A Functional Role for CCR6 on Proallergic T Cells in the Gastrointestinal Tract

ANA BELÉN BLÁZQUEZ,* ADINA KAY KNIGHT,* HOHETEBERHAN GETACHEW,* JONATHAN S. BROMBERG,‡ SERGIO A. LIRA,‡ LLOYD MAYER,‡ and M. CECILIA BERIN*,‡

Jaffe Food Allergy Institute and ‡Immunology Institute, Mount Sinai School of Medicine, New York, New York

BACKGROUND & AIMS: CCL20 is a chemokine that regulates the homeostatic and inflammatory trafficking of leukocytes to the small intestine and regulates the development of the gastrointestinal lymphoid architecture. T cells expressing T helper cell (Th) 2 cytokines are critical for experimental food allergy, and we hypothesized that CCL20 is involved in the localization of these cells to the gut. **METHODS:** We evaluated the role of CCR6 in allergic diarrhea induced by sensitization and oral challenge with ovalbumin (OVA) using $CCR6^{+/+}$ and $CCR6^{-/-}$ mice. **RESULTS:** $CCR6^{-/-}$ mice were protected from OVA-induced diarrhea but surprisingly were not impaired in mastocytosis or allergen-specific immunoglobulin E. $CCR6^{-/-}$ mice were also protected from T cell-mediated diarrhea induced by anti-CD3 antibody. Allergic diarrhea was associated with an increased expression of Th2 cytokines within the intestinal mucosa that was significantly reduced in $CCR6^{-/-}$ mice. Inhibition of lymphocyte homing by treatment with FTY720 did not impair allergic diarrhea, indicating that reactivation of T cells could occur locally within the small intestine. Finally, T-cell transfer studies demonstrated that CCR6 was required both on the transferred T cells and in the recipient mouse to manifest allergic disease in the gastrointestinal tract. **CONCLUSIONS: These studies highlight a mast cell– and immunoglobulin E–independent role for CCR6-bearing T cells in the pathogenesis of gastrointestinal allergic disease.**

Food allergic reactions are initiated by allergen cross-linking of immunoglobulin (Ig) E bound to intestinal mast cells, mast cell degranulation, and release of mast cell products that act directly on the intestinal epithelium, or indirectly through enteric nerves, to induce changes in intestinal ion secretion and barrier function.[1,2] Mice systemically sensitized to ovalbumin (OVA) and repeatedly orally challenged with OVA develop a mast cell and IgE-dependent acute diarrhea associated with a T helper cell (Th) 2 inflammation in the small intestine.[3] We have previously shown that mesenteric lymph node (MLN) $CD4^+$ T cells from mice with allergic diarrhea can transfer allergic disease to naïve mice,[4] highlighting the role of T lymphocytes in an IgE- and mast cell–driven model system. Forbes et al recently showed that transgenic expression of the single T-cell cytokine interleukin (IL)-9 within the intestine could lead to a local mastocytosis and diarrhea replicating experimental models of allergen-driven experimental food allergy.[5] Inhibition of IL-4 and IL-13 given early during repeated oral allergen challenge can also inhibit allergic symptoms.[6] Allergen-specific T cells producing Th2 cytokines have been shown to be present in the intestinal mucosa of human subjects with food allergic diseases,[7,8] including non-IgE–mediated food allergic disease. The factors responsible for recruitment of pathogenic T cells to the intestine in food allergic disorders are not known, and we hypothesized that mucosally expressed chemokines would be critical for the homing of T cells to the gut in experimental food allergy.

CCL20 (MIP-3α) is a chemokine that is expressed by gastrointestinal epithelium,[9] is regulated by nuclear factor (NF)-κB,[10] and is overexpressed in inflammatory bowel disease.[10,11] We have recently shown that ligation of the low-affinity IgE receptor on intestinal epithelial cells leads to release of functional CCL20.[12] Expression of CCL20 is highest in the follicle-associated epithelium of the Peyer's patch,[13,14] but it is also expressed by mouse and human enterocytes.[9] The cognate receptor for CCL20 is CCR6 and is expressed on memory T cells, B lymphocytes, and dendritic cells (DCs). $CCR6^{-/-}$ mice have impaired mucosal but not systemic humoral responses to immunization and rotavirus infection.[15] In addition, $CCR6^{-/-}$ mice have alterations in the architecture of organized lymphoid tissue in the gastrointestinal tract, including Peyer's patches, isolated lymphoid follicles, and cryptopatches.[16–18] We hypothesized that this ubiquitous mucosal chemokine would play a role in the homing of T lymphocytes to the gastrointestinal tract in

Abbreviations used in this paper: CFSE, carboxyfluoroscein succinimidyl ester; DC, dendritic cells; ELISA, enzyme-linked immunosorbent assay; IFN, interferon; Ig, immunoglobulin; IL, interleukin; mRNA, messenger RNA; MLN, mesenteric lymph node; NF, nuclear factor; OVA, ovalbumin; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; RT, reverse-transcription; Th, T helper cell; TNF, tumor necrosis factor.

