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Early Phase Bronchoconstriction in the Mouse Requires Allergen-Specific IgG

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Allergen provocation of allergic asthma patients is often characterized by an initial period of bronchoconstriction, or early phase reaction (EPR), that leads to maximal airway narrowing within 15–30 min, followed by a recovery period returning airway function to baseline within 1–2 h. In this study, we used a defined OVA provocation model and mice deficient for specific leukocyte populations to investigate the cellular/molecular origins of the EPR. OVA-sensitized/challenged wild-type (C57BL/6J) mice displayed an EPR following OVA provocation. However, this response was absent in gene knockout animals deficient of either B or T cells. Moreover, transfer of OVA-specific IgG, but not IgE, before the OVA provocation, was capable of inducing the EPR in both strains of lymphocyte-deficient mice. Interestingly, an EPR was also observed in sensitized/challenged mast cell-deficient mice following an OVA provocation. These data show that the EPR in the mouse is an immunologically based pathophysiological response that requires allergen-specific IgG but occurs independent of mast cell activities. Thus, in the mouse the initial period of bronchoconstriction following allergen exposure may involve neither mast cells nor IgE-mediated events.

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2 Abbreviations used in this paper: EPR, early phase reaction; LPR, late phase reaction.

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EPR occurs in mice deficient of mast cells following allergen provocation. Collectively, these data show that the EPR in the mouse, unlike asthma patients, is a pathophysiological response requiring neither IgE nor mast cell activities.

Materials and Methods

Animals

Wild-type C57BL/6J mice, gene knockout animals deficient of B cells (C57BL/6-IgG-6-10mGm (24)), T cells (C57BL/6-Tcr7/H9262 (25), αβ TCR T cells (C57BL/6-Tcr7/H9262 (25), γδ TCR T cells (C57BL/6-Tcr8/H9262 (26)), and mouse deficient of mast cells (WBBOB/β-B-Ki/H9262 (25)) were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were maintained in microisolator cages housed within a specific pathogen-free animal facility. The sentinel mice within this animal colony surveyed negative for known mouse pathogens. Protocols and studies involving animals were conducted in accordance with National Institutes of Health and Mayo Foundation institutional guidelines.

Experimental protocol

The allergen-provocation protocol used in this study was previously described (23). Briefly, 8–14 wk-old mice were immunized by two i.p. injections (100 μl) of OVA (20 μg; grade IV (Sigma-Aldrich, St. Louis, MO)) complexed with 2.25 mg Imject Alum (AL(OH)3/Mg(OH)2; Pierce, Rockford, IL) on days 0 and 14 of the protocol. The mice were challenged on days 24, 25, and 26 by 20-min inhalations of an aerosol generated by nebulization of a 1% OVA solution prepared in saline. Control mice received i.p. injections of saline (days 0 and 14) and 20 min aerosol challenge on days 24, 25, and 26. All mice were provoked with an OVA aerosol (5% in saline) for 20 min 48 h after the last of the three OVA (or saline) challenges (i.e., day 28) and continuous measurements of inspiratory/expiratory flow were recorded on conscious mice using whole-body plethysmography (Buxco Electronics, Troy, NY). In some studies, the highest Penh values following OVA provocation (i.e., the early phase kinetic maxima (KA)) were used to determine the increase in Penh as a percentage of baseline (BL). Penh values for each group of mice (PenhKA − PenhBL)/PenhBL × 100.

