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IL-9 Promotes IL-13-Dependent Paneth Cell Hyperplasia and Up-Regulation of Innate Immunity Mediators in Intestinal Mucosa

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IL-9 contributes to lung inflammatory processes such as asthma, by promoting mast cell differentiation, B cell activation, eosinophilia, and mucus production by lung epithelial cells. The observation that IL-9 overexpressing mice show increased mast cell numbers in the intestinal mucosa suggests that this cytokine might also play a role in intestinal inflammation. In colons from IL-9 transgenic mice, the expression of Muc2, a major intestinal mucin gene, was up-regulated, together with that of CLCA3 chloride channel and resistin like α, which are goblet cell-associated genes. Additional IL-9 up-regulated genes were identified and included innate immunity genes such as angiogenin 4 and the PLA2g2a phospholipase A2, which are typical Paneth cell markers. Histochemochemical staining of Paneth cells by phloxine/tartrazine showed that IL-9 induces Paneth cell hyperplasia in Lieberkühn glands of the small intestine, and in the colonic mucosa, where this cell type is normally absent. Expression of Paneth cell markers, including angiogenin 4, PLA2g2a, and cryptdins, was induced in the colon of wild-type mice after two to four daily administrations of IL-9. By crossing IL-9 transgenic mice with IL-13−/− mice, or by injecting IL-9 into IL-4R−/− mice, we showed that IL-13 was required for the up-regulation of these Paneth cell-specific genes by IL-9. Taken together, our data indicate that Paneth cell hyperplasia and expression of their various antimicrobial products contribute to the immune response driven by TH2 cytokines, such as IL-9 and IL-13 in the intestinal mucosa. The Journal of Immunology, 2009, 182: 4737–4743.

The lung and gastrointestinal mucosae are both composed of a single layer of interconnected epithelial cells, which form a first line of defense against external aggressors by outer microorganisms. Depending on further differentiation, epithelial cells directly contribute to mucosal immunity either mainly by establishing a physical barrier, or by producing a large spectrum of bioactive molecules. Goblet cells are responsible for mucus production, which confers a physical protection by trapping pathogens and interfering with their adhesion to the mucosa. In contrast, Paneth cells, which are actually mediated by up-regulation of IL-13 expression by T and mast cells (5–7).

Like most effector cells from the innate immune system, lung and intestinal epithelial cells act as an efferent arm of adaptive immune responses. In particular, cytokine production by TH2 lymphocytes is well known to affect epithelial cell physiology, including mucus production, which is a hallmark of nematode infections and allergic asthma. Such conditions can be mimicked by overexpression of TH2 cytokines such as IL-9 and IL-13 in the lungs. Indeed, IL-9 transgenic (Tg)3 and IL-13 Tg mice show increased mucus production by lung goblet cells (1, 2) and airway hyperresponsiveness (2–4). Further studies of IL-9 Tg mice showed that IL-9 activities on lung epithelial cells are actually mediated by up-regulation of IL-13 expression by T and mast cells (5–7).

In the gastrointestinal mucosa, IL-13 and IL-9 production similarly contribute to the resistance against nematode infections (8–10). Although the actual effector mechanisms by which TH2-polarized immune responses allow for parasite expulsion are not completely elucidated, epithelial cells seem to be actively involved in this process, for instance as a result of accelerated cell turnover (11). Paneth cell hyperplasia is also associated with nematode infections (12, 13). Although there is no evidence that intestinal epithelial cells respond to IL-9, the similarities between the lung and intestinal mucosae, as well as the fact that IL-9-induced mastocytosis seemed mainly restricted to these tissues, prompted us to investigate the potential activity of IL-9 on colonic epithelial cells in vivo. In this study, we show that IL-9 up-regulates the expression of innate immunity genes including Paneth cell markers such as angiogenin 4, cryptdins, and phospholipase A2, and that this activity requires IL-13. Histochemochemical staining confirmed that IL-9 induces Paneth cell hyperplasia in small intestine and in colon.

