Hapten Facilitates Food Allergen-Related Intestinal Hypersensitivity

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Abstract: *Background:* Haptens can bind to proteins to elicit antigenicity. Whether haptens play a role in the pathogenesis of food allergy remains to be investigated. This article aims to elucidate the role a hapten plays in food antigen-related T helper 2 (T_h2) pattern inflammation in the intestine. *Methods:* The effect of trinitrobenzene sulfonic acid (TNBS; as a hapten) on the properties of dendritic cells was assessed by a cell culture model. BALB/c mice were sensitized with a mixture of TNBS and ovalbumin (OVA; as a model antigen). Intestinal T_h2 response, OVA-specific immunoglobulin E and histamine were analyzed with the mouse model. In addition to the infiltration of the intestinal inflammatory cells, cytokine expression profiles were determined. *Results:* TNBS increased the expression of T-cell immunoglobulin and mucin domain-4 and CD80 and decreased the levels of interleukin-12 in dendritic cells. Higher serum levels of OVA-specific immunoglobulin E, histamine expression and skewed antigen-specific T_h2 polarization in the intestinal tissue were detected in mice sensitized with TNBS + OVA as compared with those treated with either OVA or TNBS alone. In addition, the TNBS-OVA-treated mice also showed an increased number of inflammatory cells, high levels of interleukin-4 and a decreased expression of interferon-γ in the lamina propria mononuclear cells. *Conclusions:* Hapten TNBS can facilitate the initiation of food antigen-related T_h2 pattern inflammation, such as food allergy, in the intestine.

Key Indexing Terms: Food allergy; Intestine; Antigen; Hapten; Helper T cells. **[Am J Med Sci 2013;345(5):375–379.]**

Food allergy is defined as a disorder in which antigenic food proteins elicit skewed immune response by the abrogation of oral tolerance. The prevalence of food allergy is estimated to be about 3% to 4% in adults and approximately 6% to 8% in young children and infants.[1] Commonly, food allergy is the immunoglobulin E (IgE)-mediated reactions to food antigens, such as cow's milk, eggs, peanuts, soybeans, wheat, fish and tree nuts, with a skewed T helper 2 (T_h2) response.[2] The clinical symptoms of food allergy vary from mild abdominal-irritating to life-threatening anaphylactic shock.[3] So far, no definitive therapies are available for food allergy; the only effective remedy is to avoid ingesting the offending food. Yet, our knowledge is still limited about how food allergy is initiated.

A hapten is a small molecule that cannot elicit an immune response alone, but it does when attached to a large carrier such as a protein; the carrier may be one that also does not elicit an immune response by itself.[4] In recent years, with the huge increase in atopic diseases, there has been a noted increase in dietary hapten exposure through processed food, formula milk, and oral antibiotic and drug use.[5] In fact, there is an established association between hapten and several atopic conditions such as allergic dermatitis.[6–8] Therefore, haptens may be also involved in the pathogenesis of food allergy, which yet remains to be investigated.

The T-cell immunoglobulin and mucin domain (TIM)-1 is expressed by activated helper T cells. TIM4 is the ligand of TIM1 and is expressed by activated dendritic cells (DCs). The interaction of TIM1 and TIM4 plays a major role in the initiation of an aberrant T_h2 response. Exposure to some microbial products (such as staphylococcal enterotoxin B) can induce the expression of TIM4 in DCs. DCs can be activated by trinitrobenzene sulfonic acid (TNBS); yet, whether TNBS-activated DCs can express TIM4 is unclear.

In this study, we induced a food antigen-related intestinal T_h2 inflammation by treating mice with a mixture of a model hapten, TNBS, and ovalbumin (OVA), a model food allergen, without any other adjuvants. This suggests that haptens might be an environmental triggering factor in the pathogenesis of food allergy.

