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Interleukin-15 and NK1.1$^+$ Cells Provide Innate Protection against Acute *Salmonella enterica* Serovar Typhimurium Infection in the Gut and in Systemic Tissues$^\nabla$

Ali A. Ashkar,[1]†* Sarah Reid,[1] Elena F. Verdu,[2] Kun Zhang,[3] and Brian K. Coombes[3]†*

Department of Pathology and Molecular Medicine,[1] Department of Medicine,[2] and the Michael DeGroote Institute for Infectious Disease Research, Department of Biochemistry and Biomedical Sciences,[3] McMaster University, Hamilton, ON L8N 3Z5, Canada

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Control of bacterial colonization at mucosal surfaces depends on rapid activation of the innate immune system. Interleukin-15 (IL-15) directs the development, maturation, and function of a population of cells positive for NK1.1, such as natural killer (NK) cells, which are critical components of the innate immune defense against several viral and bacterial pathogens. Using IL-15-deficient mice, in vivo depletion of NK1.1$^+$ cells from wild-type mice, and in vivo overexpression of IL-15 from a recombinant adenovirus, we tested the role of IL-15 and NK1.1$^+$ cells in innate protection of the murine gut and reticuloendothelial system from *Salmonella enterica* serovar Typhimurium infection. IL-15 and the NK1.1$^+$ cell population provided innate protection from serovar Typhimurium in mice at the enteric mucosae and in the reticuloendothelial system during murine typhoid. Interestingly, serovar Typhimurium extensively colonized the gut of IL-15$^{-/-}$ mice and wild-type C57BL/6 mice depleted of NK1.1$^+$ cells prior to infection, even though the animals were not pretreated with antibiotics to reduce colonization resistance and there was an absence of overt inflammation in the colon and cecum. Enhanced dissemination of *Salmonella* from the gut of mice depleted of NK1.1$^+$ cells correlated with a localized disruption of IL-17 in the colon. These data suggest a relationship between the gut ecosystem and the innate mucosal immune system, which may be linked via IL-15 and NK1.1$^+$ cells.

Drug resistance continues to erode the efficacy of conventional antibiotics against many serious bacterial pathogens, contributing to a doubling of the death rate due to infectious disease in the past two decades (33). This problem is particularly germane to *Salmonella enterica* serovar Typhimurium infections, where multidrug resistance has been on the rise globally for almost a decade (10). These gram-negative bacteria infect humans using virulence factors that promote invasiveness and immune system avoidance (8). A notable concern is the multidrug-resistant strain of serovar Typhimurium DT104, which has emerged in North America (and globally) as a serious threat to public health due to its association with increased morbidity (31) and higher rates of death (18, 19).

One approach to bridge the widening innovation gap in anti-infectives involves harnessing innate immune defenses of the susceptible host. Although this strategy is in its infancy, the potential revolutionary impact for the treatment of infectious diseases and for improving human and animal health has called for a critical exploration of its potential (35). The thinking behind this paradigm is that immune enhancement could reduce reliance on traditional antibiotic therapies, thus preserving their useful life span and engendering a more resilient host environment for pathogenic microbes.

The vast majority of pathogens, including *Salmonella*, enter hosts and initiate infection at mucosal surfaces. Besides physical barriers limiting the ability of microbial pathogens to attach and transit through epithelial cells, the inducible innate immune response is a central first line of defense, comprising many cell types and cytokines, of which NK cells and interleukin-15 (IL-15) are of particular interest. NK cells are well known for their early innate contribution to antiviral defenses and their ability to kill tumor cells without prior exposure. The development, maturation, and function of these cells are dependent on IL-15, identified for its ability to stimulate proliferation of the IL-2-dependent CTLL-2 T-cell line in the presence of neutralizing anti-IL-2 antibodies (4). IL-15 is produced by a variety of myeloid and stromal cell types (2, 5, 9, 22) and is required for proper NK and NKT cell development and function. Mice lacking IL-15 or IL-15 receptor alpha subunit have no NK cells and few NKT cells (23, 29). Overexpression of IL-15 in IL-15-transgenic mice leads to an increase in NK cells and NK-cell-derived gamma interferon (IFN-γ), which is associated with resistance to tumors (47), *Mycobacterium bovis* (44), *Listeria monocytogenes* (46), and toxic shock induced by *Escherichia coli* (20). In humans, IL-15 is more prominently elevated in those suffering from systemic salmonellosis than in those suffering from infection localized strictly to the gastrointestinal tract (32). Previous work using a mouse model of infection showed that IL-15 was implicated in the host defense

