Innate Lymphoid Cells Promote Anatomical Containment of Lymphoid-Resident Commensal Bacteria

Gregory F. Sonnenberg *et al.*

Science **336**, 1321 (2012);
DOI: 10.1126/science.1222551

interface might disturb placental development or function, dysregulation of this pathway might also contribute to a variety of pregnancy complications. Conversely, altered chemokine silencing may influence the susceptibility of the decidua to infection. More generally, however, our results demonstrate that genes encoding Th1/Tc1–attracting chemokines are subject to epigenetic regulation in tissue stromal cells and that such regulation can significantly influence a tissue's capacity for T cell accumulation. This demonstration raises questions regarding how the repressive H3K27me3 histone mark is targeted to select chemokine genes and whether related pathways control T cell access to the stroma of infected, autoimmunity-afflicted, or cancer-bearing tissues.

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Acknowledgments: We thank S. K. Dey and K. Johnson for advice, and A. Frey and J. Ernst for comments on the manuscript. The Histopathology and Vaccine and Cell Therapy core facilities of the New York University Cancer Institute provided histology services and tetramer reagents and were supported by NIH, National Cancer Institute (P30CA016087). P.N., E.T., and C.-S. T. performed experiments; P.A. and D.E.L. provided critical expertise and reagents; P.N. and A.E. analyzed data; and P.N. and A.E. designed experiments and wrote the manuscript. The data reported in the manuscript are tabulated in the main paper and in the supplementary materials. This work was supported by grants from NIH (RO1AI062980) and the American Cancer Society to A.E. (RSG-10-158-01-LIB).

Supplementary Materials

www.sciencemag.org/cgi/content/full/336/6086/1317/DC1
Materials and Methods
Figs. S1 to S7
References (*20*–*29*)

3 February 2012; accepted 24 April 2012
10.1126/science.1220030

Innate Lymphoid Cells Promote Anatomical Containment of Lymphoid-Resident Commensal Bacteria

Gregory F. Sonnenberg,[1] Laurel A. Monticelli,[1] Theresa Alenghat,[1] Thomas C. Fung,[1] Natalie A. Hutnick,[2] Jun Kunisawa,[3,4] Naoko Shibata,[3,4] Stephanie Grunberg,[1] Rohini Sinha,[1] Adam M. Zahm,[5] Mélanie R. Tardif,[6] Taheri Sathaliyawala,[7] Masaru Kubota,[7] Donna L. Farber,[7] Ronald G. Collman,[8] Abraham Shaked,[9] Lynette A. Fouser,[10] David B. Weiner,[2] Philippe A. Tessier,[6] Joshua R. Friedman,[5] Hiroshi Kiyono,[3,4,11] Frederic D. Bushman,[1] Kyong-Mi Chang,[8,12] David Artis[1,13]*

The mammalian intestinal tract is colonized by trillions of beneficial commensal bacteria that are anatomically restricted to specific niches. However, the mechanisms that regulate anatomical containment remain unclear. Here, we show that interleukin-22 (IL-22)–producing innate lymphoid cells (ILCs) are present in intestinal tissues of healthy mammals. Depletion of ILCs resulted in peripheral dissemination of commensal bacteria and systemic inflammation, which was prevented by administration of IL-22. Disseminating bacteria were identified as *Alcaligenes* species originating from host lymphoid tissues. *Alcaligenes* was sufficient to promote systemic inflammation after ILC depletion in mice, and *Alcaligenes*-specific systemic immune responses were associated with Crohn's disease and progressive hepatitis C virus infection in patients. Collectively, these data indicate that ILCs regulate selective containment of lymphoid-resident bacteria to prevent systemic inflammation associated with chronic diseases.

Colonization of the mammalian gastrointestinal tract by commensal bacteria is essential for promoting normal intestinal physiology (*1*–*3*). In healthy mammals, commensal bacteria are anatomically restricted to either the intestinal lumen, the epithelial surface, or within the underlying gut-associated lymphoid tissues (GALTs) (*1*–*5*). Anatomical containment is essential to limit inflammation and maintain normal systemic immune cell homeostasis (*1*, *2*). Loss of containment and subsequent dissemination of commensal bacteria to peripheral organs promotes inflammation and is a hallmark of multiple chronic human infectious and inflammatory diseases, including progressive HIV infection, hepatitis virus infection, and inflammatory bowel disease (*6*–*10*). Therefore, understanding the pathways that promote anatomical containment of commensal bacteria and prevent systemic inflammation may provide targets for treatment and prevention of chronic human diseases.

