Eosinophilia is induced in the colon of Th2-sensitized mice upon exposure to locally expressed antigen

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Eosinophilia is induced in the colon of Th2-sensitized mice upon exposure to locally expressed antigen. Am J Physiol Gastrointest Liver Physiol 293: G383–G390, 2007. First published April 12, 2007; doi:10.1152/ajpgi.00341.2006.—Eosinophilic inflammation is a hallmark of allergic disease orchestrated by Th2-type cytokines (16). In addition to participating in pulmonary allergic diseases (e.g., asthma), eosinophils as resident and recruitable cells in the gastrointestinal (GI) mucosa play a critical role in responses to parasitic infection as well as in the regulation of gastrointestinal allergy (9, 45). Eosinophil accumulation is a profound cellular inflammation, often seen as a component of eosinophil-associated GI disorders (EGIDs). EGIDs involve most parts of the GI tract, including disorders such as eosinophilic esophagitis, eosinophilic gastritis, and gastroenteritis. This series of disorders can result in marked eosinophilic infiltration into the GI tract. The alterations peaked around day 5 and resolved over the next 5–10 days, and no epithelial cell damage was detected throughout the entire course. Administration of a control (empty) adenovirus vector did not lead to any pathological changes. These data suggest that colonic eosinophilia can be induced by exposure to an antigen associated with preexisting Th2-skewed responses. Thus the model established here may provide a useful tool to study GI and, in particular, colonic inflammation with respect to underlying mechanisms involved in the recruitment and the immediate function of eosinophils.

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Innovative Methodology

MATERIALS AND METHODS

Animals and cell lines. Female BALB/c (H-2d) mice aged 6–8 wk were purchased from Charles River Laboratories (Troy, NY) and housed in pathogen-free conditions at Central Animal Facility at McMaster University. All animal experiments were approved by the Animal Ethics Research Board of McMaster University and conducted according to regulations of the Canadian Council on Animal Care.

Adenovirus vectors. AdLuc is a replication-deficient adenovirus vector that expresses firefly luciferase (1). AdOVA is an adenovirus vector that expresses chicken OVA (Zhu Q, Thomson CW, Rosenthal K, McDermott MR, Collins SM, and Gauldie J, unpublished observations). Adenovirus vector were propagated in 293 cells and purified using adenovirus vector that expresses firefly luciferase (1). AdOVA is an adenovirus vector that expresses firefly luciferase (1). Compared with histological lung sections taken from sensitized mice challenged with AdBHG (a control adenovirus vector without heterologous gene), which were identical to those from naive mice (Fig. 1B, left), AdOVA-transfected lungs of sensitized mice showed a marked immune response, characterized by lymphocytic infiltration surrounding the pulmonary blood vessels and bronchi (Fig. 1B, right). Eosinophils were rarely seen in control lungs (Fig. 1B, left) but were readily visualized by Congo red stain in AdOVA challenged lungs (Fig. 1B, right). Lymphocytosis and increased eosinophil counts were also observed in the BAL fluid taken from these AdOVA-challenged mice (data not shown). Thus IN antigen challenge of Th2-sensitized mice using adenovirus vectors specifically induced antigen-specific inflammatory responses in a similar manner to repeated antigen aerosolization to the upper airway as previously described (41).

Colonic Th2 precondition and adenovirus-mediated gene transfer to the colon. We next investigated whether mice sensitized with IP injections of OVA and Al(OH)3 had a Th2-type response in the colon and, if so, whether Th2 sensitization would cause differences in quantity of gene transfer to the colon mucosa compared with naive controls. Similar to observations in the lung, levels of OVA-specific IgG1 antibodies in colonic tissue increased after sensitization, whereas
IgG2a antibodies were undetectable (Fig. 1). This suggests that, similar to the lung, the colonic mucosal immune system can also be preconditioned to develop Th2-type responses specific for the antigen after sensitization by IP route.

