggest that both inflammation and the activating Ag are required for the T cell-mediated destruction of β -cells in pancreatic islets. The presence of Ag alone does not lead to islet destruction in most murine models of insulin-dependent diabetes mellitus. Inflammatory cytokines are required for up-regulation of T cell costimulation and for prevention of Ag-induced anergy (48, 49). These important studies suggest that both Ag and inflammation are required for the activation of a full immune response; however, whether it is Ag or inflammation that is specifically required for the recruitment of the T cells has not been addressed in these studies. We have shown here that recruitment of Ag-experienced Th2 cells is not obligatorily linked to cell activation. In other studies that use autoimmune tissue destruction as a readout, it is difficult to distinguish recruitment functions from effector functions. The studies of Xie et al. (3) of adoptively transferred transgenic cells have indicated that Th1 cells could be recruited into the peritoneal cavity by the inflammatory stimulus IFA and the IFN-induced chemokine IFN-inducible protein 10, suggesting that Ag is not required for recruitment into this tissue compartment either. In our studies, we are able to separate T cell recruitment from T cell effector functions. Under some conditions (for example, after stimulation of IgE-bearing mast cells using the T cell irrelevant Ag BSA-DNP₂₂; Fig. 3), large numbers of Th2 cells are recruited with a resting phenotype similar to their status at the time of adoptive transfer. This demonstrates clearly that activation and recruitment are not inextricably linked. Under conditions of natural exposure to inflammation-inducing pathogens, T cell activation and recruitment generally occur simultaneously, because pathogens contain both T cell-activating protein Ags and components that induce local inflammation. Activation is also important because the low frequency of naive T cells specific for any individual pathogen generally necessitates activation and clonal expansion of these cells to elicit a protective response. Whether T cell recruitment is tied to T cell activation is particularly critical in the cases of autoimmunity and allergy in which recruitment of activated T cells may determine whether pathological tissue injury occurs.

It has been suggested that activation of mast cells and basophils can lead directly to production of Th2 cytokines and recruitment of eosinophils (50, 51). Studies using anti-IgE blocking Abs have shown a role for IgE and Th2 cytokines in eosinophil recruitment (50). In murine asthma models that use a small number of repetitive airway Ag challenges and in those in which both priming and challenge are via the airway, *c-kit*-deficient (*W/W^v*) mice with congenitally low numbers of tissue mast cells have impaired eosinophil recruitment (52). In other models, this contribution of mast cells to eosinophil recruitment has not been observed (53). It appears that stronger priming and more frequent airway Ag challenges can overcome the eosinophil recruitment defect in mast cell-deficient mice (27). Notably, stronger stimuli also increase Th2 cell recruitment to the airway (23, 31).

The present set of experiments was designed in such a way that we could distinguish between direct and indirect effects of mast cells on eosinophil recruitment. Systemic administration of anti-DNP IgE followed by airway challenge with BSA-DNP₂₂, which activates only mast cells and basophils without activating the T

cells, does not induce eosinophil recruitment. This indicates that recruitment of eosinophils is not a direct consequence of IgE-stimulated mast cells. This study and others support an indirect model in which mast cell activation can increase Th2 cell recruitment and that activation of these immigrant Th2 cells can in turn recruit eosinophils.

The observation that P- or E-selectin contributes to Th2 cell recruitment to the lungs is consistent with the observation by Lukacs et al. (54) and Broide et al. (55) who observed decreased eosinophilia at early time points after challenge in models of allergic airway inflammation similar to that reported here. Lukacs et al. observed similar levels of Th2 cytokines in the lungs of both E-selectin-deficient and wild-type mice 8 h after aerosol Ag challenge, suggesting that recruitment of Th2 cells was not dependent on E-selectin in this model. Together with our data in P- and E-selectin-deficient mice, this may point to a primarily P-selectin-mediated event in Th2 recruitment to the lung. Alternatively, it may suggest that the difference in T cell recruitment in selectin-deficient mice is not apparent until later in the response because the peak of T cell recruitment and cytokine production in our system is ~24–48 h after challenge.