Studies in murine models identified a critical role for the cytokine interleukin-22 (IL-22) in regulating intestinal immunity, inflammation, and tissue repair (*11*, *12*). $CD4^+$ T cells and innate lymphoid cells (ILCs) are sources of IL-22 (*11*–*14*); however, whether T cell– or ILC-derived IL-22 contributes to the anatomical containment of commensal bacteria and prevention of systemic inflammation in the steady state has not been investigated. To address this issue, we sought to identify the IL-23–responsive cell populations that express IL-22 in intestinal tissues and GALTs of healthy human donors (see supplementary materials and methods). After ex vivo stimulation with recombinant (r) IL-23, a population of $IL\text{-}22^+$ cells was found in intestinal samples from healthy human donors that lacked expression of lineage markers CD20, CD56, and CD3 (Fig. 1A) and was $CD127^+$, CD45-intermediate ($CD45^{INT}$), and $ROR\gamma t^+$ (Fig. 1B), a phenotype consistent with ILCs in humans (*11*, *14*). $IL\text{-}22^+$ cells in the mesenteric lymph node (mLN) of healthy human donors also exhibited an ILC phenotype (fig. S1, A and B). Examination of tissues from healthy nonhuman primates revealed an analogous population of $IL\text{-}22^+$ cells that exhibited an ILC phenotype in rectal tissues (Fig. 1, C and D) and inguinal LNs (fig. S1, C and D). A population of

[1]Department of Microbiology and Institute for Immunology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA. [2]Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA. [3]Division of Mucosal Immunology, Institute of Medical Science, The University of Tokyo, Tokyo 108-8639, Japan. [4]Department of Medical Genome Science, Graduate School of Frontier Science, The University of Tokyo, Chiba 277-8562, Japan. [5]Department of Pediatrics, Division of Gastroenterology, Hepatology, and Nutrition, Perelman School of Medicine, University of Pennsylvania, Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA. [6]Centre de Recherche en Infectiologie, Centre Hospitalier de l'Université Laval, Faculty of Medicine, Laval University, Quebec, Canada. [7]Department of Surgery and the Columbia Center for Translational Immunology, Columbia University Medical Center, New York, NY 10032, USA. [8]Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA. [9]Department of Surgery, University of Pennsylvania, Philadelphia, PA 19104, USA. [10]Inflammation and Immunology Research Unit, Biotherapeutics Research and Development, Pfizer Worldwide R&D, Cambridge, MA 02140, USA. [11]Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Tokyo 102-0075, Japan. [12]Philadelphia VA Medical Center, Philadelphia, PA 19104, USA. [13]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA.

*To whom correspondence should be addressed. E-mail: dartis@mail.med.upenn.edu

IL-22$^+$ cells was also constitutively present in intestinal tissues or mLNs from naïve mice that lacked expression of lineage markers CD3 or NK1.1 (Fig. 1E and fig. S1E) but were CD127$^+$, CD45^{INT}, RORγt$^+$, and CD90.2 (Thy1)$^+$ (Fig. 1F and fig. S1F), indicating that they were ILCs (*11*, *14*). The presence of IL-22–producing ILCs in mice was independent of commensal bacteria, as their frequencies were similar in conventional versus germ-free mice (fig. S2, A and B). Collectively, these data identify that ILCs are a dominant IL-23–responsive, IL-22–producing cell population constitutively present in the intestine and GALTs of healthy mammals.

To test whether ILCs contribute to the anatomical containment of commensal bacteria in the steady state, control or anti-CD90.2 monoclonal antibody (mAb) was administered to naïve *Rag1*$^{-/-}$ mice to deplete ILC populations. Before depletion, *Rag1*$^{-/-}$ mice exhibited a population of IL-22–producing CD90.2$^+$ ILCs in the intestine and mLN (fig. S2, C and D). Notably, whereas peripheral tissues from isotype-treated or anti-NK1.1 mAb-treated *Rag1*$^{-/-}$ mice were sterile,