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IL-9-INDUCED PANETH CELL EXPANSION IN INTESTINAL MUCOSA

Materials and Methods

Mice

IL-9-Tg mice, obtained in the FVB/N background and expressing high levels of IL-9 in all organs, were described previously (14). IL-13-deficient mice (129 × C57BL/6 genetic background) were obtained by insertion of a cassette containing a LacZ reporter gene and a neomycin resistance gene into exon 1 of the IL-13 locus, as described previously (15). IL-9 Tg mice were bred with IL-13 knock-out mice (IL-13−/−) to generate F1 mice that were backcrossed for one generation with IL-13−/− mice. Cells were screened by PCR on genomic DNA for the IL-13 gene and with a TaqI bioassay for circulating IL-9. The sequences of the primers were: IL-13 WT: 5′-GGGTTGACTGCACTGGTCG-3′ (forward) and 5′-GGTGTGC AGCTCTCTCAAAAAGC-3′ (reverse); IL-13−/−: 5′-GGGGCAGTGAG GOCATTTTCCTG-3′ (forward) and 5′-GGCGGAAGGCGGCTGC CGCG-3′ (reverse). The IL-13−/− and IL-9−/− mice were generated in the C57BL/6 background by deletion of the exons 2–6 of the IL-9-Rα-chain, corresponding to the extracellular part of the receptor as described in Ref. 5. For the current study, IL-9−/− mice were backcrossed for five generations onto BALB/c background. FVB and IL-9Tg mice were bred with BALB/c mice to obtain F1 mice (respectively designated FVB/B6 and IL-9 Tg/c) used as cell donors for transfer experiments.

Microarray and subtractive hybridization experiments

Total RNA was extracted from the distal colon of four FVB or IL-9 Tg mice using TriPure isolation reagent (Roche). Four micrograms of total RNA, pooled for each mouse group, were amplified according to the One-cycle cDNA Synthesis Kit from Affymetrix. The expression of 14,000 genes was assessed on the GeneChip mouse expression Array 430A. Staining, washing, and scanning procedures were conducted as described in the GeneChip Expression Analysis technical manual (Affymetrix). All intensity values were scaled to an average value of 100 per GeneChip according to the method of global scaling, or normalization, provided in the Affymetrix Microarray Suite software, version 5.0 (MAS5.0). Differential expression between two arrays was then calculated with the GeneChip Operating software from Affymetrix. Complete microarray data have been deposited in the GEO public repository under the series record GSE14249.

For representational difference analysis experiments, pooled total RNA was prepared from colon of five FVB and five IL-9 Tg mice, using guanidine isothiocyanate lysis and CsCl gradient centrifugation. Polyadenylated RNA was purified from total RNA with oligo(dT) cellulose columns (Pharmacia). Double-stranded cDNA was generated from 5 μg poly(A)+ RNA using oligo(dT) primer and the SuperScript Choice System for cDNA synthesis, according to the manufacturer’s recommendations (Life Technologies). Representational difference analysis was performed as described by Hubank and Schatz (17). After three rounds of subtraction, final differenct products were digested with DpnII and cloned into the BamHI site of pTZ19R. Double-stranded plasmid DNA was prepared and sequenced with a Thermo-sequenase Sequencing kit (Amersham Biosciences). Sequence comparisons with GenBank and EMBL databases were performed with the BLAST search program (National Center for Biotechnology Information).

In vivo treatments

C57BL/6, IL-4−/−, and IL-4R−/− mice were injected i.p. with IL-9 (200 ng), which had been purified from the serum of IL-9 Tg mice using a rat anti-mouse IL-9 Ab and ion exchange chromatography. Four days later, the mice were given a second i.p. injection (200 ng) and were sacrificed the following day. Control mice received the same volume of buffer (PBS plus 1% mouse serum) as used for cytokine injections. In the experiments with FVB mice, the animals received two or four daily injections, and were sacrificed the day following the last injection.