Of particular interest was the finding that Th2 cell recruitment to the lung parenchyma and the airway lumen was not completely parallel. One possible explanation is that the lack of selectins on lung endothelial cells in P- and E-selectin-deficient mice may limit the rate at which Th2 cells enter the lung parenchyma from the circulation. The residual influx of Th2 cells from the blood in the selectin double-deficient mice may be mediated by other receptors such as $\alpha 4$ integrins (reviewed in Ref. 44). If the rate of movement of Th2 cells from the parenchyma out into the airway lumen is not affected by the selectin defect, then the number of cells accumulating in the parenchyma may be substantially reduced.

Several studies suggest that Th1 cells exhibit a lower threshold for migration into target tissues than do Th2 cells (14). We show here that the barrier to Th2 cell migration can be overcome by induction of a variety of types of inflammation in the tissue target. IgE is a component of Th2-type inflammation, whereas the bacterial component LPS is generally associated with Th1-type responses. Interestingly, both can elicit a permissive environment for recruitment of Th2 cells. Our data imply that Ag-nonspecific inflammatory signals are sufficient for recruitment of Th2 cells to the lung and show that the T cell's cognate Ag is not necessary to overcome the threshold for recruitment of Th2 cells. Two important principles emerge from our experiments. First, we suggest that recruitment of Th2 cells in this system is analogous to recruitment of neutrophils and other cells of the innate immune system, depending primarily on local inflammatory signals and independent of specific Ag. Second, we have shown that activation of Ag-experienced Th2 cells and their migration into a site of inflammation are not necessarily linked. In the second observation lies a lesson for future studies of Th2-type responses in that measurable effector functions are the consequence of both recruitment and activation of the effector Th2 cells. Our studies describe a novel mechanism by which a pool of activated Ag irrelevant Th2 cells may impact the quality of a tissue-specific inflammatory response.