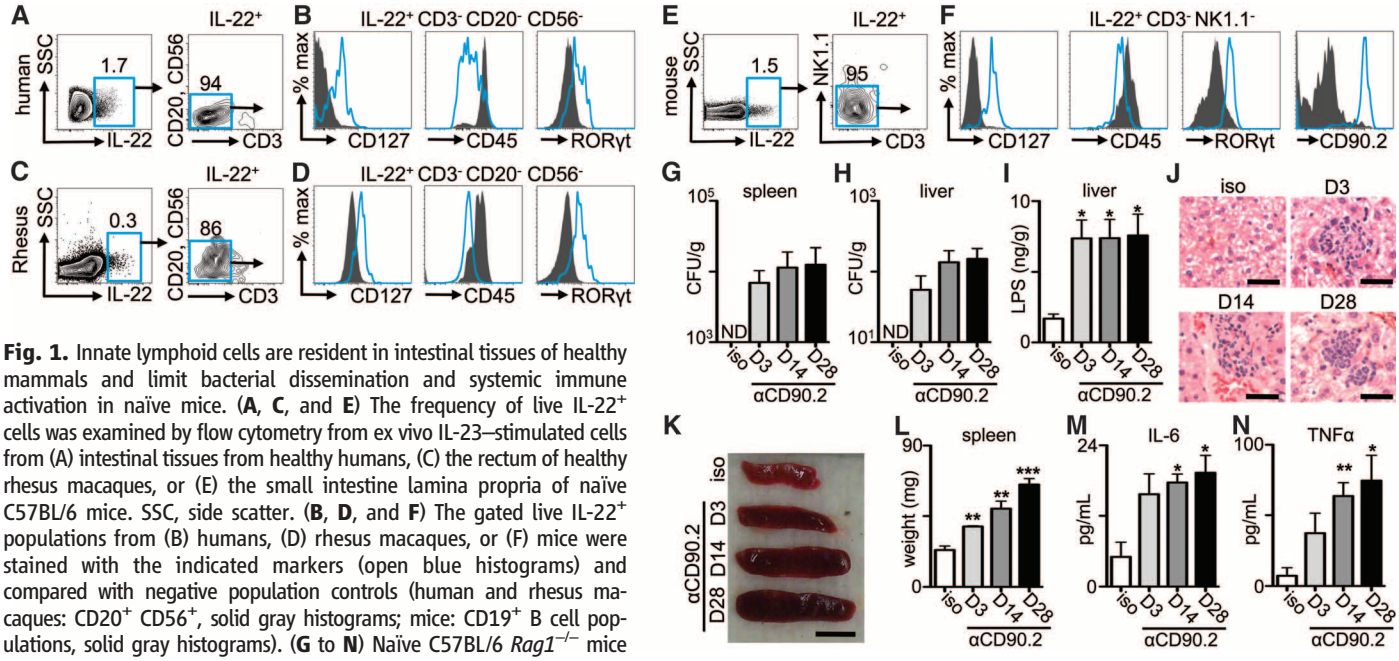

Fig. 1. Innate lymphoid cells are resident in intestinal tissues of healthy mammals and limit bacterial dissemination and systemic immune activation in naïve mice. (**A**, **C**, and **E**) The frequency of live IL-22$^+$ cells was examined by flow cytometry from ex vivo IL-23–stimulated cells from (A) intestinal tissues from healthy humans, (C) the rectum of healthy rhesus macaques, or (E) the small intestine lamina propria of naïve C57BL/6 mice. SSC, side scatter. (**B**, **D**, and **F**) The gated live IL-22$^+$ populations from (B) humans, (D) rhesus macaques, or (F) mice were stained with the indicated markers (open blue histograms) and compared with negative population controls (human and rhesus macaques: CD20$^+$ CD56$^+$, solid gray histograms; mice: CD19$^+$ B cell populations, solid gray histograms). (**G** to **N**) Naïve C57BL/6 *Rag1*$^{-/-}$ mice were administered an isotype control or anti-CD90.2 mAb starting on day 0 and sacrificed on day 3, 14, or 28. Colony-forming units (CFUs) present in homogenates from the (G) spleen and (H) liver of antibody-treated mice. (I) LPS concentrations in homogenates from the liver of antibody-treated mice. (J) Hematoxylin and eosin (H&E)–stained histological sections of the liver of antibody-treated mice. Scale bars, 5 μm. Spleen (K) size and (L) weight from antibody-treated mice. Scale bar, 5 mm. Serum concentrations of (M) IL-6 and (N) TNF-α from antibody-treated mice. All data are representative of three independent experiments of three individual mice per group, five total individual human donors, or two total individual Rhesus macaques. Data shown are the mean ± SEM (error bars). Statistics compare days postdepletion versus isotype using the Student's *t* test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. ND, none detected.