Serum Ig levels

Serum IgE levels were determined using an immunoassay for mouse IgE (OPT EIA Mouse IgE set, catalog no. 26551k; BD Pharmingen, San Diego, CA). Anti-mouse IgE mAb (capture) was coated on flat-bottom microtiter plates (Nalge Nunc International, Naperville, IL) and incubated overnight. Standards and serum (diluted 1/2) were incubated followed by detection with biotinylated anti-mouse IgE, avidin-HRP, and tetramethylbenzidine substrate (Pierce). The limit of detection associated with this assay is ~2 ng/ml. Total IgG was determined using a mouse Ig radial immunodiffusion kit (RN272, The Binding Site, Birmingham, U.K.) as per the manufacturer’s instructions (limit of detection −1 mg/ml). OVA-specific IgG1 serum levels were determined as previously described (27). Briefly, microtiter plates were coated overnight with 20 μg/ml chicken egg OVA. The coated plates were washed several times with PBS and blocked with 0.2% gelatin buffer (pH 8.2) for 3 h at 37°C. Serum diluted 1/10 was incubated in duplicate overnight, washed in PBS, and incubated with an alkaline phosphatase-conjugated rat anti-mouse IgG1 mAb (BD Pharmingen) for 2 h. Plates were developed with a phosphatase substrate (Sigma Fast P-Nitrophenyl Phosphate (Sigma-Aldrich)) and the absorbance of each sample was measured at 405 nm using a Vmax kinetic microplate reader (Molecular Devices, Sunnyvale, CA). The detection limit of OVA-specific IgG1 using this assay system was ~0.2 OD405 U.

OVA-specific IgG isolation

Wild-type C57BL/6J mice were subjected to the OVA protocol described above. On day 28, before the 5% OVA provocation, the animals were euthanized and serum was collected from pools of 10 mice, sterile filtered (0.45-μ filter; Millipore, Bedford, MA), and subjected to affinity chromatography using a 5-ml HiTrap protein G column (Amersham Biosciences, Uppsala, Sweden). The flow through from the column was collected and once again run over the column to quantitatively deplete from the serum all the IgG present. The bound IgG was washed with five-column volumes of 20 mM sodium phosphate (pH 7.0) and eluted with 0.1 M glycine-HCl (pH 2.7). The fractions eluted from the column were assayed for protein content by Bio-Rad protein assay (Bio-Rad, Hercules, CA) and pooled together. The buffer in the pooled IgG fractions and the IgG-depleted serum were each changed to PBS using Slide-Alyzers (Pierce), and equilibrated at 4°C overnight in PBS. Assessments of IgG and IgE levels in these final preparations (using the assays described above) demonstrated that IgG was absent in IgG-depleted serum and that purified mouse IgG preparations were devoid of IgE. The per mouse recovery of purified IgG was ~30 mg, whereas the amount of IgE present in IgG-depleted serum was ~300 ng/mouse.

Adaptive transfer of IgG into mice

In studies assessing the role of OVA-specific Iggs, animals were injected (i.p.) with either purified IgG or IgG-depleted serum Ig from OVA-treated wild-type mice on days 22 and 24 (1 h before the 1% OVA challenge) of the provocation protocol. The amount of Ig administered was set to the amount recoverable from an OVA-sensitized/aerosol-challenged wild-type mouse (i.e., 30 mg purified IgG/mouse and IgG-depleted serum equivalent to 300 ng of IgE). Control groups of mice were administered either non-specific mouse IgG (Sigma-Aldrich) or ragweed-specific IgGs purified as described above.

Statistical analysis

Data presented are the means (±SE). Statistical analysis was performed on parametric data using Student t tests with differences between means considered significant when p < 0.05.

Results and Discussion

The EPR requires both B and T lymphocytes

The involvement of B and T lymphocytes in the EPR was assessed using knockout mice deficient of either cell type. Airflow was continuously measured in groups of OVA-sensitized/challenged mice following OVA provocation and plotted as a function of the time postprovocation (Fig. 1). Saline sensitized/challenged C57BL/6J mice did not display an increase in Penh (i.e., airway resistance) in the first hour following allergen provocation. However, OVA-sensitized/challenged mice showed an increase within 5 min of provocation, reached maximal levels ~15 min post-OVA provocation, and returned to baseline levels within 60 min (Fig. 1A). This result was similar to those obtained previously with BALB/c mice (23), suggesting that the appearance of the EPR is not an inbred strain-dependent phenomenon. In contrast to wild-type mice, OVA-sensitized/challenged knockout animals deficient of either B (C57BL/6-Igh-6 tm1Cgn (24)), T cells (C57BL/6-Tcr7/H9262 (25)), or TCR-β deficient (C57BL/6-Tcr7/H9262 (26)) cells were unable to develop an EPR following OVA provocation (Fig. 1B). In addition, further studies using knockout mice deficient of either the αβ TCR (25) or the γδ TCR (26) subpopulations of T cells showed that mice deficient of either subpopulation were not capable of eliciting an EPR (Fig. 1C).

