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Interleukin 15 and CD4$^+$ T Cells Cooperate to Promote Small Intestinal Enteropathy in Response to Dietary Antigen

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BACKGROUND & AIMS: CD4$^+$ T cells specific for dietary gluten and interleukin 15 (IL15) contribute to the pathogenesis of celiac disease. We investigated whether and how they interact to damage the intestine using mice that overexpress human IL15 in the intestinal epithelium and have CD4$^+$ T cells specific for ovalbumin, a dietary antigen. **METHODS:** We crossed mice with CD4$^+$ T cells specific for ovalbumin (OTII) with mice that overexpress human IL15 under an intestine-specific promoter (B6 × IL15Tge). The offspring (OTII × IL15Tge mice) received control or ovalbumin-containing diets until 3 months of age. Enteropathy was monitored by weight, ratio of villous:crypt length, and the number of intestinal lymphocytes. Phenotype, cytokine production, and degranulation of mucosal and spleen lymphocytes were analyzed by multicolor flow cytometry or enzyme-linked immunosorbent assay. Regulatory T-cell function and CD8$^+$ T-cell activation were analyzed in co-culture assays. **RESULTS:** Exposure to ovalbumin reduced growth and led to enteropathy in OTII × IL15Tge mice but not in control OTII × B6 littermates. Enteropathy was associated with expansion of mucosal granzyme B$^+$ CD8$^+$ T cells, and developed despite increased frequency of functional ovalbumin-specific regulatory T cells. Ovalbumin-activated CD4$^+$ T cells secreted IL2, which along with IL15 stimulated expansion of noncognate intestinal cytotoxic CD8$^+$ T cells, which did not respond to regulatory T cells and induced epithelial damage. **CONCLUSIONS:** We observed that in mice given food antigen, cooperation between IL15 and CD4$^+$ T cells is necessary and sufficient to activate CD8$^+$ T cells and damage the small intestine. We propose that this process is involved in the development of celiac disease.

Keywords: Gluten Allergy; Treg Cells; Immune Regulation; Mouse Model.

In physiologic conditions, robust immunologic mechanisms avoid adverse responses to food antigens.[1] In contrast, in celiac disease (CD), abnormal activation of intestinal lymphocytes by dietary gluten leads to severe small intestinal enteropathy. In CD, intestinal lesions depend on the activation of lamina propria (LP) CD4$^+$ T lymphocytes (TLs) by gluten peptides selectively presented by HLA-DQ2/8 molecules, the main genetic risk factor.[2] This mechanism is necessary, however, it is not sufficient to break tolerance to the dietary antigen and to induce tissue damage.[2] Previous studies, including ours, also have implicated interleukin 15 (IL15), a cytokine up-regulated in the CD intestine. IL15 promotes the survival, accumulation, and activation of cytotoxic CD8$^+$ intraepithelial lymphocytes (IEL), which can induce epithelial lesions.[3–6] IL15 also might impair local immune regulation, either by making TLs resistant to suppression by transforming growth factor-β and Forkhead box P3 (FOXP3$^+$) regulatory T cells (Tregs),[7–9] or by hampering the generation of Tregs.[10] Whether and how gluten-specific CD4$^+$ TLs and IL15 interact to activate CD8$^+$ IELs and to drive tissue damage, however, is unknown.

To address this question, we have developed a mouse model. Transgenic CD4$^+$ T cells specific for ovalbumin (OTII) mice with CD4$^+$ TL specific for an epitope of ovalbumin (OVA) were crossed with heterozygous B6 × IL15Tge mice, which express a secreted form of human IL15 in the intestinal epithelium.[11] We next compared the consequences of OVA-containing diet in the created OTII × IL15Tge mice and in OTII × B6 littermates, which also possess OVA-specific CD4$^+$ TL but do not overexpress IL15. In OTII × IL15Tge mice, but not in OTII × B6 mice, early and chronic exposure to dietary OVA led to growth retardation, and to an enteropathy associated, as in CD, with expansion of Granzyme B$^+$ CD8$^+$ TL in LP and epithelium. We further show that IL15 cooperates with IL2 produced by OVA-activated CD4$^+$ TLs to stimulate the expansion of cytotoxic CD8$^+$ TL.