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References

- Chen, Z. M., and M. K. Jenkins. 1998. Revealing the in vivo behavior of CD4⁺ T cells specific for an antigen expressed in *Escherichia coli*. *J. Immunol.* 160: 3462.
- Ingulli, E., A. Mondino, A. Khoruts, and M. K. Jenkins. 1997. In vivo detection of dendritic cell antigen presentation to CD4⁺ T cells. *J. Exp. Med.* 185:2133.
- Xie, H., Y. C. Lim, F. W. Luscinskas, and A. H. Lichtman. 1999. Acquisition of selectin binding and peripheral homing properties by CD4⁺ and CD8⁺ T cells. *J. Exp. Med.* 189:1765.
- Roman, L. M., L. F. Simons, R. E. Hammer, J. F. Sambrook, and M. J. Gething. 1990. The expression of influenza virus hemagglutinin in the pancreatic β cells of transgenic mice results in autoimmune diabetes. *Cell* 61:383.
- Miyazaki, Y., K. Araki, C. Vesin, I. Garcia, Y. Kapanci, J. A. Whitsett, P. F. Piguet, and P. Vassalli. 1995. Expression of a tumor necrosis factor- α transgene in murine lung causes lymphocytic and fibrosing alveolitis: a mouse model of progressive pulmonary fibrosis. *J. Clin. Invest.* 96:250.
- d'Ostiani, C. F., G. Del Sero, A. Bacci, C. Montagnoli, A. Spreca, A. Mencacci, P. Ricciardi-Castagnoli, and L. Romani. 2000. Dendritic cells discriminate between yeasts and hyphae of the fungus *Candida albicans*: implications for initiation of T helper cell immunity in vitro and in vivo. *J. Exp. Med.* 191:1661.
- MacDonald, A. S., A. D. Straw, B. Bauman, and E. J. Pearce. 2001. CD8⁺ dendritic cell activation status plays an integral role in influencing Th2 response development. *J. Immunol.* 167:1782.
- Whelan, M., M. M. Harnett, K. M. Houston, V. Patel, W. Harnett, and K. P. Ringley. 2000. A filarial nematode-secreted product signals dendritic cells to acquire a phenotype that drives development of Th2 cells. *J. Immunol.* 164:6453.
- Dustin, M. L., S. K. Bromley, Z. Kan, D. A. Peterson, and E. R. Unanue. 1997. Antigen receptor engagement delivers a stop signal to migrating T lymphocytes. *Proc. Natl. Acad. Sci. USA* 94:3909.
- Springer, T. A. 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 76:301.
- Bonecchi, R., G. Bianchi, P. P. Bordignon, D. D'Ambrosio, R. Lang, A. Borsatti, S. Sozzani, P. Allavena, P. A. Gray, A. Mantovani, and F. Sinigaglia. 1998. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J. Exp. Med.* 187:129.
- Clissi, B., D. D'Ambrosio, J. Geginat, L. Colantonio, A. Morrot, N. W. Freshney, J. Downward, F. Sinigaglia, and R. Pardi. 2000. Chemokines fail to up-regulate $\beta 1$ integrin-dependent adhesion in human Th2 T lymphocytes. *J. Immunol.* 164: 3292.
- Sebastiani, S., P. Allavena, C. Albanesi, F. Nasorri, G. Bianchi, C. Traidl, S. Sozzani, G. Girolimoni, and A. Cavani. 2001. Chemokine receptor expression and function in CD4⁺ T lymphocytes with regulatory activity. *J. Immunol.* 166: 996.
- D'Ambrosio, D., A. Iellem, L. Colantonio, B. Clissi, R. Pardi, and F. Sinigaglia. 2000. Localization of Th-cell subsets in inflammation: differential thresholds for extravasation of Th1 and Th2 cells. *Immunol. Today* 21:183.
- Sallusto, F., A. Lanzavecchia, and C. R. Mackay. 1998. Chemokines and chemokine receptors in T-cell priming and Th1/Th2-mediated responses. *Immunol. Today* 19:568.
- Randolph, D. A., G. Huang, C. J. Carruthers, L. E. Bromley, and D. D. Chaplin. 1999. The role of CCR7 in Th1 and Th2 cell localization and delivery of B cell help in vivo. *Science* 286:2159.
- Austrup, F., D. Vestweber, E. Borges, M. Lohning, R. Brauer, U. Herz, H. Renz, R. Hallmann, A. Scheffold, A. Radbruch, and A. Hamann. 1997. P- and E-selectin mediate recruitment of T-helper-1 but not T-helper-2 cells into inflamed tissues. *Nature* 385:81.
- Bradley, L. M., V. C. Asensio, L. K. Schioetz, J. Harbertson, T. Kralh, G. Patstone, N. Woolf, I. L. Campbell, and N. Sarvetnick. 1999. Islet-specific Th1, but not Th2, cells secrete multiple chemokines and promote rapid induction of autoimmune diabetes. *J. Immunol.* 162:2511.
- Katakai, T., K. J. Mori, T. Masuda, and A. Shimizu. 1998. Differential localization of Th1 and Th2 cells in autoimmune gastritis. *Int. Immunol.* 10:1325.
- Randolph, D. A., C. J. Carruthers, S. J. Szabo, K. M. Murphy, and D. D. Chaplin. 1999. Modulation of airway inflammation by passive transfer of allergen-specific Th1 and Th2 cells in a mouse model of asthma. *J. Immunol.* 162:2375.
- Grzych, J. M., E. Pearce, A. Cheever, Z. A. Caulada, P. Caspar, S. Heiny, F. Lewis, and A. Sher. 1991. Egg deposition is the major stimulus for the production of Th2 cytokines in murine *Schistosomiasis mansoni*. *J. Immunol.* 146: 1322.
- Asnagli, H., and K. Murphy. 2001. Stability and commitment in T helper cell development. *Curr. Opin. Immunol.* 13:242.
- Randolph, D. A., R. Stephens, C. J. Carruthers, and D. D. Chaplin. 1999. Cooperation between Th1 and Th2 cells in a murine model of eosinophilic airway inflammation. *J. Clin. Invest.* 104:1021.
- Murphy, K. M., A. B. Heimberger, and D. Y. Loh. 1990. Induction by antigen of intrathymic apoptosis of CD4⁺CD8⁺TCR^{lo} thymocytes in vivo. *Science* 250: 1720.
- Barden, M. J., J. Allison, W. R. Heath, and F. R. Carbone. 1998. Defective TCR expression in transgenic mice constructed using cDNA-based α - and β -chain genes under the control of heterologous regulatory elements. *Immunol. Cell Biol.* 76:34.