MATERIALS AND METHODS

Reagents

The histamine enzyme-linked immunosorbent assay (ELISA) kit was purchased from Biotang Inc (Waltham, MA). OVA-specific IgE ELISA kit was purchased from AbD Serotec (Raleigh, NC). CD80, interleukin (IL)-12p70 and IL-4 ELISA kits were purchased from R&D Systems (Burlington, Ontario, Canada). Antibodies against IL-4, interferon (IFN)-γ and Foxp3 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Phycoerythrin-CY7 anti-mouse IL-4, phycoerythrin anti-mouse IFN-g and allophycocyanin anti-mouse CD4 were purchased from eBioscience (San Diego, CA). RNeasy Mini Kit and iScript cDNA Synthesis Kit were purchased from Bio-Rad (Mississauga, Ontario, Canada). HotStarTaq Master Mix Kit was purchased from Qiagen (Mississauga, Ontario, Canada). Recombinant Flt3 ligand (Flt3L) was purchased from R&D Systems (Missisauga, Ontario, Canada). The rest of the reagents were purchased from Sigma-Aldrich (Oakville, Ontario, Canada).

Generation of Bone Marrow-Derived Dendritic Cells

Bone marrow (BM) cells were obtained by flushing the femurs of BALB/c mice with phosphate-buffered saline. The BM cells were resuspended in lysis buffer for 2 to 4 minutes to lyse red blood cells. The remaining BM cells were washed 2 times in RPMI 1640 medium. BM cells were then cultured at 1×10^6 cells/mL in 6-well plates in RPMI 1640 culture medium supplemented with 200 ng/mL of recombinant murine Flt3L. The culture medium was changed on days 3 and 5, and replaced with fresh medium supplemented with Flt3L. The cells

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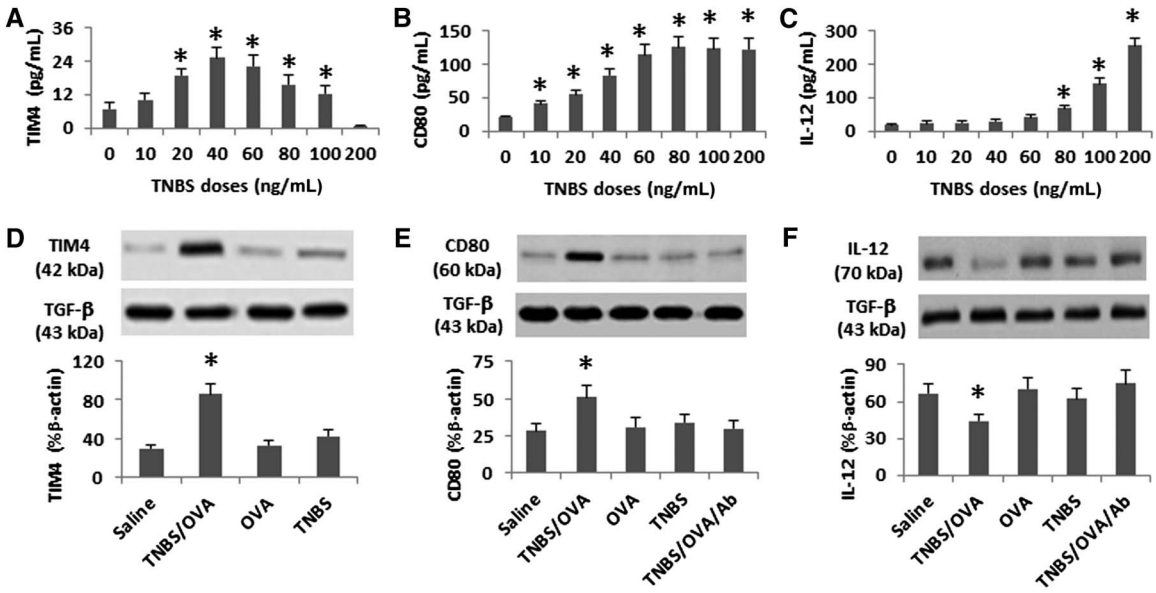