To investigate gene transfer to the colon, 10⁹ PFU of adenovirus vector expressing luciferase (AdLuc) were given by the IR route, 7 days after Th2 sensitization. At days 2, 4, 6, and 8 after vector challenge, entire colons were removed and cut into distal, middle, and proximal sections of equal length for luciferase measurement. Significant levels of luciferase expression were detected and were shown to persist for ~6–7 days (Fig. 2). However, no differences in luciferase gene expression were found between sensitized and naive mice (Fig. 2), suggesting that Th2 sensitization does not lead to alterations in gene transfer ability or modulation of gene expression systems at the colonic mucosa.

**Induction of colonic inflammatory response after antigen challenge at the colonic mucosa.** Eosinophilia of the upper GI tract and small intestine can be experimentally induced in mice through oral feeding of allergen (23, 34). We here examined whether colonic eosinophilia can be induced in these Th2-sensitized mice upon exposure to the OVA antigen at the mucosa of the large intestine. Seven days after sensitization as described above, mice were challenged IR with 1×10⁹ PFU of AdOVA and colons were removed for examination of histological changes at various time points. In AdBHG (control vector)-challenged mice, there were only one or two visible lymphoid aggregates, relatively small in size, and indistinguishable from those seen in naive animals (Fig. 3A). However, in AdOVA-challenged mice, on gross examination, we observed some degree of redness, edema, and hypotonia of the colon and the presence of vascular ectasias in the lining and wall of the colon (Fig. 3B). Histological sections of the colons of these mice showed hyperplasia of the lymphoid follicles with lymphocytic infiltration, across the lamina propria and submucosa (Fig. 3B). Enlarged lymphoid follicles were increasingly dispersed along the entire length of the colon (Fig. 3C, part of colon shown).

Further analysis was performed to assess the histological changes in the colon, including morphometric analysis of...
Animals were sensitized with 8 μg OVA absorbed to Al(OH)₃ by 2 ip injections. Seven days after the second injection, 10⁷ PFU of AdLuc was given by IR. Colon were removed at days 2, 4, 6, and 8, and segmented into 3 equal-length pieces (distal, middle, and proximal) and homogenized. Luciferase activities were measured in each homogenate. Gene transfer in sensitized mice was not statistically different from that in naive mice with respect to the level of gene expression and the length of time. Results are expressed as mean ± SD from 3 mice each group.

Eosinophilic infiltration was examined by using Congo red stain on colon sections obtained from challenged mice. Challenge of sensitized mice with the control vector AdBHGF by the IR route did not induce a significant appearance of eosinophils in the colon (Fig. 5A). However, upon IR challenge with AdOVA, a large number of eosinophils was present in the colonic lamina propria at day 5 postinfection (Fig. 5B). This suggests that colonic eosinophils can be induced in association with local antigen exposure as seen in the lung in Th2-sensitized animals.

The colonic inflammatory response was also examined in sensitized mice at days 10, 15, and 20 postchallenge with AdOVA. Analysis of these colonic tissues with respect to lymphoid follicles and eosinophils revealed that at these later time points inflammation was markedly decreased. There were no overt histological changes at day 15 after challenge compared with controls, suggesting that inflammation began to resolve by day 10–15.

Eosinophils are involved in the inflammatory response by discharging their granule-specific proteins at site of inflammation (45). We thus examined colon sections by transmission electron microscopy and asked whether infiltrated eosinophils are activated in the colonic mucosa after antigen challenge. Compared with naive (Fig. 6A) and sensitized animals with irrelevant antigen challenge (Fig. 6B), colonic eosinophils of OVA-challenged mice displayed a moderate degree of piecemeal degranulation, which is indicated by increased numbers of granules with reduced electron translucency matrix density or halos (Fig. 6C). Although these intracellular granules were significantly increased in number than those of controls (Fig. 6D), we did not observe extensive degranulation such as loss of electron dense core or morphologically altered granules.