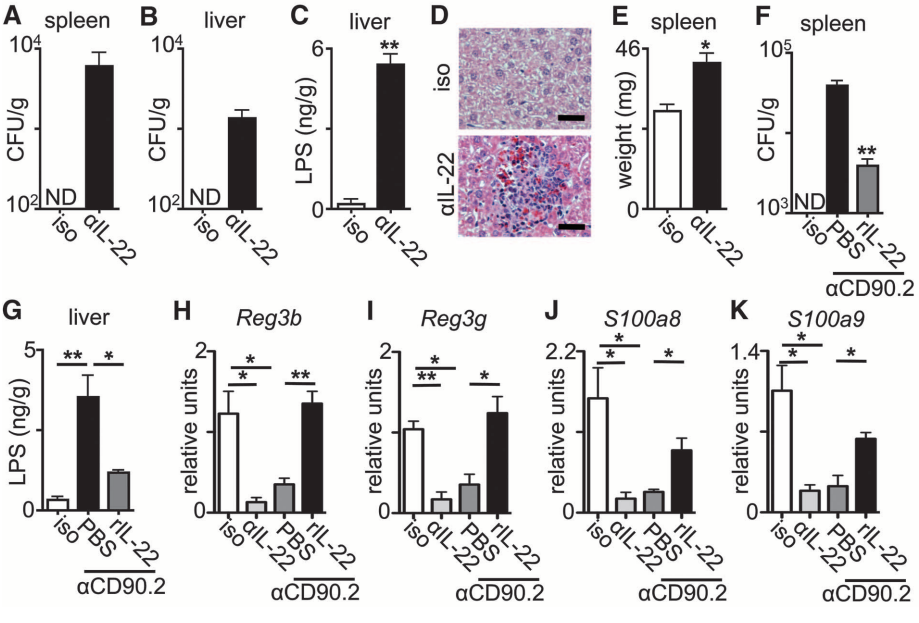

Fig. 2. ILCs regulate anatomical containment of commensal bacteria through IL-22–dependent induction of antimicrobial peptides. (**A** to **E**) Naïve C57BL/6 *Rag1*$^{-/-}$ mice were administered an isotype control or anti–IL-22 mAb starting on day 0 and were sacrificed on day 14. CFUs present in homogenates from the (A) spleen and (B) liver of antibody-treated mice. (C) LPS concentrations in homogenates from the liver of antibody-treated mice. (D) H&E-stained histological sections of the liver of antibody-treated mice. Scale bars, 5 μm. (E) Spleen weight from antibody-treated mice. (**F** to **K**) Naïve C57BL/6 *Rag1*$^{-/-}$ mice were administered an isotype control or anti-CD90.2 mAb with PBS control or rIL-22 starting on day 0 and were sacrificed on day 14. (F) CFUs present in homogenates from the spleen of antibody-treated mice. (G) LPS concentrations in homogenates from the liver of antibody-treated mice. Relative fold change of (H) *Reg3b*, (I) *Reg3g*, (J) *S100a8*, and (K) *S100a9* transcript in terminal ileum epithelial RNA from treated mice. All data are representative of two or more independent experiments with a minimum of three to four mice per group. Data shown are the mean ± SEM (error bars). Statistics compare treatment versus isotype unless otherwise noted using the Student's *t* test. *$P < 0.05$; **$P < 0.01$. ND, none detected.

spleen and liver from anti-CD90.2 mAb-treated *Rag1*$^{-/-}$ mice contained culturable bacteria and significantly increased levels of lipopolysaccharide (LPS) in the liver at days 3, 14, and 28 postdepletion (Fig. 1, G to I, and fig. S3, A to D). Collectively, these data indicate a requirement for ILCs in the anatomical containment of commensal bacteria under steady-state conditions.

We sought to test whether depletion of ILCs and subsequent bacterial dissemination elicited systemic immune activation in healthy mice. In comparison to isotype mAb-treated *Rag1*$^{-/-}$ mice, examination of peripheral organs from anti-CD90.2 mAb-treated *Rag1*$^{-/-}$ mice revealed hepatic inflammation characterized by foci of neutrophils, increased spleen size and weight, and elevated serum levels of IL-6 and tumor necrosis factor–α (TNF-α) at days 3, 14, and 28 postdepletion (Fig. 1, J to N). Further, anti-CD90.2 mAb-treated *Rag1*$^{-/-}$ mice that were given oral antibiotics to deplete intestinal commensal bacteria (*15*) did not exhibit peripheral dissemination of culturable bacteria or systemic inflammation (fig. S4, A to H), collectively implicating a critical role for ILC-mediated containment of commensal bacteria to prevent systemic inflammation in lymphocyte-deficient mice.

To test whether ILC-mediated anatomical containment of commensal bacteria was dependent on IL-22/IL-22R interactions, naïve *Rag1*$^{-/-}$ mice were treated with isotype or anti–IL-22 mAb. Anti–IL-22 mAb-treated mice, but not isotype mAb-treated mice, exhibited culturable bacteria in the spleen and liver (Fig. 2, A and B) and significantly increased levels of hepatic LPS (Fig. 2C). Anti–IL-22 mAb-treated mice also exhibited signs of systemic inflammation (Fig. 2, D and E), indicating that neutralization of IL-22 in *Rag1*$^{-/-}$ mice is sufficient to promote bacterial dissemination and systemic inflammation.

To determine whether therapeutic delivery of exogenous IL-22 could restore anatomical containment of commensal bacteria in ILC-depleted mice, anti-CD90.2 mAb-treated *Rag1*$^{-/-}$ mice were treated with either phosphate-buffered saline (PBS) control or rIL-22. ILC-depleted mice that received rIL-22 exhibited decreased amounts of culturable bacteria in the spleen (Fig. 2F) and significantly decreased levels of hepatic LPS (Fig. 2G) compared with control anti-CD90.2 mAb-treated mice. Examination of intestinal epithelial cells from anti–IL-22 mAb-treated or anti-CD90.2 mAb-treated *Rag1*$^{-/-}$ mice demonstrated a significant reduction in expression of the IL-22 regulated antimicrobial proteins *Reg3b*, *Reg3g*, *S100a8*, and *S100a9* (*11*–*13*, *16*, *17*), which could be restored with delivery of rIL-22 to ILC-depleted mice (Fig. 2, H to K). Collectively, these data indicate that ILCs are critical in promoting IL-22–dependent pathways that limit peripheral dissemination of commensal bacteria and systemic inflammation.