Cell transfer

Bone marrow was aseptically flushed from femora and tibias of FVB/c or IL-9 Tg/c 10- to 12-wk-old mice. Cells were resuspended and filtered through a 70-μm nylon cell strainer. Red cells were removed by osmotic shock. Hematopoietic cells were washed, counted, and resuspended in DMEM for injection. For cell transfer experiments, IL-9−/− recipient mice were exposed to two doses of 550 rad of ionizing radiation, given 3 h apart, followed by i.v. injection of 4 × 106 bone marrow cells isolated from IL-9−/− mice. Cells were injected in a volume of 100 μl of sterile DMEM. Control recipient mice received an equivalent volume of medium. Mice were killed 3 wk after the transfer.

Mouse MCP (mMCP)-1 ELISA

Serum mMCP-1 concentrations were measured in duplicate in 96-well microtiter plates using a commercial mMCP-1 kit (Moredun Scientific) according to the manufacturer’s instructions.

RT-PCR

Distal colon and small intestine were removed and submersed in RNA later (Ambion) between 12 or 48 h. Lungs were frozen in liquid nitrogen. Total RNA was isolated from tissues using TriPure isolation reagent (Roche) according to the manufacturer’s instructions. Reverse transcription was performed on 2 μg of total RNA with an oligo(dT) primer (Roche) and M-MLV RT (Invitrogen). PCR amplifications were performed from cDNA corresponding to 20 ng of total RNA. Quantitative PCR were performed using primers sets corresponding to murine angiogenin 4 (Ang4), phospholipase C-β4 (PLA2g4c), MCPC-1 (MCP), mMCPs, and β-actin with qPCR Mastermix for SYBR Green I (Eurogentec). The sequences of the primers (final concentration: 300 nM) were: Ang4: 5′-CTCGGGTCTCAGAATGTAAGTGCA-3′ (forward) and 5′-GAATCTTCTAATAAAGGTTCGGTACC-3′ (reverse); PLA2g4c: 5′-CAC TCCCTATCCTCATGTCCG-3′ (forward) and 5′-GAACAGATGATAGTCGACACCAAG-3′ (reverse); MCP: 5′-GCTGACAGCAT GAAAGCTCCTC-3′ (forward) and 5′-GGTGAAGCTAGCAGGGG-3′ (reverse); and β-actin: 5′-TCTCTGGGCAAAGTACTTCTG-3′ (forward) and 5′-CTGATCCAC ATCTGCTGAAG-3′ (reverse). Samples were first heated 10 min at 95°C. cDNA was amplified as follows: 40 cycles of a two-step PCR program at 95°C for 15 s and 60°C for 1 min (63°C for PLA2g4c), except for mMCPs for which the hybridization and elongation steps were performed respectively at 65°C for 30 s and at 72°C for 1 min. Melting point analysis was conducted by heating the amplitcon from 60–65°C to 95°C. Muc2 qPCR was performed with the Premix Ex Taq from TaKaRa using SYBR Green I (Eurogentec). The sequences of the primers (final concentration: 300 nM) were: 5′-TGAGAAAGAAGATGTCCTACA-3′ (forward) and 5′-AOGCTGTGTATCTTCTGCA-3′ (reverse). The samples were first heated at 95°C for 30 s and then amplified with 45 cycles of a three-step PCR program at 95°C for 10 s, 60°C for 1 min, and 72°C for 15 s, followed by a melting point analysis.

Semiquantitative PCR for IL-13 and β-actin were performed with the TaKaRa Mix for PCR. The sequences of the primers (final concentration: 200 nM) were: β-actin: 5′-ATGTTGAGATCATATGCCTG-3′ (forward) and 5′-GCTGGAAGGTCGACAGTOA-3′ (reverse) and IL-13-5′-TCTG GCTCCTGACAGTCCT-3′ (forward) and 5′-GGTTGCTTGTTGTTA GCGTGAAG-3′ (reverse). cDNA was amplified as follows: 18 cycles (β-actin) or 36 cycles (IL-13) of a three-step PCR program at 95°C for 1 min, 57°C for 1 min, and 72°C for 2 min.