* Corresponding author. Mailing address for Brian K. Coombes: Biochemistry & Biomedical Sciences, McMaster University, HSC-4H17, 1200 Main St. West, Hamilton, Ontario L8N 3Z5, Canada. Phone: (905) 525-9140, ext. 22159. Fax: (905) 522-9033. E-mail: coombes@mcmaster.ca. Mailing address for Ali Ashkar: Pathology & Molecular Medicine, McMaster University MDCL-4015, 1200 Main St. West, Hamilton, ON L8N 3Z5, Canada. Phone: (905) 525-9140. Fax: (905) 522-6750. E-mail: ashkara@mcmaster.ca.
† Contributed equally.
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against the swine pathogen *Salmonella enterica* serovar Choleraesuis (21). However, an avirulent strain was used in these studies, and so it was not possible to assess the role of this cytokine in defense against virulent microbes that can actively subvert innate immunity (8). The murine model of *Salmonella* colitis employs an antibiotic pretreatment regimen prior to infection that produces a qualitative shift in the gut microbiota in favor of the *Firmicutes* and *Cytophaga-Flavobacterium-Bacteroidetes* phyla (41), the effect of which is reduced innate colonization resistance of the animal. The ability of *Salmonella* serovar Typhimurium to colonize the gut of antibiotic-pretreated animals is enhanced by the ensuing inflammatory process, leading to colitis and typhlitis (1). It is also thought that this underlying inflammation is necessary to allow serovar Typhimurium to outcompete the normal microbiota and colonize the gut to high levels (43). However, whether IL-15 and/or NK cells are important in innate protection of the nonperturbed gut (absence of antibiotic treatment) and systemic tissues following infection with virulent serovar Typhimurium has not been studied. We used a well-characterized model of murine typhoid to study the role of IL-15 and NK1.1$^+$ cells in innate microbial defense against serovar Typhimurium. Three different experimental approaches, involving IL-15 knockout mice, in vivo depletion of NK1.1$^+$ cells, and in vivo overexpression of IL-15, provide evidence that IL-15 and NK1.1$^+$ cells are required for innate control of *Salmonella* infection in the gut and at systemic sites of infection targeted by this pathogen.

MATERIALS AND METHODS

Bacterial strains and manipulations. Wild-type *Salmonella enterica* serovar Typhimurium strain SL1344 was used throughout this study. Bacteria were routinely cultured in Luria broth (LB) supplemented with streptomycin at 50 μg/ml. Prior to infection, SL1344 was cultured overnight in LB with shaking at 225 rpm. Overnight cultures were washed in 100 mM HEPES (pH 8.0)–0.9% NaCl and then resuspended in the same buffered solution.

Animal experiments. Experiments were performed in accordance with protocols approved by the Animal Ethics Committee at McMaster University and by the Canadian Council on Animal Care. Mice were housed under specific-pathogen-free conditions in a level II barrier facility. Female C57BL/6 (B6) mice or IL-15$^{-/-}$ mice (Taconic) were infected orally with 0.5×10^7 to 1×10^7 CFU of serovar Typhimurium suspended in 0.1 ml of 100 mM HEPES (pH 8.0)–0.9% NaCl. At 48 to 72 h after infection, the colon, cecum, spleen, and liver were collected separately into 1 ml of sterile phosphate-buffered saline (PBS) on ice and homogenized (MixerMill 400; Retsch). Spleen and liver tissue homogenates were diluted in PBS and plated on LB agar containing streptomycin for determination of total *Salmonella* CFU. Tissue homogenates from the cecum and the colon were plated on bismuth sulfite agar. Plates were incubated at 37°C, and CFU were enumerated after 16 h.

NK1.1$^+$-cell depletion experiments. Seven- to 9-week old female C57BL/6 mice were injected intraperitoneally with 200 μg of anti-mouse NK1.1 antibody (PK136 mouse immunoglobulin G2a hybridoma HB191; ATCC) daily for 2 days prior to challenge. On day 3 after the first injection, mice were infected by oral gavage with 5×10^6 CFU serovar Typhimurium and sacrificed on day 2 postinfection. To verify NK1.1$^+$-cell depletion, half of the spleen was used for fluorescence-activated cell sorting (FACS) analysis of splenocytes (see below) and the other half was used for bacterial load determinations. Cecum and colon tissues were also harvested for bacterial enumeration. For cytokine determination experiments, two groups of four B6 mice were depleted of NK1.1$^+$ cells as described above and were inoculated with serovar Typhimurium or sterile buffer. As a control, two groups of B6 mice were treated identically but were left NK1.1$^+$ cell replete. The small intestine and colon were collected at 48 h after infection, and the feces were removed. Tissues were flash frozen in liquid nitrogen at the site of surgery and then homogenized in 1 ml PBS containing protease inhibitor cocktail. Tissue homogenates were centrifuged, and murine IL-17 and IFN-γ were measured in the supernatant using a DuoSet enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems) according to the manufacturer's instructions.

In vivo expression of IL-15 using recombinant adenovirus. A recombinant adenovirus expressing human IL-15 was generated in the parental virus, Ad-d170.3, obtained from the Robert E. Fitzhenry Vector Laboratory (Centre for Gene Therapeutics, Hamilton, Canada). Seven- to nine-week-old female C57BL/6 mice were administered 5×10^8 PFU of recombinant adenovirus (Ad-IL-15) or wild-type virus (Ad) by intravenous tail injection on days 1 and 4. Mice were challenged on day 5 with 5×10^6 CFU of serovar Typhimurium and sacrificed on day 7. For IL-15 cytokine analysis, blood samples were collected from mice at the time of sacrifice and serum was isolated after 24 h at 4°C. IL-15 levels were quantified using a hIL-15 DuoSet ELISA kit (R&D Systems) according to the manufacturer's instructions.

FACS. Spleens were isolated from mice, and single-cell suspensions were prepared. Splenocytes were enumerated using a hemacytometer and resuspended in 0.2% bovine serum albumin-PBS for FACS analysis. Cells were plated at 1×10^6 per well in 96-well round-bottom plates. Each well was washed and blocked using CD16/CD32 antibody (eBioscience) for 15 min on ice. Cells were then washed and surface stained for 30 min on ice with phycoerythrin-conjugated anti-mouse NK1.1 antibody (clone PK136; BD Pharmingen). Stained cells (100,000 gated events) were analyzed on a FACSCanto flow cytometer. All FACS data was analyzed using the FlowJo flow cytometry analysis software program (Tree Star Inc., Ashland, OR).