26. Frenette, P. S., T. N. Mayadas, H. Rayburn, R. O. Hynes, and D. D. Wagner. 1996. Susceptibility to infection and altered hematopoiesis in mice deficient in both P- and E-selectins. *Cell* 84:563.
27. Hamelmann, E., A. Oshiba, J. Schwarze, K. Bradley, J. Loader, G. L. Larsen, and E. W. Gelfand. 1997. Allergen-specific IgE and IL-5 are essential for the development of airway hyperresponsiveness. *Am. J. Respir. Cell Mol. Biol.* 16:674.
28. Eisen, H. N., M. E. Carsten, and S. J. Belman. 1954. Studies of hypersensitivity to low molecular weight substances. III. The 2,4-dinitrophenyl group as a determinant in the precipitin reaction. *J. Immunol.* 73:296.
29. Haskins, K., R. Kubo, J. White, M. Pigeon, J. Kappler, and P. Marrack. 1983. The major histocompatibility complex-restricted antigen receptor on T cells. I. Isolation with a monoclonal antibody. *J. Exp. Med.* 157:1149.
30. Ishizaka, K., H. Tomioka, and T. Ishizaka. 1970. Mechanisms of passive sensitization. I. Presence of IgE and IgG molecules on human leukocytes. *J. Immunol.* 105:1459.
31. Cohn, L., R. J. Homer, A. Marinov, J. Rankin, and K. Bottomly. 1997. Induction of airway mucus production by T helper 2 (Th2) cells: a critical role for interleukin 4 in cell recruitment but not mucus production. *J. Exp. Med.* 186:1737.
32. Bradley, L. M., J. Harbertson, and S. R. Watson. 1999. Memory CD4 cells do not migrate into peripheral lymph nodes in the absence of antigen. *Eur. J. Immunol.* 29:3273.
33. Cohn, L., R. J. Homer, H. MacLeod, M. Mohrs, F. Brombacher, and K. Bottomly. 1999. Th2-induced airway mucus production is dependent on IL-4R α , but not on eosinophils. *J. Immunol.* 162:6178.
34. Townsend, J. M., G. P. Fallon, J. D. Matthews, P. Smith, E. H. Jolin, and N. A. McKenzie. 2000. IL-9-deficient mice establish fundamental roles for IL-9 in pulmonary mastocytosis and goblet cell hyperplasia but not T cell development. *Immunity* 13:573.
35. Temann, U. A., G. P. Geba, J. A. Rankin, and R. A. Flavell. 1998. Expression of interleukin 9 in the lungs of transgenic mice causes airway inflammation, mast cell hyperplasia, and bronchial hyperresponsiveness. *J. Exp. Med.* 188:1307.
36. Zhu, Z., R. J. Homer, Z. Wang, Q. Chen, G. P. Geba, J. Wang, Y. Zhang, and J. A. Elias. 1999. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J. Clin. Invest.* 103:779.
37. Lloyd, C. M., T. Delaney, T. Nguyen, J. Tian, A. C. Martinez, A. J. Coyle, and J. C. Gutierrez-Ramos. 2000. CC chemokine receptor (CCR)3/eotaxin is followed by CCR4/monocyte-derived chemokine in mediating pulmonary T helper lymphocyte type 2 recruitment after serial antigen challenge in vivo. *J. Exp. Med.* 191:265.
38. Borges, E., W. Tietz, M. Steegmaier, T. Moll, R. Hallmann, A. Hamann, and D. Vestweber. 1997. P-selectin glycoprotein ligand-1 (PSGL-1) on T helper 1 but not on T helper 2 cells binds to P-selectin and supports migration into inflamed skin. *J. Exp. Med.* 185:573.
39. van Wely, C. A., A. D. Blanchard, and C. J. Britten. 1998. Differential expression of α 3 fucosyltransferases in Th1 and Th2 cells correlates with their ability to bind P-selectin. *Biochem. Biophys. Res. Commun.* 247:307.
40. Knibbs, R. N., R. A. Craig, P. Maly, P. L. Smith, F. M. Wolber, N. E. Faulkner, J. B. Lowe, and L. M. Stoolman. 1998. α (1,3)-Fucosyltransferase VII-dependent synthesis of P- and E-selectin ligands on cultured T lymphoblasts. *J. Immunol.* 161:6305.