Northern blot

Tissue expression of mCLCA3/gob5 was determined by Northern blot analysis of RNA extracted from colon using the TriPure isolation reagent (Roche). Five micrograms of poly(A)+ mRNA were fractionated by electrophoresis in 1.3% agarose gels containing 2.2 mol/L formaldehyde and photo-dried into Hybond-C Extra membranes (Amersham Biosciences). Filters were hybridized to mCLCA3/gob5 probes, 32P-labeled by using Rediprime II labeling kit (Amersham Biosciences). β-Actin probes were used as controls for equal loading. Phosphorimager or InstantImager quantifications were used to determine the level of signal.

Histochemical staining

Distal colons were fixed with buffered formalin and embedded in paraffin. For Paneth cell-specific staining, 7-μm sections were cut, dewaxed, and hydrated. Nuclei were stained with hemalum for 45 s. After a brief wash in water, sections were stained in phosphate solution (0.5 g of phosphate B and 0.5 g of calcium chloride in 100 ml of distilled water) for 20 min, successively rinsed in water and in Cellosize (2-ethoxy ethanol, Aldrich), and differentiated with a saturated tetrathionate solution (2.5 g of tetrathionate in 100 ml of Cellosize) for 4–6 min. After rinsing and mounting, sections were stained by light microscopy with 0.2% toluidine blue, 0.6% Neutral red, and 0.5% naphthol AS-Bi phosphate salt in 0.05% alkaline (5 min), periodic acid (10 min), Schiff reagent (20 min), and counterstained with hematoxylin (30 s).

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IL-13 and mCLCA3 expression in the colon

Distal colon total RNA was extracted either from IL-9 Tg or from FVB mice (A) or from IL-9 Tg, IL-9 Tg/IL-13−/−, IL-9 Tg/IL-13−/−, and control littermates (B and C). A, RT-PCR for IL-13 and β-actin. Each lane corresponds to one mouse. B, Quantitative RT-PCR for Muc2 normalized with β-actin. Results were expressed as mean values and SEM (n = 4–5). C, Northern blot analysis of mCLCA3 expression was quantified by InstantImager analysis of blots hybridized with a mCLCA3 probe, before reprobing with β-actin ( arbitrary units).

Results

IL-9 increases IL-13 and mucus production in the intestinal mucosa

IL-9 overexpression was previously shown to induce mucus production and to up-regulate the expression of various mucus-associated genes by lung epithelial cells, and this effect of IL-9 was mediated by IL-13 (5–7). As shown in Fig. 1A, IL-13 expression was also up-regulated in the colons from IL-9 transgenic mice. To determine whether mucus production was similarly up-regulated by IL-9 in this tissue, we analyzed the expression of Muc2, which is expressed by mucin-secreting cells (19) and was recently shown to be induced by IL-13 in lung epithelial cells (20). In line with this observation, IL-9 administration up-regulated Fcgbp expression in the colon of wild-type (WT), but not IL-4Rα−/− mice (data not shown).

For immunostaining, euthanized mice were perfused with PBS. Freshly collected colons were immersed in buffered formaldehyde for 24 h at room temperature and embedded in paraffin. Tissue sections of 7 μm in thickness were cut, placed on SuperFrost Plus slides, dried at 37°C overnight, and processed by standard methods for immunohistochemistry. In brief, sections were deparaffinized, permeabilized for 10 min in PBS containing 0.1% Triton X-100, treated for 5 min in PBS containing 1.5% H2O2 to block endogenous peroxidase activity, and washed in PBS. Sections were treated for 90 min at 97°C in sodium citrate buffer 0.01 M (pH 5.8) to unmask Ags. Blocking was performed by incubating sections for 30 min with TNB (PerkinElmer, TSA Fluorescein system, NEL70). Immunolabeling was done in blocking solution containing the Abs. PLA2g2a was detected with a goat Ab at a dilution of 1/50 (Santa Cruz Biotechnology, sc-14472). The secondary Ab (at 1/800) was a donkey anti-goat Ab IgG-HRP (Santa Cruz Biotechnology, sc-2033). The immunostaining was revealed with DAB substrate reaction (Brown staining) (Roche, ref. 718 096), and the sections were stained with Hemalun (45 s, blue staining).