Murine histopathology. Colons and ceca from experimental animals were fixed in paraformaldehyde and transferred to 70% ethanol until processed for paraffin embedding. The samples were sectioned at 5 μm and stained with hematoxylin and eosin. For pathological scoring, six fields per sample were examined and scored as described previously to facilitate comparisons (6). This scoring system was as follows: for lumen tissue, sum of empty, score = 0; necrotic epithelial cells, scant = 1, moderate = 2, and dense = 3; and polymorphonuclear cells (PMNs), scant = 2, moderate = 3, and dense = 4; for surface epithelium, sum of no pathological change, score = 0; regenerative change, mild = 1, moderate = 2, and severe = 3; desquamation, patchy = 1, diffuse = 2; for PMNs in epithelium, score = 1; ulceration, score = 1; for mucosa, sum of no pathological change, score = 0; crypt abscesses, rare (<15%) = 1, moderate (15% to 50%) = 2, and abundant (>50%) = 3; presence of mucinous plugs = 1; and presence of granulation tissue = 1; for submucosa, sum of no pathological change = 0; mononuclear cell infiltrate, 1 small aggregate = 0, more than one aggregate = 1, and large aggregates plus increased single cells = 2; PMN infiltrate, none = 0, single = 1, and aggregates = 2; and edema, mild = 0, moderate = 1, and severe = 2.

Statistical tests. Survival plots were analyzed using the log-rank (Mantel-Cox) test. Bacterial loads were expressed as the means with standard errors and compared between groups using a two-tailed Mann-Whitney nonparametric test. *P* values of <0.5 were considered significant.

RESULTS

IL-15$^{-/-}$ mice are more susceptible to *Salmonella* infection than wild-type animals. IL-15 is a pleiotropic cytokine involved in innate immune protection against some bacterial and viral infections. However, the role of IL-15 in innate protection against serovar Typhimurium infection is not known. We began our work using the well-characterized model of murine salmonellosis, which offers the ability to examine infection outcomes at both enteric and systemic sites. IL-15$^{-/-}$ mice and congenic wild-type mice (B6) were infected with wild-type serovar Typhimurium by oral gavage and monitored following infection. All IL-15$^{-/-}$ mice infected with serovar Typhimurium died by day 4 after infection (Fig. 1A), with a median survival of 3.5 days, whereas wild-type mice had a prolonged survival time after infection compared to IL-15$^{-/-}$ mice ($P = 0.0084$, log rank, Mantel-Cox). The median survival time of 6.5 days for infected B6 mice was characteristic of the infection dynamic for wild-type serovar Typhimurium, suggesting that mice deficient in IL-15 had a defect in innate control of early pathogen colonization. To test this, the bacterial load in the spleen and liver was measured 2 days after infection and was

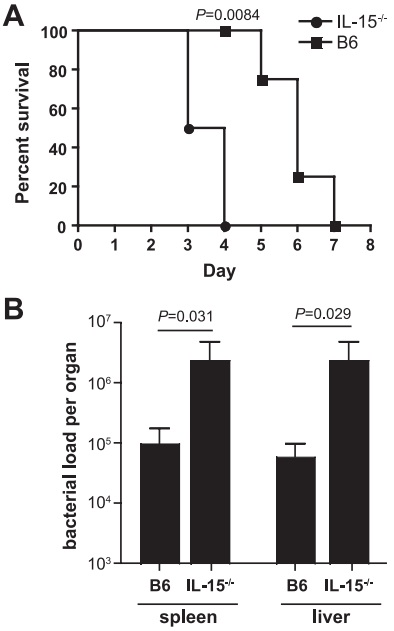

FIG. 1. IL-15$^{-/-}$ mice are more susceptible to *Salmonella* infection. (A) Survival plots of wild-type mice (B6) and IL-15 knockout mice (IL-15$^{-/-}$) ($n = 8$ per group) infected with serovar Typhimurium by the oral route (for mean survival time for IL-15$^{-/-}$ mice versus that for B6 mice, $P = 0.0084$. (B) Bacterial loads in the spleen and liver of B6 mice and IL-15$^{-/-}$ mice ($n = 8$ per group). Data are the means with standard errors from two separate experiments (B6 versus IL-15$^{-/-}$ mice: spleen, $P = 0.031$; liver, $P = 0.029$.

found to be ~50 times higher in IL-15$^{-/-}$ mice than in B6 mice (Fig. 1B).