FIGURE 1. TNBS increases the expression of TIM4, CD80 and IL-12 in dendritic cells (DCs). (A–C) Bone marrow-derived dendritic cells were prepared and exposed to TNBS in the culture at graded doses for 72 hours. The supernatants were collected and analyzed by enzyme-linked immunosorbent assay. The bars indicate the levels of TIM4 (A), CD80 (B) and IL-12p70 (C). (D–F) Mice were gavage-fed with saline, TNBS-OVA, OVA alone or TNBS alone, or pretreated with anti-TIM4 antibody (Ab) and then gavage-fed with TNBS-OVA. The immunoblots show the levels of DC-derived TIM4 (D), CD80 (E) and IL-12 (F). The bars below gels show the summarized integrated density of the immunoblots. Data are presented as mean ± standard deviation in bar graphs (n = 3 in A–C; n = 6 in D–F). IL, interleukin; OVA, ovalbumin; TGF-β, transforming growth factor-β; TIM4, T-cell immunoglobulin and mucin domain-4; TNBS, trinitrobenzene sulfonic acid. *$P < 0.01$, compared with dose "0" group in A–C, compared with saline group in D–F.

were harvested on day 7. The purity of CD11c+ DCs was >95%, as assessed by flow cytometry.

Effect of TNBS on the Properties of DCs

Bone marrow-derived dendritic cells (BmDCs) were cultured in 96-well plates (1×10^5/well) in the presence of TNBS at graded doses (from 0 to 200 ng/mL) for 3 days. The supernatants were collected and analyzed by ELISA for the levels of TIM4, CD80 and IL-12p70. In addition, CD11c + DCs were isolated from the mice jejunum (see following). Total proteins were extracted from the DCs and analyzed by Western blot analysis for the levels of TIM4, CD80 and IL-12p70.

Animals

Male BALB/c mice (6–8 weeks old, body weight around 25 g per mouse) were purchased from Charles River Canada (St. Constant, Quebec, Canada) and maintained in a pathogen-free environment. The experimental procedures were approved by the Animal Care Committee at the McMaster University.

A Murine Model of Hapten-Facilitated Intestinal Allergy

Male BALB/c mice (6–8 weeks old) were fed with a mixture of TNBS (1 mg per mouse) and OVA (100 μg per mouse) in 0.3 mL of saline, TNBS alone, or OVA alone on days 0, 1, 2, 3 and 4. The mice were challenged with OVA (1 mg per mouse) in 0.3 mL of saline intragastrically on days 9, 11 and 13 using a ball-ended feeding needle. Control groups were treated with normal saline. Twenty-four hours after the last challenge, the mice were euthanized by cervical dislocation and the jejunal segments collected for analysis.

Enzyme-Linked Immunosorbent Assay

The levels of IL-4, IFN-γ, TIM4, OVA-specific IgE antibody, CD80, IL-12p70 and histamine were determined by ELISA with commercial reagent kits following the manufacturer's instruction.

Intestinal Tissue Inflammatory Cell Counts

Following our routine procedures,[9,10] the jejunal segments were excised and fixed with 4% paraformaldehyde, and processed for paraffin sections. The sections were stained with hematoxylin and eosin (for mononuclear cell and eosinophil counting), and 0.5% toluidine blue (for mast cell counting). The numbers of mononuclear cells, eosinophils and mast cells were counted under a light microscope. Twenty fields (200×) were counted for each mouse and the slides coded. The observers were blinded to the codes to avoid observer bias.

Assessment of Antigen-Specific T_h2 Response

Lamina propria mononuclear cells (LPMCs) were isolated from the small intestine following our routine procedure.[11] Jejunal segments were opened and washed with phosphate-buffered saline; mucus was removed by incubation with predigestion buffer. After grinding the tissue between 2 sterile glass slides to obtain a single cell suspension, the cells were passed through a cell strainer (100 μm). The cells were centrifuged over a Percoll gradient for mononuclear cell enrichment. LPMCs (1×10^6/well) were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) and cultured in the presence or absence of OVA for 4 days. The cells were collected at the end of culture and stained with fluorescence-labeled antibodies of CD3, CD4 and IL-4. The cells were analyzed first by CFSE dilution; then, the gated proliferating cells were further