Peripheral dissemination of intestinal commensal bacteria is commonly associated with impaired intestinal epithelial barrier integrity, resulting in the translocation of commensal bacteria from the intestinal lumen (*1*, *2*, *5*, *6*, *18*–*20*). However, both isotype and anti-CD90.2 mAb-treated *Rag1*$^{-/-}$ mice exhibited no significant differences in levels of serum fluorescein isothiocyanate (FITC) after oral administration of FITC-dextran (*21*), fecal albumin, or intestinal expression

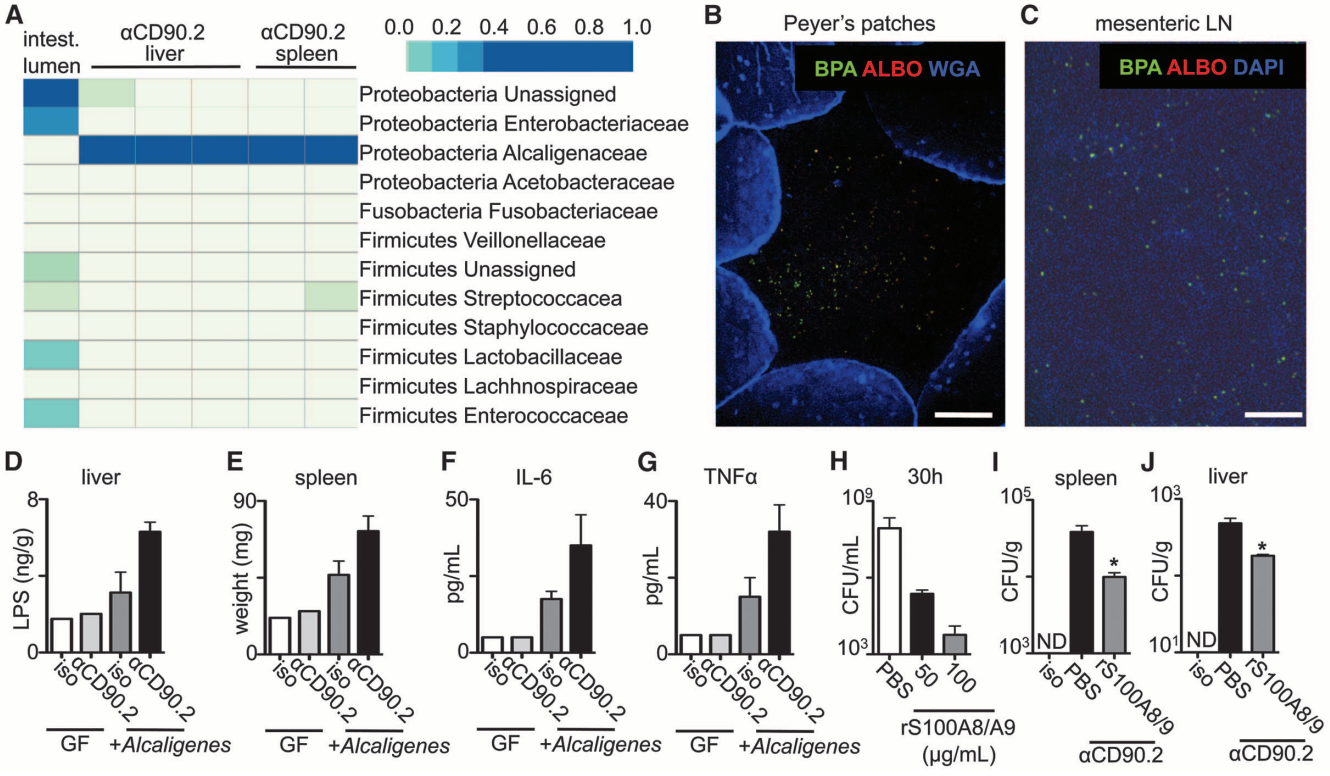

Fig. 3. Innate lymphoid cells regulate selective anatomical containment of *Alcaligenes* species to limit systemic inflammation. (**A** to **C**) Naïve C57BL/6 *Rag1*$^{-/-}$ mice were administered an isotype control or anti-CD90.2 mAb starting on day 0 and were sacrificed on day 14. Tissues from the liver and spleen were homogenized and cultured in LB broth. (A) Pyrosequencing of contents from the intestinal lumen or tissue cultures from anti-CD90.2 mAb-treated *Rag1*$^{-/-}$ mice. The top right bar represents sequence frequency. (B) Peyer's patches and (C) mesenteric lymph nodes from control mice were analyzed by FISH using probes to identify *Alcaligenes spp.* (BPA and ALBO) and epithelial cells (wheat germ agglutinin) or DNA (4′,6-diamidino-2-phenylindole). Scale bars, 100 μm. (**D** to **G**) Naïve germ-free (GF) or *Alcaligenes* monoassociated *Rag1*$^{-/-}$ mice were administered an isotype control or anti-CD90.2 mAb starting on day 0 and were sacrificed on day 5. (D) LPS concentrations in homogenates from the liver of antibody-treated mice. (E) Spleen weight from antibody-treated mice. Serum concentrations of (F) IL-6 and (G) TNF-α from antibody-treated mice. (**H**) *Alcaligenes* was cultured in the presence of PBS or rS100A8/rS100A9, and CFUs were measured after 30 hours. (**I** and **J**) Naïve *Rag1*$^{-/-}$ mice were administered an isotype control or anti-CD90.2 mAb with PBS control or rS100A8/S100A9 on day 0 and were sacrificed on day 5. CFUs present in homogenates from the (I) spleen and (J) liver of antibody-treated mice. All data are representative of two independent experiments with a minimum of two to three mice per group. Data shown are the mean ± SEM (error bars). Statistics compare PBS versus rS100S8/A9 treatments using Student's *t* test **P* < 0.05. ND, none detected.