Serovar Typhimurium reaches high bacterial load in gut of IL-15-deficient mice. In the absence of antibiotic pretreatment, serovar Typhimurium reaches a low bacterial density in the murine cecum and colon due to innate colonization resistance and infection leads to scant inflammatory response in gut tissues (1). The increased systemic bacterial load in IL-15$^{-/-}$ mice prompted us to examine the role of IL-15 in innate protection at the gut mucosae. For these experiments, we orally infected specific-pathogen-free non-antibiotic-treated IL-15$^{-/-}$ mice and B6 mice and determined bacterial loads in the cecum and colon 48 h postinfection. In accord with previous data (1, 7), wild-type mice that were not pretreated with antibiotics prior to infection had low-level colonization of enteric tissues (Fig. 2A). In contrast, IL-15$^{-/-}$ mice had a ~1,000-fold increase in the bacterial load in the cecum and colon ($P = 0.0159$) (Fig. 2A). This greater bacterial colonization of the gut was not accompanied by overt inflammation in either the colon or the cecum (Fig. 2B). The sum inflammatory scores were similar for both B6 mice and IL-15$^{-/-}$ mice in the cecum (2.0 ± 1.2 and 2.3 ± 0.8, respectively) and colon (2.8 ± 0.8 and 3.4 ± 0.5, respectively). Using a similar scoring matrix, these values were comparable to those reported previously for *Salmonella*-infected B6 mice not treated with antibiotic prior to infection (1). For comparison, inflammatory scores following *Salmonella* infection of antibiotic-pretreated animals are ~20 using similar metrics (1, 6, 7). The overall structure of gut tissues from infected mice with extensive bacterial loads was preserved, with little to no infiltration of polymorphonuclear cells and mononuclear cells in the lumen, epithelium, and submucosa (Fig. 2C). These data indicated that IL-15 participates in innate control of *Salmonella* colonization of the gut and that in this genetic background, inflammation is not required for serovar Typhimurium to overcome colonization resistance as reported for wild-type animals (30, 43).

NK1.1$^+$ cells are a major effector-cell population controlling acute salmonellosis. IL-15 is a key mediator of NK1.1$^+$-cell development and function (11). NK cells are a source of IFN-γ during *Salmonella* infection of streptomycin-pretreated mice (17), but the role of NK1.1$^+$ cells in protecting the murine gut without prior antibiotic treatment has not been studied. To examine the role of NK1.1$^+$ cells in controlling early stages of serovar Typhimurium infection, we depleted NK1.1$^+$ cells from wild-type C57BL/6 mice using a depleting monoclonal antibody and measured cecal pathology, splenic bacterial load, and colonization of the cecum and colon following oral infection. We first verified NK1.1$^+$-cell depletion in our experimental animals by quantifying NK1.1-positive splenocytes at necropsy. FACS analysis of the splenocyte population showed an expected number of NK1.1$^+$ cells (4.38%) from B6 mice (Fig. 3A) and a reduction of this proportion to 0.15% in NK1.1$^+$-cell-depleted animals (Fig. 3B). To examine the role of the NK1.1$^+$ cell population in early colonization dynamics, we quantified the bacterial load in the spleen and in the gut 48 h postinfection. Similar to what we observed for IL-15-deficient mice, depletion of NK1.1$^+$ cells resulted in a ~100-fold increase in numbers of *Salmonella* serovar Typhimurium bacteria in the spleen (Fig. 3C) and a ~500-fold increase in numbers in both the cecum and the colon (Fig. 3D).

Delivery of a recombinant adenovirus expressing IL-15 enhances innate resistance and delays systemic colonization by *Salmonella* serovar Typhimurium. The previous experiments with IL-15$^{-/-}$ animals and NK1.1$^+$-cell-depleted B6 mice provided evidence for IL-15 and NK cells in providing innate protection from *Salmonella* infection both in the gut and in systemic tissues. Based on this, we reasoned that overexpression of IL-15 above endogenous levels in wild-type mice should provide additional protection from early colonization by *Salmonella*, which it did. To do this, we delivered to mice a recombinant nonreplicating adenovirus expressing human IL-15 (Ad-IL-15) or a control virus not expressing IL-15 (Ad) and then infected the animals with *Salmonella* by oral gavage. As expected, delivery to mice of Ad-IL-15 but not of Ad alone increased the proportion of NK1.1-positive cells in the spleen (Fig. 4A and B) and led to enhanced serum IL-15 levels (Fig. 4C). To test whether this increased IL-15 and NK1.1-positive cell population affected bacterial colonization, we quantified the bacterial load in systemic tissues and in the gut 2 days after infection, a time point corresponding to maximal NK cell numbers and circulating IL-15. Mice treated with Ad-IL-15 had a significantly lower bacterial load in the spleen and liver at 2 days postinfection (Fig. 4D) than mice treated with the Ad control. Treatment with Ad-IL-15 prior to *Salmonella* infection also reduced the bacterial load in the cecum by approximately 1,000-fold, to levels that were near the limit of detection of our experimental setup (Fig. 4E). These data corroborate a role for IL-15 and NK1.1$^+$ cells in providing innate protection from