**Statistical analysis**

Statistical significance was analyzed using the Instat3 program. Mann-Whitney U test or unpaired Student’s t test were run to determine the p value when comparing two groups. Differences with p < 0.05 were considered statistically significant. Values were presented as means ± SEM.

**Figure 1.** A functional IL-13 gene is required for IL-9-induced Muc2 and mCLCA3 expression in the colon. Distal colon total RNA was extracted either from IL-9 Tg or from FVB mice (A) or from IL-9 Tg, IL-9 Tg/IL-13−/−, and control littermates (B and C). A, RT-PCR for IL-13 and β-actin. Each lane corresponds to one mouse. B, Quantitative RT-PCR for Muc2 normalized with β-actin. Results were expressed as mean values and SEM (n = 4–5). C, Northern blot analysis of mCLCA3 expression was quantified by InstantImager analysis of blots hybridized with a mCLCA3 probe, before reprobing with β-actin ( arbitrary units).

**Figure 2.** Angiogenin 4 basal expression and induction by IL-9 require IL-13. A, Quantitative RT-PCR for Ang4 was performed on colon total RNA from IL-9 Tg, IL-13−/−, IL-9 Tg/IL-13−/−, and control littermates. B, Total RNA was extracted from colon and small intestine of WT and IL-13−/− mice, and Ang4 expression was measured by quantitative RT-PCR. C, IL-4−/− and IL-4Rα−/− mice received two i.p. injections of IL-9 (200 ng) or sterile PBS, 5 days and 1 day before the analysis of Ang4 expression by quantitative RT-PCR on colon and small intestine total RNA (upper and middle panel). Results were normalized with β-actin. Serum MMCP-1 concentrations were measured by ELISA (lower panel). D, Quantitative RT-PCR for Ang4 was performed on small intestine of total RNA from irradiated IL-9Rα−/− mice reconstituted with 4 × 106 bone marrow cells from WT or IL-9 Tg mice. Mice were sacrificed 3 wk after the transfer of cells. Mean values and SEM were calculated from at least four mice of each group. *p ≤ 0.05.
Ang4 is mainly produced by Paneth cells, belongs to the RNase A superfamily, and has angiogenic and bactericidal activities (21–23). As shown on Fig. 2A, Ang4 expression in the colon is up-regulated in IL-9 Tg mice in an IL-13-dependent way. Interestingly, Ang4 basal expression in colon, but not in small bowel, was dramatically lower in IL-13−/− mice as compared with wild-type mice, even in the absence of IL-9 overexpression (Fig. 2B). This observation indicates that IL-13 is required for constitutive expression of Ang4 in colon, probably in response to commensal microflora, which was previously shown to induce the expression of this gene (23). Similar results were observed with mice deficient in IL-4Rα, which is involved in responses to IL-4 and IL-13. These mice showed a decrease of basal Ang4 expression in the colon, but not in the small bowel, and no up-regulation of Ang4 in response to IL-9 administration (Fig. 2C).

Induction of PLA2g4c and PLA2g2a by IL-9 in the colon. Quantitative RT-PCR was performed on colon total RNA from WT and IL-9 Tg mice for PLA2g4c (A) or for PLA2g2a (B). Results were normalized with β-actin. The data were expressed as mean values and SEM (n = 4–8). Differences between IL-9 Tg and wild-type mice were statistically significant (p ≤ 0.0286; Mann-Whitney U test).

![FIGURE 3](image)

**FIGURE 3.** Induction of PLA2g4c and PLA2g2a by IL-9 in the colon. Representative sections of colon (A–D) from control FVB (WT) and IL-9 Tg mice stained with hemalun, phloxine B, and tartrazine. Sections were examined by light microscopy (×40 objective). Paneth cells granules are stained in red, and are indicated by arrows when they are located at the base of the crypts or by arrowheads when located nearby the apex of the glands. E–H, PLA2g2a expression detected in colon by immunohistochemistry in wild-type (E and F) or in IL-9 Tg mice (G and H) (magnification, ×20). E and G, Longitudinal sections. F and H, Transversal sections.