OVA-induced increases in total IgE and OVA-specific IgG1 do not occur in either B or T cell knockout mice

The lack of an EPR in B cell-deficient mice suggested that this bronchoconstrictive response was mediated by Ig, consistent with asthma patients where the EPR has been shown to result, in part, from the presence of allergen-specific IgE (28). Total serum IgE and IgG levels were measured in saline challenged control wild-type mice as well as OVA-treated wild-type, B cell, and T cell-deficient knockout mice (Fig. 2, A and B). These data show that although total IgE and IgG levels increase significantly in response to OVA treatment, neither Ig subtype was detectable in OVA-treated knockout mice deficient of B cells. Moreover, total serum IgE levels were undetectable in T cell-deficient mice and total IgG was reduced to a level below saline control wild-type mice. However, the presence of low levels of IgG in the serum of T cell-deficient mice, suggested that production of OVA-specific IgG in these mice was possible. In wild-type mice, OVA sensitization/challenge led to a >10-fold increase in OVA-specific IgG1, a Th2-associated (15) Ig subtype. However, OVA-specific IgG1 was not detectable in T cell-deficient mice (Fig. 2C). The absence of an
EPR and OVA-specific IgE and IgG1 in both B and T cell-deficient mice suggested that the EPR is a Th2-mediated pathophysiologic response elicited by resident pulmonary cells and/or mechanisms using one or both of these IgGs. In addition, the loss or significant decrease of IgE/IgG1 production in OVA-treated knockout mice deficient of either B or T cells (29) or IgG-depleted serum from OVA-sensitized/challenged wild-type animals. The Ag specificity of this response was demonstrated by the inability of serum IgG or IgG-depleted serum (i.e., IgE) from ragweed-sensitized/aerosol-challenged mice to induce an EPR following OVA provocation or OVA-sensitized/aerosol-challenged mice (Fig. 3B). The recovery of the EPR also did not require prior exposure to allergen; transfer of OVA-specific IgG into naive B cell-deficient mice also induced an EPR following OVA provocation (Fig. 3C). These data thus limit T cell participation in the EPR to helper functions necessary for allergen-specific Ig production by B cells. This conclusion was supported by Ig transfer experiments using T cell-deficient (αβ−/−γδ−/−) mice (Fig. 4). Transfer of IgG from OVA-sensitized/challenged wild-type mice into OVA-sensitized/challenged T cell-deficient animals was again sufficient to induce an EPR following OVA provocation equivalent to the responses observed in wild-type animals.
The observation that the EPR occurred in the presence of allergen-specific IgG, and was not induced following transfer of allergen-specific IgE, requires that any mechanism to explain the EPR in the mouse must rely exclusively on allergen-specific IgG. The implicit assumption is that this IgG-dependent mechanism will be analogous to IgE pathways in humans and is still mast cell-mediated, requiring allergen-specific IgG and the FcεRI known to be on mast cells (for example, see Ref. 31). However, this extrapolation is not supported by the observation that OVA provocation of sensitized/challenged mast cell-deficient mice resulted in an EPR (Fig. 5). Thus, in this model system mast cells are not necessary for the EPR. Unfortunately, the definitive character of this result is complicated by the controversial role of mast cells in the lungs of mice. These leukocytes are not a prevalent resident cell population of the lungs of mice (32, 33) and OVA sensitization/challenge of mast cell-deficient mice, similar to wild-type animals, leads to a host of pulmonary pathologies, including goblet cell metaplasia/mucus production and the development of airway hyperresponsiveness.