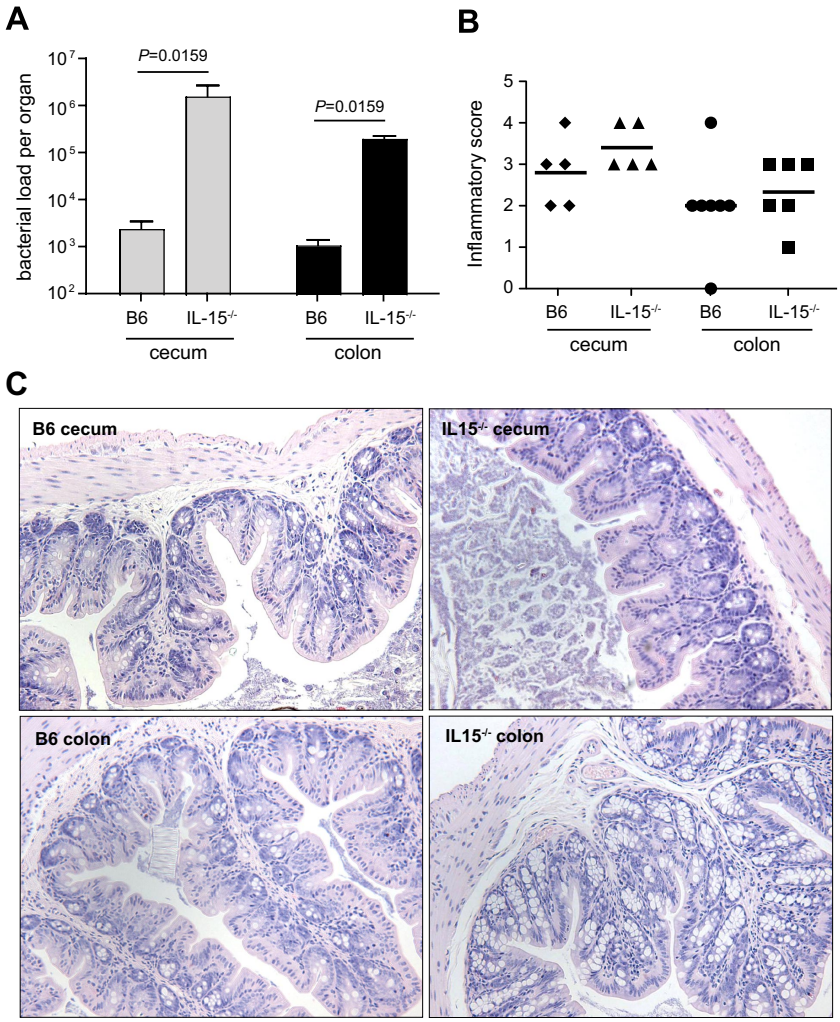

FIG. 2. Serovar Typhimurium colonizes the gut of IL-15-deficient mice in the absence of inflammation. (A) Bacterial load in the cecum and colon of wild-type B6 mice and IL-15$^{-/-}$ mice at day 3 after oral infection ($n = 8$ per group). Data are the means with standard errors from two separate experiments (B6 versus IL-15$^{-/-}$ mice: cecum, $P = 0.0159$; liver, $P = 0.0159$). (B) Immunological scores in the cecum and colon from mice colonized with *Salmonella* serovar Typhimurium. Ceca and colons were harvested from infected mice at day 3 after infection and processed for histopathological examination as outlined in Methods. Data are aggregate scores of pathology from five tissue sections from five to seven individual mice per group. Each data point represents an individual animal and shows the scatter of data, with horizontal lines representing the means. (C) Sections of cecum and colon from infected mice stained with hematoxylin and eosin. Representative images are shown from those animals scored in panel B.

serovar Typhimurium infection at systemic and enteric sites targeted by this pathogen.

Enhanced dissemination of *Salmonella* in NK1.1$^+$-cell-depleted mice correlates with blunted IL-17 response in the colon. Previous work with rhesus macaques demonstrated that an IL-17 deficiency induced by chronic viral infection promotes dissemination of *Salmonella* from the gut. To test whether the enhanced dissemination of *Salmonella* from the gut that we observed in NK1.1$^+$-cell-depleted B6 mice was related to IL-17, we depleted two groups of B6 mice of NK1.1$^+$ cells and inoculated them with serovar Typhimurium or left them uninfected. Nondepleted B6 mice were used as controls and treated identically. In the absence of NK1.1$^+$-cell depletion, *Salmonella* infection resulted in an increase in IL-17 and IFN-γ in the small intestine, consistent with previous data (Fig. 5A and B) (38). In mice uninfected but depleted of NK1.1$^+$ cells, we noted a marked increase in basal IL-17 levels compared to results for control mice that were not depleted of NK1.1$^+$ cells, suggesting that NK1.1$^+$ cells may serve to negatively regulate IL-17 production in the gut. IL-17 protein levels in the small intestine did not change appreciably at 48 h following *Salmonella* infection in mice depleted of NK1.1$^+$ cells. However, in the colon of mice depleted of NK1.1$^+$ cells, this increase in IL-17 was normalized following infection with *Salmonella* (Fig. 5C) and reached undetectable levels in two infected mice. IFN-γ levels in the colon or small intestine did not correlate with bacterial loads, consistent with previous data showing that regional IFN-γ production in the gut is insufficient for control of *Salmonella* infection (13). Together, these data suggest that the enhanced dissemination of *Salmonella* from the gut of NK1.1-depleted mice may be related to a localized IL-17 deficiency in the colon following *Salmonella* infection.