![FIGURE 4](image)

**FIGURE 4.** Role of IL-13 in PLA2g4c and PLA2g2a basal expression and induction by IL-9. WT and IL-4Rα−/− mice received i.p. injections of IL-9 (200 ng) or sterile PBS, 5 and 1 day before analysis of PLA2g4c (upper panel) and PLA2g2a (middle panel) expression by quantitative RT-PCR on colon total RNA. Results were normalized with β-actin. Serum mMCP-1 concentrations were measured by ELISA (lower panel). Mean values and SEM were calculated from four to five mice of each group. *, p ≤ 0.03; Mann-Whitney U test.

![FIGURE 5](image)

**FIGURE 5.** IL-9 induces hyperplasia of Paneth cells in intestinal mucosa. A–D, Representative sections of colon (B and D) and duodenum (A and C) from control FVB (WT) and IL-9 Tg mice stained with hemalun, phloxine B, and tartrazine. Sections were examined by light microscopy (×40 objective). Paneth cells granules are stained in red, and are indicated by arrows when they are located at the base of the crypts or by arrowheads when located nearby the apex of the glands. E–H, PLA2g2a expression detected in colon by immunohistochemistry in wild-type (E and F) or in IL-9 Tg mice (G and H) (magnification, ×20). E and G, Longitudinal sections. F and H, Transversal sections.

A microarray experiment was performed to further identify differentially expressed genes in the colon of IL-9 Tg or wild-type mice. Beside mast cell-specific genes such as mMCP-1, −2, −4, and FceRI, B cell-specific genes such as the Ig heavy and light chains, CD19, and CD79, two members of the phospholipase A2 (PLA2) family were found to be up-regulated by IL-9, namely PLA2g2a and PLA2g4c. PLA2s hydrolyze glycerophospholipids to produce free fatty acids, such as arachidonic acid and lysophospholipids.

**IL-13 is required for phospholipase A2 induction by IL-9**

A microarray experiment was performed to further identify differentially expressed genes in the colon of IL-9 Tg or wild-type mice. Beside mast cell-specific genes such as mMCP-1, −2, −4, and FceRI, B cell-specific genes such as the Ig heavy and light chains, CD19, and CD79, two members of the phospholipase A2 (PLA2) family were found to be up-regulated by IL-9, namely PLA2g2a and PLA2g4c. PLA2s hydrolyze glycerophospholipids to produce free fatty acids, such as arachidonic acid and lysophospholipids.
PLA2g2a is a small secreted protein released by Paneth cells, and has antimicrobial activity against Gram-positive bacteria probably conferred by the highly cationic nature of this protein and its preference for cleaving anionic phosphatidylglycerol which is highly represented in bacterial membranes (26, 27). By contrast, PLA2g4c is a cytosolic, membrane-associated protein that contributes to arachidonic acid metabolism and phospholipid remodeling in response to inflammatory stimuli, such as IL-1 (28).

As illustrated on Fig. 3, both PLA2s were up-regulated in the colon of IL-9 Tg. IL-9 administration induced the expression of these genes in the colon of wild-type, but not of IL-4R/H1100/H1100 mice, whereas Ang4 is clearly induced in the colon after two IL-9 injections (Fig. 6). PLA2g2a and cryptdins up-regulation required four IL-9 injections. The up-regulation of these Paneth cell-specific genes correlated with that of mast cell specific genes such as mMCPs.