0016-5085/10/$36.00
doi:10.1053/j.gastro.2009.09.016

experimental food allergy and tested this using $CCR6^{+/+}$ and $CCR6^{-/-}$ mice.

Materials and Methods

Allergic Diarrhea

$CCR6^{-/-}$ mice were generated previously by S. Lira,[15] backcrossed for 10 generations to the Balb/c background, and maintained in specific pathogen-free conditions. Balb/c mice were purchased from the National Cancer Institute (Frederick, MD). All experiments were performed with the approval of the Institutional Animal Care and Use Committee. Female age-matched $CCR6^{+/+}$ and $CCR6^{-/-}$ mice (5–8 weeks of age) were sensitized to OVA as previously described.[4] Symptoms were monitored for 30 minutes after feeding, and diarrhea was marked as present or absent.

Cholera Toxin and CD3-Induced Diarrhea

Cholera toxin-induced diarrhea was generated by administering 50 μg of cholera toxin (List Biological Laboratories, Campbell, CA) by intragastric gavage to $CCR6^{+/+}$ or $CCR6^{-/-}$ mice. After 3 hours, mice were killed, and 3 intestinal loops/mouse prepared for weight/length and wet/dry weight ratios. Intestinal segments were weighed, dried in an oven (80°C, 48 hours), and reweighed. Wet/dry weight ratios are reflective of fluid secretion and edema.

T cell-mediated diarrhea was induced as previously described.[19,20] Briefly, mice were administered 0.2 mg of anti-CD3 (eBioscience, San Diego, CA; functional grade) by intraperitoneal injection or phosphate-buffered saline (PBS) as control. After 2 hours, mice were killed, and intestinal loops were prepared as above.

Cell Culture

MLN were isolated, and cells were cultured with media alone or OVA (100 μg/mL) for 72 hours. Supernatants were collected for cytokine determination by enzyme-linked immunosorbent assay (ELISA) (eBioscience).

Adoptive Transfer

Donor mice were sensitized and fed with OVA to induce allergic diarrhea. MLN cells were isolated and restimulated with OVA as above prior to washing and intravenous transfer to naïve mice (4×10^6 cells/mouse). For $CD4^+$ T-cell transfer, $CD4^+$ T cells were negatively selected (StemCell, Vancouver, BC, Canada) prior to transfer of 2×10^6 cells/mouse. Recipient mice were orally challenged with OVA every second day starting 24 hours after transfer, and diarrhea symptoms were monitored as above.

Alternatively, 3×10^6 $CD4^+$ T cells from DO11.10 mice were carboxyfluoroscein succinimidyl ester (CFSE)-labeled and transferred to naïve $CCR6^{+/+}$ or $CCR6^{-/-}$ mice. After 24 hours, recipient mice were fed with 50 mg of OVA. After 72 hours, cells in the MLN were isolated for analysis of proliferation.

FTY720 Administration

FTY720 was a gift from V. Brinkmann (Novartis Pharma AG, Basel, Switzerland) to J.S.B. To block lymphocyte egress from the lymph nodes, mice were treated orally with 0.3 mg/kg FTY720 daily beginning 1 day prior to initiation of oral OVA challenges and daily thereafter. Efficacy was verified by measurement of circulating $CD4^+$ and $CD8^+$ T cells.

Reverse-Transcription Polymerase Chain Reaction

RNA was isolated from jejunum, and real-time reverse-transcription polymerase chain reaction (RT-PCR) was performed as previously described.[4]

Histology and Immunostaining

Jejunal segments were fixed in formalin and paraffin. Jejunal mast cells were detected by chloroacetate esterase staining according to published protocols of mast cell detection.[21]

IgE Measurement

OVA-specific IgE in serum and intestinal lavage fluid was measured by capture ELISA as previously described.[4]

Results

The CCL20-CCR6 Chemokine Axis Is Necessary for Gastrointestinal Allergic Symptoms

In a screen of local chemokine expression during the course of allergic diarrhea, CCL20 messenger RNA (mRNA) expression was significantly up-regulated compared with control mice that were sensitized but unfed with OVA (Figure 1*A*). Immunostaining for CCL20 showed a diffuse positive expression pattern in enterocytes and an intense immunoreactivity in M cells as has previously been reported[14] (Figure 1*B*). CCL20 is known to be an important chemokine for mucosal lymphocyte homeostasis, and, to test the role of this chemokine in allergic diarrhea, we used mice that were genetically deficient for the receptor CCR6. Wild-type mice sensitized to OVA developed acute self-limiting symptoms beginning after the third oral challenge with OVA (>50% of mice had symptoms by the third feed and approximately 90% by the fifth feed). In contrast, $CCR6^{-/-}$ mice were significantly protected from the onset of allergic diarrhea (Figure 1*C*).

CCR6-Independent IgE and Mast Cell Responses

It has previously been demonstrated[3] that diarrhea symptoms in this model are dependent on mast cells