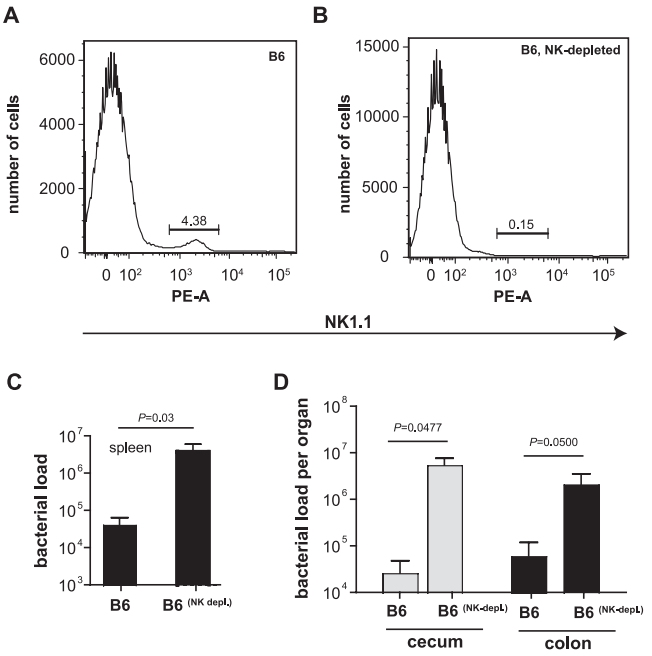

FIG. 3. NK1.1$^+$ cells are a major effector-cell group during acute salmonellosis. Analysis of NK1.1$^+$ cells from B6 mice (A) or NK-cell-depleted mice (B). The percentage of NK1.1$^+$ cells from the analyzed population is shown in each panel. (C) Bacterial loads in the spleens of wild-type mice (B6) and NK1.1$^+$-cell-depleted mice infected with serovar Typhimurium for 2 days ($n = 5$ per group). Data are the means with standard errors from two separate experiments ($P = 0.03$). (D) Bacterial loads in the cecum and colon of wild-type mice (B6) or mice depleted of NK cells and then infected with *Salmonella* for 2 days ($n = 5$ per group). Data are the means with standard errors from two separate experiments (cecum, B6 versus B6$^{\text{NK-depleted}}$, $P = 0.0477$; colon, B6 versus B6$^{\text{NK-depleted}}$, $P = 0.05$).

DISCUSSION

With the steady increase in bacterial resistance to current antibiotics, modulation of innate immunity is viewed as a promising approach to the development of antimicrobial therapies (35, 12). Toll-like-receptor agonists and cationic host defense peptides have already shown promise as agents of protection in animal models of human infectious diseases (14, 15, 40). Alternatively, direct expansion of the effector-cell population responsible for innate protection stands to offer similar advantages (26). Tackling these issues with bacterial pathogens is challenging, because these microbes have evolved strategies to subvert innate host defenses (8). Animal models capable of capturing the nuances of both the microbial and host elements of this dynamic interaction are therefore essential. Our interest in this area relates to the potential role of infectious agents in the pathobiology of inflammatory bowel disease, particularly the contribution of enteric bacterial pathogens. By using knockout animals, in vivo depletion of immune cells, and targeted overexpression of IL-15 from a recombinant adenovirus, we have shown that IL-15 and the population of NK1.1$^+$ cells are key mediators of innate defense against serovar Typhimurium at mucosal surfaces and systemic sites targeted by this pathogen.

IL-15 is implicated in the pathogenesis of human inflammatory bowel diseases (IBDs), such as Crohn's disease, ulcerative colitis, and celiac disease. A greater percentage of peripheral blood mononuclear cells from patients with Crohn's disease or ulcerative colitis produced IL-15 than was the case with normal donors, which was further augmented in vitro following lipopolysaccharide stimulation (25). Similarly, IL-15 was detected in inflamed mucosal biopsies from IBD patients but not from controls (27, 39), supporting the hypothesis that IL-15-mediated processes may underlie the chronic inflammatory conditions of IBD. The finding that IL-15 contributed to murine intestinal inflammation induced by the chemical stimulant dextran sodium sulfate was consistent with this hypothesis (48), leading to the suggestion that antagonizing IL-15 may improve IBD outcomes. However, our data may moderate this idea, because although removal of IL-15 from the system can ameliorate the symptoms of chronic colitis by reducing inflammation (48), it may in fact bring about an increased susceptibility to infectious disease at the enteric mucosal surface. This work supports a model wherein IL-15 and NK1.1$^+$-cell defense systems participate in innate resistance to serovar Typhimurium in the gut. While it is likely that NK cells are the subset of NK1.1$^+$ cells participating in this innate pathway due to their increased numbers during *Salmonella* infection relative to NKT cells (3, 24), we cannot exclude at this point the involvement of NKT cells or NK1.1$^+$ $\alpha\beta$ T cells, which may participate in an IL-4–IL-12 axis via macrophages (34).

The mechanism of enhanced dissemination of *Salmonella* serovar Typhimurium from the gut to systemic tissues is an area of interest and appears to involve both bacterial (45) and host (38) factors. In antibiotic-pretreated *Il17ra*$^{-/-}$ mice, *Salmonella* dissemination from the gut to systemic tissues is enhanced (38). In mice with a nonperturbed commensal microbiota used in our experiments, a local IL-17 deficiency in the colon was noted following *Salmonella* infection of NK1.1$^+$-cell-depleted animals and reached undetectable levels in two animals in the group. Although the group mean IL-17 level in the colon was similar to that of infected, nondepleted animals, the overall change in colon IL-17 levels following infection was more pronounced in mice depleted of NK1.1$^+$ cells, which may account for the enhanced dissemination of *Salmonella* from the gut in these animals. In the gut, IFN-γ levels were generally low and did not correlate with bacterial load or enhanced dissemination of infection, which is consistent with data that systemic IFN-γ (36) but not local IFN-γ in the gut (13) correlated with enhanced control of *Salmonella* infection. The source of this IFN-γ in animals depleted of NK1.1$^+$ cells is likely the population of intraepithelial lymphocytes, although additional work is required to establish this and to understand the interplay between NK1.1$^+$ cells and IL-17 production in the gut following *Salmonella* infection. Interestingly, we saw a marked increase in IL-17 in the colon and small intestine of mice depleted of NK1.1$^+$ cells. A recent report noted that NK cell depletion exacerbated experimental collagen-induced arthritis in mice and that NK cell depletion increased the frequency of IL-17-secreting cells in draining lymph nodes (28). These data suggest a negative regulatory function for NK cells in modulating IL-17 levels during arthritis, and a similar relationship between NK cells and IL-17 may also exist in the gut based on our depletion experiments.