PLA2g4c and resistin-like α, but not Paneth cell markers, are similarly regulated by IL-9 in lung and intestinal mucosae

To compare the effects of IL-9 on the intestinal and lung mucosae, we analyzed the expression of the IL-9-induced genes in lungs from IL-9 Tg mice. As shown in Fig. 7, PLA2g4c expression was up-regulated in IL-9 transgenic lungs, and this effect was dependent on IL-13 as previously observed in colon. By contrast, PLA2g2a and Ang4

![FIGURE 6. Kinetics of Ang4, PLA2g2a, and cryptdin induction by IL-9. Normal FVB mice received i.p. daily injections of IL-9 (200 ng) or sterile PBS, for 2 or 4 days. Mice were sacrificed the day after the last injection. Quantitative RT-PCR was performed for Ang4, PLA2g2a, cryptdins, and mMCPs on colon total RNA as described in Materials and Methods. Results were normalized with β-actin. Mean values and SEM were calculated from two to three mice of each group.](http://www.jimmunol.org/)

![FIGURE 7. Lung expression of IL-9-induced PLA2g4c is IL-13-dependent. Total RNA was extracted from lungs of control littermates, IL-9Tg, IL-13−/−, and IL-9Tg/IL13−/− mice and quantitative RT-PCR was performed on RNA for PLA2g4c. Results were normalized with β-actin and expressed as mean values and SEM (n = 4–5). *, p ≤ 0.0286; Mann-Whitney U test.](http://www.jimmunol.org/)

![FIGURE 8. Colon expression of Resistin-like α is induced by IL-9 in an IL-13-dependent way. A, Quantitative RT-PCR for Retnla on colon total RNA from IL-9Tg, IL-13−/−, and IL-9Tg/IL13−/−, and control littermates. B, IL-4−/− and IL-4Rε−/− mice received two i.p. injections of IL-9 (200 ng) or sterile PBS, and 1 day before the analysis of Retnla expression by quantitative RT-PCR on colon total RNA. Results were normalized with β-actin and expressed as mean values and SEM (n = 4). *, p ≤ 0.03; Mann-Whitney U test.](http://www.jimmunol.org/)
mRNAs were not detectable in lungs (data not shown), in line with the fact that these genes are specific Paneth cell markers.

The resistin-like molecule family contains a series of other antimicrobial proteins secreted by epithelial cells in an IL-13-dependent way, and playing a role in defenses against nematode infections (30–32). We have previously shown that Retnla was induced by IL-9 in an IL-13-dependent way in lungs (5). We therefore analyzed the colonic expression of Retnla in our model. As illustrated in Fig. 8A, Retnla was up-regulated in IL-9 Tg mice through an IL-13-dependent mechanism. In line with this observation, IL-9 administration failed to increase Retnla expression in IL-4R−/− mice, whereas its effect was maintained in IL-4−/− mice (Fig. 8B).

Incidentally, basal expression of Retnla also seems to be partially IL-13 dependent, as illustrated by the lower expression of this gene in untreated IL-4R−/− mice or in nontransgenic IL-13−/− mice, as compared with IL-4−/− and to IL-13−/− mice, respectively.

Discussion

We have previously shown that IL-9 promotes mucus production by lung epithelial cells via IL-13 induction in hematopoietic cells. In this study, we focused on the activities of IL-9 on the intestinal mucosa and found that IL-9 up-regulates not only the expression of goblet cell-associated genes, such as Muc2, mCLCA3/gob5, and Retnla, but also that of Paneth cell markers, such as Ang4, PLA2g2a, and cryptdins. These activities required the presence of a functional IL-13 or IL-4RA gene, as previously shown for IL-9 activities on lung epithelial cells (5). These observations suggest that Paneth cell hyperplasia represents one of the efferent arms of the TH2-driven immune response against intestinal pathogens such as nematode worms.