Materials and Methods

Mouse Models

B6 (C57BL/6j) mice, OTII mice, b2m$^{-/-}$ mice, and major histocompatibility complex (MHC)II$^{-/-}$ mice were purchased from

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Abbreviations used in this paper: CD, celiac disease; DC, dendritic cell; IEL, intraepithelial lymphocyte; IFN, interferon; IL, interleukin; iTreg, regulatory T cell induced in the periphery in response to exogenous antigens; LP, lamina propria; mAb, monoclonal antibody; MLN, mesenteric lymph nodes; nTreg, natural regulatory T cell generated in the thymus; OTII, CD4$^+$ T cells specific for ovalbumin; OVA, ovalbumin (albumin from chicken egg white); PBS, phosphate-buffered saline; rhIL, recombinant human interleukin; TL, T lymphocyte; TCR, T-cell receptor; Treg, regulatory T cell; WT, wild type.

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Charles River (L'Arbresle, France). OTII RAG2$^{-/-}$ Thy1.1 mice and OTI RAG2$^{-/-}$ mice were a gift from O. Lantz (Institut Curie, Paris, France). Heterozygous B6 × IL15Tge male mice, which express human IL15 under control of the intestine-specific T3b promoter, were crossed with female OTII or B6 mice.[11] A diet containing 10% OVA or an isoprotein/isocaloric control diet (Genestil, Royaucourt, France) was given to OTII or B6 mice during the last week of gestation, and to their offspring until 3 months of age. Daily intake of OVA was approximately 17 mg/g of mouse body weight. Animals were bred under specific pathogen-free conditions in accordance with European Guidelines. Experiments were approved by the local ethics committee.

Histology and Immunohistochemistry

Sections (5 μm) from formol-fixed, paraffin-embedded duodenum were stained with H&E. Granzyme B was detected using antimouse Granzyme B goat IgG (AF1865; R&D Systems, Lille, France), biotinylated rabbit antigoat IgG (E0466; Dako, Les Ulis, France), and streptavidin peroxidase (P0397; Dako).

Cell Isolation and Flow Cytometry

Isolation of LP, mesenteric lymph nodes (MLNs), and spleen cells, and staining for surface markers and intracellular Foxp3, Granzyme B, and cytokines, were performed as described.[12] Antibodies are listed in the Supplementary Materials and Methods section. Cells were analyzed on a FACS Canto II using BD FACSDiva software (BD Biosciences, Le Pont-de Claix, France).

Cell Subset Purification and Functional Assays

T-cell subsets were enriched by an AutoMACS Pro Separator (Miltenyi Biotec, Paris, France) and further purified using a FACS Aria cell sorter II (BD Biosciences). To test Treg activity, 5 × 10^4 OVA-specific responder T cells (Vβ5$^+$CD25$^-$CD4$^+$) were co-cultured at a 1:1 ratio with OVA-specific Tregs (Vβ5$^+$CD25$^+$CD4$^+$), or, as a control, with OVA-nonspecific TLs (Vβ5$^-$CD4$^+$CD25$^-$), in 96-well, round-bottom plates with 0.5 μg/mL $OVA_{323-339}$ peptide (Covalab, Villeurbanne, France) and 5 × 10^4 irradiated spleen cells from OTII mice fed the control diet. Proliferative responses were assessed in triplicate cultures by [^{3}H] thymidine incorporation. To test T-cell response to Tregs, 5 × 10^4 CD45$^+$CD25$^-$ B6 spleen cells were labeled using the Cell Trace Violet cell proliferation kit (Life Technologies, Saint Aubin, France) and co-cultured at a 1:1 ratio with unlabeled CD45$^+$CD25$^+$ cells or, as a control, with unlabeled CD45$^+$CD25$^-$ cells. To assess proliferation, cells were stimulated for 48–72 hours with 0.2 μg/mL CD3 monoclonal antibodies (mAbs) (145-2C11; BD Biosciences), and then analyzed by flow cytometry. To assess induction of interferon (IFN)-γ and Granzyme B, cells were stimulated with CD3 mAbs and 0.2 μg/mL CD28 mAbs (37.51; BD Biosciences), in the presence or absence of 20 ng/mL human IL15 (Miltenyi Biotec), and then analyzed by enzyme-linked immunosorbent assay (R&D Systems).