of the tight-junction proteins claudin-1 or claudin-2 (fig. S5, A to D), nor did they exhibit histological signs of intestinal inflammation (fig. S5E). Further, the mLNs of control and anti-CD90.2 mAb-treated mice contained equivalent frequencies of macrophages and dendritic cells, whereas anti-CD90.2 mAb-treated mice contained significantly higher frequencies of neutrophils in the mLNs (fig. S5, F to H), suggesting that depletion of ILCs does not result in a global impairment of intestinal epithelial barrier integrity and that disseminating bacteria may not originate from the intestinal lumen.

Metabolic profiling (*22*) of bacterial colonies from the liver or spleen of anti-CD90.2 mAb-treated *Rag1*$^{-/-}$ mice identified that the disseminating bacteria were *Alcaligenes* spp. (fig. S6A), a genus of Gram-negative bacteria that reside within the Peyer's patches (PPs) and mLNs of healthy humans, nonhuman primates, and mice (*4*, *23*). 16S-directed polymerase chain reaction confirmed the presence of *Alcaligenes* spp. in liver and spleen from anti-CD90.2 mAb-treated, but not isotype-treated, *Rag1*$^{-/-}$ mice (fig. S6B), and pyrosequencing of 16S recombinant DNA tags demonstrated that samples from the intestinal lumen of untreated *Rag1*$^{-/-}$ mice contained multiple phylogenetic groups of commensal bacteria, whereas cultures from the liver and spleen of ILC-depleted *Rag1*$^{-/-}$ mice exhibited a homogeneous population of *Alcaligenaceae* (Fig. 3A). Analysis of these sequences identified the species as *Alcaligenes xylosoxidians* (also referred to as *Achromobacter xylosoxidans*). To interrogate the origins of the *Alcaligenes* spp., tissues from naïve mice were analyzed by fluorescent in situ hybridization (FISH) using *Alcaligenes*-specific probes. Consistent with a previous report (*4*), we found *Alcaligenes* spp. in the interior of PPs and mLNs of healthy mice (Fig. 3, B and C, and fig. S7). Collectively, these results indicate that the loss of ILCs results in selective dissemination of lymphoid-resident *Alcaligenes* spp. to peripheral tissues.

To determine whether *Alcaligenes* spp. were sufficient to promote inflammation, *Alcaligenes* was administered systemically to *Rag1*$^{-/-}$ mice. In comparison to isotype-treated mice, both anti-CD90.2 mAb-treated *Rag1*$^{-/-}$ mice and *Rag1*$^{-/-}$ mice that received systemic *Alcaligenes* spp. exhibited significantly increased hepatic LPS and systemic inflammation (fig. S8, A to F). Furthermore, whereas germ-free *Rag1*$^{-/-}$ mice exhibited no increases in hepatic LPS or systemic inflammation after administration of anti-CD90.2 mAb, germ-free *Rag1*$^{-/-}$ mice that were monoassociated with *Alcaligenes* and treated with anti-CD90.2 mAb exhibited increased hepatic LPS, increased spleen weight, and elevated levels of serum IL-6 and TNF-α compared with isotype-treated monoassociated mice (Fig. 3, D to G).