Several reports have highlighted the key role of IL-9 and IL-13 during nematode infections. Inhibition of IL-9 impaired Trichuris muris clearance (10), whereas IL-9 overexpression accelerated expulsion of T. muris or Trichinella spiralis (9, 33). Similarly, mice treated with anti-IL-13 Abs or deficient in IL-13 fail to expel T. muris (8, 34). However, the mechanism involved in worm clearance remains controversial, and might depend on the nematode species. For T. muris, mast cells and eosinophils seem dispensable, since anti-c-kit treatment and ablation of IL-5 have no effect on development of protective immunity (35). By contrast, mast cells are required for T. spiralis expulsion. In this model, mast cells increase epithelial permeability by producing proteases such as mMCP-1, a process promoted by IL-9 (24, 36). Other antinematode effector mechanisms modulated by IL-9 or IL-13 include increased epithelial cell turnover acting like an epithelial esculator to expel Trichuris parasites (11) and jejunal muscle hypercontractility (37). Paneth cell hyperplasia has also been reported during T. spiralis infection, and this process seemed T cell-dependent (13), but the role of IL-9 and IL-13 in this process had not been described so far. The up-regulation of Paneth cell-derived antimicrobial products, including cryptdins, Ang4, and PLA2g2a might therefore directly contribute to the anti-parasite response. Interestingly, Retnla can also participate to nematode clearance, although this potential antimicrobial protein is preferentially produced by goblet cells and is up-regulated by IL-9 and IL-13 in both lungs and colon. This gene is also up-regulated during helminth infections (38), and could participate to the immune response by its profibrotic activity and tissue remodeling or by increasing smooth muscle cell proliferation (31, 39, 40).

So far, there is no direct evidence that Paneth cell-derived products such as cryptdins, Ang4, and PLA2g2a possess nematode killing activity. Cryptdins are particularly potent against bacteria such as Escherichia coli (41), but their potential cytotoxicity against parasites has not been assessed. However, another human defensin was recently shown to provoke membrane pore formation in Trypanosoma cruzi parasites (42). Ang4 belongs to the RNase A family and has both angiogenic and microbialic activities (22, 23). Ang4 preferentially kills Gram-positive bacteria by unknown mechanisms and its activity on nematodes has not been tested. However, another member of this RNase family, eosinophil cationic protein or RNase 3, has been characterized as a membrane-disruptive cationic toxin with nonspecific toxicity to bacteria, helminthes, and various eukaryotic cellular targets (43).

PLA2g2a also shows antimicrobial properties (27, 44). PLA2g2a catalyzes the hydrolysis of glycerophospholipids but has a preference for anionic phosphatidylglycerol, conferring specificity for the bacterial cell membranes, usually rich in phosphatidylglycerol (26, 45). The large number of cationic residues of PLA2g2a confers to this enzyme a global positive charge, allowing its penetration into the negatively charged bacterial cell wall (27). Another function of proteins from the PLA2 family consists in producing free fatty acids, such as arachidonic acid and lysophospholipids, which are the respective precursors of eicosanoids and platelet-activating factor. Before being secreted, PLA2g2a can promote the release of such mediators of inflammation (46–48). However, this function is mainly attributed to cytosolic PLA2s from group IV subfamily, such as PLA2g4c, which is also up-regulated by IL-9 in the intestinal mucosa (28). So, both PLA2g2a and PLA2g4c can mediate the production of eicosanoid progenitors in response to IL-9 and IL-13. In contrast, PLA2g4c could contribute to the exocytosis of granules containing antimicrobial products by its lysophospholipase and transacylase properties, which mediate phospholipid remodeling and vesicular trafficking (49–52). Finally, PLA2g4c might participate in PLA2g2a induction like another cytosolic PLA2; PLA2g4a, which was shown to be required for PLA2g2a up-regulation by TGF-α and IL-1 in rat gastric mucosal cells (48).

Kinetics experiments show that a few days of IL-9 exposure are sufficient to induce an up-regulation of Paneth cell markers in the colon mucosa, and that this observation correlates with IL-9-induced mast cell expansion in this organ. As IL-9 induces IL-13 expression by mast cells and that this cell population is expanded in the intestinal mucosa of IL-9 transgenic mice (5–7), it is likely that these cells are at least partly responsible for this IL-13-dependent activity of IL-9 on Paneth cells. T lymphocytes represent another source of IL-9-induced IL-13 in these mice, although this population was not expanded in the intestinal mucosa of IL-9 transgenic mice (data not shown). Further studies will be needed to unravel the molecular mechanisms by which IL-13 promotes Paneth cell differentiation and hyperplasia.

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References


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