**DISCUSSION**

Previous studies imply that food allergy contributes to the development of GI eosinophilia (36, 48). Eosinophil infiltration can be triggered by hypersensitivity to cow’s milk, and the tissue eosinophilia decreases on withdrawal of milk from the diet (21, 28, 32, 48) and Th2, but not Th1, cytokine profiles were found in patients with milk hypersensitivity (3). Rothenburg and colleagues (23, 33, 34) developed a murine model of eosinophil-associated allergy, in which mice sensitized with OVA and aluminum hydroxide are fed with the same antigen by oral route. They identified eosinophils in the upper GI mucosa and related the findings to the presence of Th2-type cytokines and specific chemokines such as eotaxin (23, 24).

Naturally, the presence of luminal bacteria probably drives immune responses toward either a Th1 or Th2 cytokine bias, and such cytokine imbalance might promote the disease (19, 20). The recognition of microbial components by intestinal epithelial cells through Toll-like receptors may play an important role in establishing the polarized cytokine profile (26), and CD4⁺ T cells may be essential in producing these cytokines (25, 58). We here demonstrate that Th2-sensitized mice, but not naive mice, could develop colonic eosinophilic inflammation after antigen challenge by the rectal route. Therefore, preexisting biased Th2 immune response and expression of associated antigen appear responsible for the development of colonic eosinophilic inflammation and may also apply to a number of human GI conditions.

Studies (11, 55; Zhu Q, Thomson CW, Rosenthal K, McDermott MR, Collins SM, and Gauldie J, unpublished observation) have shown that adenovirus vector transfer of antigen genes to the colon results in efficient cell-associated gene expression in the epithelial layer with some expression extending to the lamina propria. In this regard, adenovirus vector proved to be a useful tool for antigen transfer and expression at the lower GI mucosa. In the present study, we challenged mice sensitized to OVA with adenovirus vector encoding the OVA gene by the intrarectal route and showed that antigen transfer and expression induced significant lymphocytic and eosinophilic infiltration, whereas an identical vector that lacks OVA gene (AdBHGF) had no appreciable histological effects. Thus antigen expression in the large intestine mucosa of Th2-sensitized mice induces colonic inflammation. We found that adenovirus vector-transferred gene product was expressed and detectable for up to 1 wk in the colon. Peak antigen expression was detected during the initial 1–3 days after IR administration, after which the levels reduced to baseline by 1 wk. The presence of eosinophilic inflammation is dependent on the presence of antigen as the inflammation subsided coincident with the decreased antigen presence in the colon. Antigen presence triggers colonic inflammation and, very likely, is responsible for the entire course of pathological alterations.
Eosinophils can be activated to release a series of cytotoxic granule proteins causing tissue damage, and accumulation of these eosinophils is thought to be detrimental (15, 54) and to be central to the pathogenesis of colitis (10, 39). We did not see the profound inflammation sustained for more than 1 wk nor the development of colonic ulceration, significant weight loss, or diarrhea, although granules with electron translucent matrix were significantly increased in the infiltrated eosinophils. However, no extensive eosinophil degranulation was found. These data suggest that induced eosinophils in the colon alone do not progress to tissue damage and require at least a “second” signal for activation/degranulation. Moreover, the pathological alterations (eosinophilia) are reversible and do not lead to prolonged tissue destruction or remodeling.

Several factors might be contributory to the results. First, exposure to only one antigen might be not sufficient to induce

Fig. 3. Inflammatory responses in the colon of sensitized mice 5 days after antigen challenge with adenovirus vector by IR route. Seven days after OVA (8 μg) sensitization, mice were challenged IR with 10^9 PFU of AdOVA or AdBHG. At day 5 after IR challenge, animals were killed and colons were paraffin embedded, sectioned, and stained with H&E and were examined with a light microscope. A: gross examination (top) and colon section (bottom) from sensitized mice challenged with AdBHG. B: gross examination (top) and colon section (bottom) from sensitized mice challenged with AdOVA. C: part of colon is shown for distribution of lymphoid follicles. In AdBHG-infected mice, there were only 1 or 2 visible lymphoid nodules, relatively small in size, and indistinguishable from those seen in naive animals. In AdOVA-infected mice, lymphocytes infiltrated the colon. The lymphoid follicles were enlarged and more than 2 follicles found. Results represent 1 of 5 independent experiments. Magnification of ×50 in diameter.