To examine whether decreased expression of IL-22–regulated antimicrobial peptides (Fig. 2, H to K) affects *Alcaligenes*, we added rS100A8/S100A9 (calprotectin) (*24*) to cultures and found that it inhibits the growth of *Alcaligenes* and limits colony formation in a dose-dependent manner (Fig. 3H and fig. S9). Furthermore, delivery of rS100A8/S100A9 in vivo significantly reduced burdens of *Alcaligenes* in the spleen and liver of anti-CD90.2 mAb-treated *Rag1*$^{-/-}$ mice (Fig. 3, I and J). Collectively, these results suggest that in healthy mice, ILCs promote anatomical containment of *Alcaligenes* spp., in part through promoting expression of calprotectin to limit disruption of systemic immune homeostasis.

To test whether ILCs prevent dissemination of *Alcaligenes* in lymphocyte-replete mice, we generated CD90-disparate *Rag1*$^{-/-}$ chimeric mice that permit the selective depletion of CD90.2^{+} ILCs without depleting CD90.1^{+} lymphocytes (fig. S10A) (*13*). Administration of anti-CD90.2 mAb to CD90-disparate *Rag1*$^{-/-}$ chimeric mice resulted in peripheral dissemination of *Alcaligenes* to the spleen and liver at day 3 postdepletion (Fig. 4, A and B, and fig. S10B). Chimeric mice exhibited elevated levels of hepatic LPS and inflammation, increased spleen size, and elevated levels of serum IL-6 and TNF-α at days 3, 14, and 28 postdepletion (Fig. 4, C to G), as well as significantly higher frequencies of splenic Ki-67^{+} CD4^{+} T cells, Ki-67^{+} CD8^{+} T cells, and Ki-67^{+} CD19^{+} B cells (fig. S10, C to E). Splenocyte cultures were restimulated with *Alcaligenes*-derived antigens, and significantly higher frequencies of IL-6^{+} CD4^{+} T cells and TNF-α^{+} CD4^{+} T cells were observed in ILC-depleted chimeric mice (fig. S10F). Anti-CD90.2 mAb-treated chimeric mice also exhibited significantly elevated serum immunoglobulin G (IgG) responses specific for *Alcaligenes*-derived antigens, but not luminal-resident *Escherichia coli*–derived antigens (fig. S10G) or opportunistic viruses (table S1). The inability to culture *Alcaligenes* at days 14 and 28 was associated with the development of systemic IgG specific for *Alcaligenes* spp. (Fig. 4H), indicating that despite persistent systemic