Innate colonization resistance is thought to result from the dense microbial community residing in the gut, providing com-

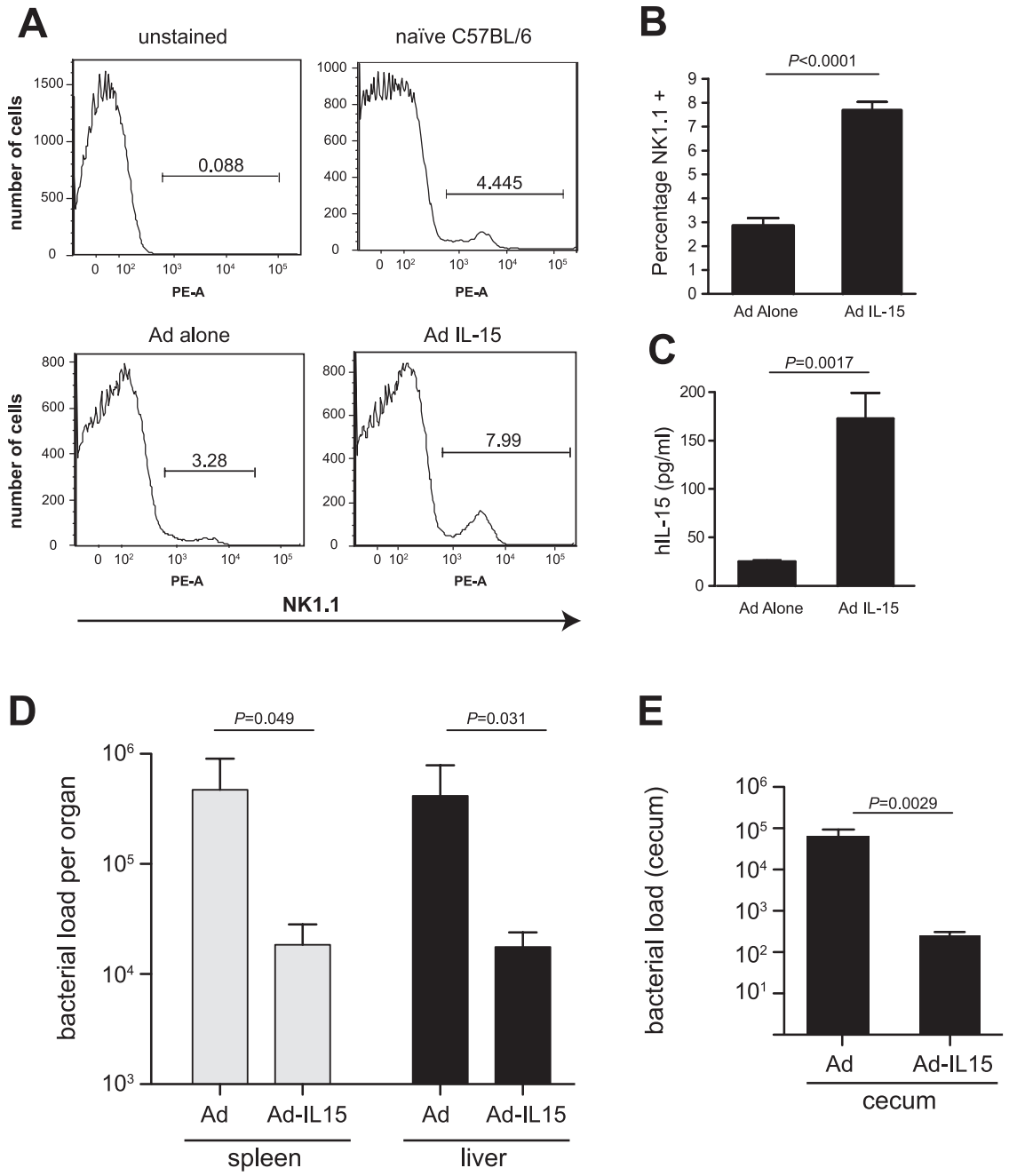

FIG. 4. Delivery of a recombinant adenovirus expressing IL-15 enhances mucosal and systemic innate defense against colonization by serovar Typhimurium. C57BL/6 mice were administered 5×10^8 PFU of adenovirus (Ad) alone,or recombinant adenovirus expressing human IL-15 (Ad IL-15) via intravenous tail vein injection as described in Materials and Methods. Mice were challenged with serovar Typhimurium and then sacrificed on day 2 postinfection. (A) FACS analysis of splenocytes isolated from naive mice or mice receiving injections of either Ad or Ad IL-15 and stained for the NK-cell marker NK1.1. (B) Average percentages of $NK1.1^+$ populations in Ad alone-treated and Ad-IL-15-treated mice (five mice per group) were calculated from FACS data ($P < 0.0001$ for $NK1.1^+$ cells from Ad-alone-treated mice versus those from mice receiving Ad-IL-15). (C) Serum from mice treated with Ad alone or with Ad -L-15 was analyzed by ELISA for human IL-15 (hIL-15). Data shown are the means with standard errors from five mice per group ($P = 0.0017$). (D) Bacterial load in the spleen and liver from mice pretreated with either Ad or Ad-IL-15 prior to infection with serovar Typhimurium ($n = 5$ per group). Data are mean with standard errors from five mice per group (Ad alone versus Ad-IL-15: spleen, $P = 0.049$; liver, $P = 0.031$). (E) Bacterial load in the cecum from mice pretreated with either Ad or Ad-IL-15 prior to infection with serovar Typhimurium ($n = 5$ per group). Data are means with standard errors from five mice per group (Ad alone versus Ad-IL-15: $P = 0.0029$).