Fig. 4. Increased number and total area of lymphoid nodules after antigen challenge with adenovirus vector by IR in sensitized mice. OVA (8 μg)-sensitized mice were challenged with AdOVA or AdBHG 7 days after sensitization. Five days later, colons were removed and analyzed under a microscope. A: number of visible lymphoid nodules were counted on one histological section from each group. Increasing number of lymphoid nodules was observed after AdOVA IR challenge compared with controls (**P < 0.01, n = 5/group). B: cross-sectional area of all lymphoid nodules (total area) were analyzed morphometrically by using image analysis software. There was an increase in total area of lymphoid nodules after AdOVA IR challenge (**P < 0.01, n = 5/group).
activation or degranulation. An early observation in human disease revealed that sequential dietary eliminations reduced clinical symptom each time (31), implying that multiple allergens are involved to cause disease. Second, the antigen is only present for up to 1 wk after challenge and this might be not long enough to promote disease development (ulceration). Third, the route of challenge might result in different pathological changes. It was shown that challenge of systemic sensitized mice by the intragastric route induced diarrhea, which was accompanied by a dramatic infiltration of eosinophils, mast cells, and CD4+ Th2 cells into the large but not the small intestine (29). It remains to be determined whether these colonic eosinophils can generate severe or irreversible pathological changes upon a more chronic antigen exposure. Because the efficacy of gene expression on repeated adenovirus gene transfer is dramatically reduced owing to the immunogenicity of the vector, different serotypes of adenovirus or other types of antigen-delivery vehicles may be used for chronic antigen challenge. Also, it warrants investigation whether these cells participate only in antigen presentation, which can potentially promote local inflammation and destroy mucosal cells as shown in the respiratory system (50).

In addition to colonic eosinophilia, we also identified lymphoid hyperplasia and the development of multiple lymphoid

Fig. 5. Colonic eosinophilia after antigen challenge with adenovirus vector in sensitized mice. Animals sensitized to OVA were challenged IR with 10⁶ PFU of AdOVA or AdBHG 7 days after sensitization. Five days later, Congo red/hematoyxlin stain for eosinophils was applied on paraffin-embedded sections of the colon. A: sensitized animals were challenged IR with AdBHG. Eosinophils were rarely found in the colon. B: sensitized animals were challenged with AdOVA. A significant eosinophilia was detected in the lamina propria. Results represent 1 of 5 independent experiments. A magnification of 400 in diameter.

Fig. 6. Granules in colonic eosinophils. Representative electron microscopy sections of naive mice (A), sensitized mice 5 days after IR challenge with AdBHG (B), or AdOVA (C), are shown. D: eosinophil granules with reduced electron translucent matrix density (black arrow) or halos (white arrow) were calculated as percentage of total granules counted. Results are expressed as mean ± SD from 5 mice each group. The magnification of the photomicrographs presented is ×9,300.
nODULES IN COLONIC EOSINOPHILIA MODEL

MOUSE COLONIC EOSINOPHILIA MODEL

G389

In conclusion, Th2-sensitized mice challenged with antigen via the rectal route can induce colonic inflammatory responses, featured by significant eosinophil infiltration and lymphoid hyperplasia in the lamina propria, which is similar to human EGID. This murine model might provide a new understanding concerning the influence of a preexisting antigen-specific Th2-type responses in the lung that mice lacking secondary lymphoid organs can generate mucosal lymphoid aggregates, called inducible bronchus-associated lymphoid tissue, beneath the epithelium at the branches of the bronchi, allowing the development of protective local immunity upon influenza infection (38). Adoptively transferred CD4+ T cells can undergo clonal expansion in the lamina propria and the epithelial layer of both small and large intestines (47). Thus scattered lymphocytes might also be able to expand in the lamina propria after antigen challenge, leading to a significant number of lymphoid follicles in the mucosa.

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REFERENCES

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