To analyze interactions between CD8$^+$ and CD4$^+$ TLs, CD8$^+$ and CD4$^+$ TLs purified from indicated sources were stained using Cell Trace Violet, added with spleen CD11c$^+$ dendritic cells (DCs) (DC/T ratio, 1:10), and stimulated with $OVA_{253-267}$, $OVA_{323-339}$ (2 μg/mL), or OVA protein (400 μg/mL) plus 20 ng/mL IL15. After 4 days, proliferation and activation markers were assessed by flow cytometry. Degranulation of CD8$^+$ TL was analyzed by stimulating cells for 4 hours with 1 μg/mL CD3 mAb, and then staining for CD107a-PE (1D4B; eBioscience, Paris, France).

Adoptive Cell Transfer

Spleen and MLN cells from OTII RAG2$^{-/-}$ Thy1.1 transgenic mice (2 × 10^6 cells in 100 μL phosphate-buffered saline [PBS]) were injected intravenously into B6 or B6 × IL15Tge mice. On days 1 and 2 after transfer, mice were gavaged with 50 mg OVA in 100 μL PBS, or with PBS alone. CD25$^+$Foxp3$^+$Thy1.1$^+$ Tregs were assessed by flow cytometry in spleen and MLN on day 5.

Western Blot

Detection of Granzyme B in duodenal tissue was performed as described,[7] using goat anti–Granzyme B (AF1865; R&D Systems). Granzyme B was quantified relative to actin, using a LAS-1000 CCD camera and Image Gauge software (Fujifilm Lifesciences, Bois d'Arcy, France).

Messenger RNA Expression

RNA was purified from duodenal biopsy specimens using an RNeasy Mini kit (Qiagen France S.A.S., Courtaboeuf, France) and reverse-transcribed.[12] Quantitative polymerase chain reaction was performed using TaqMan gene expression assays (Life Technologies). Data were normalized to Hypoxanthine-guanine phosphoribosyltransferase.

IL2 Treatment

B6 and B6 × IL15Tge mice were treated for 5 days by daily intraperitoneal injections of 200,000 IU recombinant human IL2 (rhIL2) (Proleukin, Novartis, France), generously provided by D. Klatzmann (CHU Pitié-Salpêtrière, Paris, France).

Statistical Analysis

Statistical analysis was performed with GraphPad Prism version 6.00 (GraphPad Software, Inc., La Jolla). Weight curves were compared using repeated analysis of variance followed by the Tukey post hoc test. Other comparisons used the Mann–Whitney *U* test. A *P* value less than .05 was considered statistically significant.

Results

Chronic Oral Exposure to OVA Causes Small Intestinal Lesions in OTII × IL15Tge Mice

Whether fed with control diet or OVA diet, OTII × B6 mice showed similar weight gain and a similar villous/crypt length ratio (Figure 1*A* and *B*), indicating that the presence of many CD4$^+$ TLs specific for the dietary antigen (Supplementary Figure 1*A*) is not sufficient to induce an enteropathy. In contrast, 3-month-old OTII × IL15Tge mice fed the OVA diet showed slower growth; increased cellularity in LP, epithelium, and submucosa; villous blunting; and crypt elongation in the proximal duodenum (Figure 1*A*–*F* and Supplementary Figure 2*A*). Epithelial lesions were patchy and their intensity varied. They were particularly severe in 7 of 11 OVA-fed