41. Bentley, A. M., S. R. Durham, D. S. Robinson, G. Menz, C. Storz, O. Cromwell, A. B. Kay, and A. J. Wardlaw. 1993. Expression of endothelial and leukocyte adhesion molecules intercellular adhesion molecule-1, E-selectin, and vascular cell adhesion molecule-1 in the bronchial mucosa in steady-state and allergen-induced asthma. *J. Allergy Clin. Immunol.* 92:857.
42. Labow, M. A., C. R. Norton, J. M. Rumberger, K. M. Lombard-Gillooly, D. J. Shuster, J. Hubbard, R. Bertko, P. A. Knaack, R. W. Terry, M. L. Harbison, et al. 1994. Characterization of E-selectin-deficient mice: demonstration of overlapping function of the endothelial selectins. *Immunity* 1:709.
43. Butcher, E. C., and L. J. Picker. 1996. Lymphocyte homing and homeostasis. *Science* 272:60.
44. Lusinskas, F. W., and M. A. Gimbrone, Jr. 1996. Endothelial-dependent mechanisms in chronic inflammatory leukocyte recruitment. *Annu. Rev. Med.* 47:413.
45. Finotto, S., M. F. Neurath, J. N. Glickman, S. Qin, H. A. Lehr, F. H. Y. Green, K. Ackerman, K. Haley, P. R. Galle, S. J. Szabo, et al. 2002. Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet. *Science* 295:336.
46. Mathew, A., B. D. Medoff, A. D. Carafone, and A. D. Luster. 2002. Cutting edge: Th2 cell trafficking into the allergic lung is dependent on chemoattractant receptor signaling. *J. Immunol.* 169:651.
47. Mathew, A., J. A. MacLean, E. DeHaan, A. M. Tager, F. H. Green, and A. D. Luster. 2001. Signal transducer and activator of transcription 6 controls chemokine production and T helper cell type 2 cell trafficking in allergic pulmonary inflammation. *J. Exp. Med.* 193:1087.
48. Guerdier, S., E. E. Eynon, and R. A. Flavell. 1998. Autoimmunity without diabetes in transgenic mice expressing β cell-specific CD86, but not CD80: parameters that trigger progression to diabetes. *J. Immunol.* 161:2128.
49. Ohashi, P. S., S. Oehen, P. Aichele, H. Pircher, B. Odermatt, P. Herrera, Y. Higuchi, K. Buerki, H. Hengartner, and R. M. Zinkernagel. 1993. Induction of diabetes is influenced by the infectious virus and local expression of MHC class I and tumor necrosis factor- α . *J. Immunol.* 150:5185.
50. Haile, S., J. Lefort, S. Y. Eum, C. Dumarey, M. Huerre, C. Heusser, and B. B. Vargaftig. 1999. Suppression of immediate and late responses to antigen by a non-anaphylactogenic anti-IgE antibody in a murine model of asthma. *Eur. Respir. J.* 13:961.
51. Nolte, H. 1996. The role of mast cells and basophils in immunoregulation. *Allergy Asthma Proc.* 17:17.
52. Kung, T. T., D. Stelts, J. A. Zurcher, H. Jones, S. P. Umland, W. Kreutner, R. W. Egan, and R. W. Chapman. 1995. Mast cells modulate allergic pulmonary eosinophilia in mice. *Am. J. Respir. Cell Mol. Biol.* 12:404.
53. Nagai, H., S. Yamaguchi, Y. Maeda, and H. Tanaka. 1996. Role of mast cells, eosinophils and IL-5 in the development of airway hyperresponsiveness in sensitized mice. *Clin. Exp. Allergy* 26:642.
54. Lukacs, N. W., A. John, A. Berlin, D. C. Bullard, R. Knibbs, and L. M. Stoolman. 2002. E- and P-selectins are essential for the development of cockroach allergen-induced airway responses. *J. Immunol.* 169:2120.
55. Broide, D. H., S. Sullivan, T. Gifford, and P. Sriramarao. 1998. Inhibition of pulmonary eosinophilia in P-selectin- and ICAM-1-deficient mice. *Am. J. Respir. Cell Mol. Biol.* 18:218.
56. Stephens, R., D. A. Randolph, G. Huang, M. J. Holtzman, and D. D. Chaplin. 2002. Antigen-nonspecific recruitment of Th2 cells to the lung as a mechanism for viral infection-induced allergic asthma. *J. Immunol.* 169:5458.