peting conditions for invading pathogens (42). However, the molecular basis of colonization resistance is still poorly understood. Previous work revealed that manipulating the ecology of the gut with antibiotics prior to infection leads to a loss of colonization resistance, high bacterial loads of *Salmonella* in the cecum and colon, and colitis (1, 7, 16). More recently it has been suggested that inflammation is necessary and sufficient for enteropathogenic bacteria to successfully compete with the

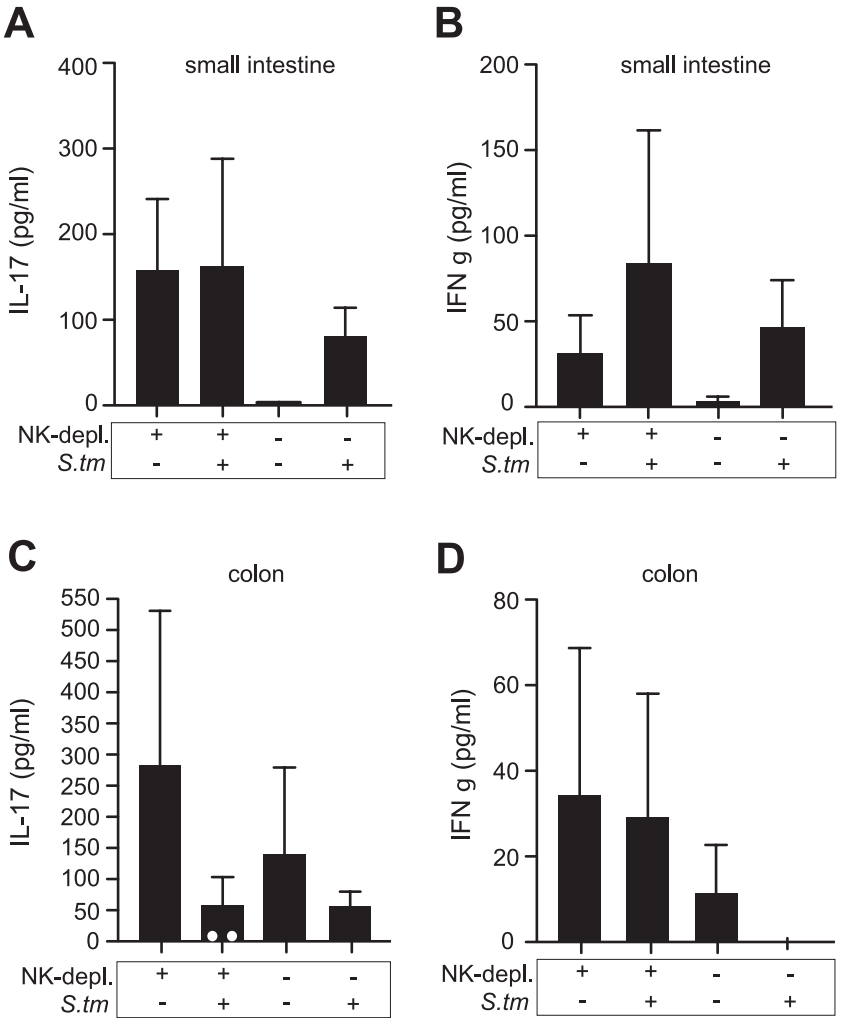

FIG. 5. A marked decrease in colon IL-17 following infection of NK1.1-depleted mice correlates with enhanced bacterial dissemination from the gut. Two groups of B6 mice were depleted of NK1.1 cells and then infected orally with *Salmonella* or left uninfected. Two groups of control mice were not depleted of NK1.1 cells but otherwise were treated identically. At 48 h following infection, IL-17 levels (A and C) or IFN-γ levels (B and D) were measured in the small intestine and colon using an ELISA. Data are the means with standard errors. White circles in panel C denote two animals with undetectable levels of IL-17.

microbiota and overcome colonization resistance (43). However, *Salmonella* serovar Typhimurium colonized the gut of IL-15$^{-/-}$ mice and wild-type mice depleted of NK1.1^{+} cells just prior to infection in the absence of inflammation. Although evidence from the literature supports a link between the microbiota and innate defenses in maintaining intestinal homeostasis and ergo innate resistance mechanisms, the details of this cross talk are not fully understood. Of relevance, the probiotic *Lactobacillus casei* elicited murine and human NK cell activities and augmented IL-15 gene expression in mucosal epithelial cells (37), suggesting that the microbiota can influence NK cell and IL-15 activities. The relationship between the microbiota and the IL-15-directed innate defenses reported here is the subject of follow-up studies ongoing in our laboratory. In light of the protective role we have identified for IL-15 and NK1.1^{+} cells following acute serovar Typhimurium infection, further studies to quantify the cytokine networks and cellular components of the inflammatory response to infectious triggers following chemical or genetic perturbation in the gut are warranted. Our work here has identified two new candidates, IL-15 and NK1.1-positive cells, that would be expected to provide innate protection in these models.

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