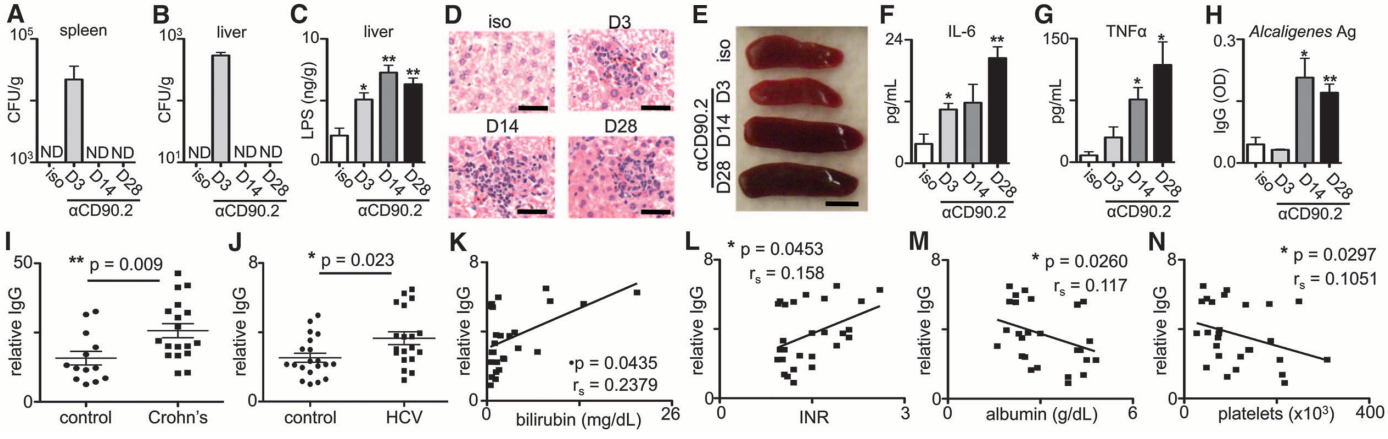

Fig. 4. ILCs regulate anatomical containment of *Alcaligenes* in lymphocyte-replete hosts, and *Alcaligenes*-specific responses are associated with chronic human disease. (**A** to **H**) Naïve CD90-disparate chimeric mice were administered an isotype control or anti-CD90.2 mAb starting on day 0 and were sacrificed on day 3, 14, or 28. CFUs present in homogenates from the (A) spleen and (B) liver of antibody-treated chimeric mice. (C) LPS concentrations in homogenates from the liver of antibody-treated mice. (D) H&E-stained histological sections of the liver of antibody-treated chimeric mice. Scale bar, 5 μm. (E) Spleen size from antibody-treated chimeric mice. Scale bars, 5 mm. Serum concentrations of (F) IL-6 and (G) TNF-α from antibody-treated chimeric mice. (H) Relative optical density (OD) values of serum IgG specific to *Alcaligenes* crude antigens in treated chimeric mice. All data are representative of three independent experiments with a minimum of three to five mice per group. Statistics compare days postdepletion versus isotype using the Student's *t* test. (**I** to **N**) Relative serum IgG (OD values normalized to total serum IgG) specific to *Alcaligenes* crude antigens in (I) control (n = 13) versus pediatric Crohn's disease patients (n = 18) or (J) control (n = 20) versus cirrhotic HCV-infected patients awaiting orthotopic liver transplantation (n = 19). Statistics compare disease status using the Mann-Whitney test. Relative serum IgG specific for *Alcaligenes* crude antigen in chronically HCV-infected individuals (n = 27) was correlated with levels of serum (K) bilirubin, (L) INR of prothrombin time, (M) albumin, and (N) platelets. The association between *Alcaligenes*-specific IgG levels and clinical parameters was compared by nonparametric Spearman's rank correlation coefficient (r_s).

inflammation, the adaptive immune system can limit the presence of live bacteria in the periphery. Collectively, these data suggest that ILCs are essential to promote anatomical containment of *Alcaligenes* to lymphoid tissues and limit the induction of systemic inflammation in lymphocyte-replete hosts.

Loss of containment of commensal bacteria and chronic systemic inflammation is associated with several chronic human diseases (*6*–*8*). To determine whether these diseases were also associated with a loss of containment of *Alcaligenes* spp., we analyzed serum samples from cohorts of pediatric Crohn's disease patients or chronically hepatitis C virus (HCV)–infected adults for the presence of *Alcaligenes*-specific IgG. In comparison to age-matched controls, serum from pediatric Crohn's disease patients and plasma from cirrhotic HCV-infected individuals awaiting liver transplantation exhibited significantly elevated levels of relative IgG specific for *Alcaligenes* spp. (Fig. 4, I and J). Although further analysis of HCV-infected individuals with and without cirrhosis demonstrated no correlations between *Alcaligenes*-specific IgG levels and patient age or serum alanine transaminase (fig. S11, A and B), there were significant correlations between plasma levels of *Alcaligenes*-specific IgG and laboratory measures of liver disease, including increased serum bilirubin and international normalized ratio (INR) of prothrombin time as well as decreased serum albumin and platelets (Fig. 4, K to N).

Mammals have evolved multiple immunologic and physiologic mechanisms to promote the anatomical containment of commensal bacteria to intestinal sites, including promoting physical barriers (via epithelial cell tight junctions), biochemical barriers (via production of mucus layers and antimicrobial peptides), and immunologic barriers (via IgA-mediated immune exclusion; intraepithelial lymphocytes; and innate pathways involving phagocytosis, Toll-like receptor–mediated sensing, and oxidative bursts) (*1*, *2*, *18*, *19*, *25*). The demonstration that depletion of ILCs results in the selective dissemination and survival of *Alcaligenes* spp. in peripheral tissues of mice indicates that, in addition to established pathways that nonselectively maintain intestinal barrier function, more discriminatory processes may have evolved to promote the selective anatomical containment of phylogenetically defined communities of lymphoid-resident commensal bacteria (fig. S12). It is notable that *Alcaligenes* spp. has recently been identified as a dominant lymphoid-resident commensal species colonizing the PPs and mLNs of mammals (*4*). Moreover, peripheral dissemination of *Alcaligenes* spp. has been reported in patients with HIV infection, cancer, and cystic fibrosis (*26*–*29*). The identification of a pathway through which IL-22–producing ILCs can prevent dissemination of lymphoid-resident *Alcaligenes* spp. and limit systemic inflammation highlights the selectivity of immune-mediated containment of defined commensal bacterial species and could offer therapeutic strategies to limit inflammation associated with multiple debilitating chronic human diseases.

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Acknowledgments: We thank members of the Artis laboratory for discussions and critical reading of the manuscript. We also thank S. Olland, R. Zollner, K. Lam, and A. Root at Pfizer for the preparation of IL-22 cytokine and antibodies. The research is supported by the NIH (grants AI061570, AI087990, AI074878, AI083480, AI095466, and AI095608 to D.A.; T32-AI007532 to G.F.S and L.A.M.; T32-AI055428 to G.F.S.; T32-RR007063 and K08-DK093784 to T.A.; and AI47619 to K.-M.C.); the NIH-funded Penn Center for AIDS Research (grant P30 AI 045008 to G.F.S. and D.A.); the Burroughs Wellcome Fund Investigator in Pathogenesis of Infectious Disease Award (to D.A.); the Philadelphia VA Medical Research and Merit Review and American Gastroenterological Association (to K.-M.C.); the Ministry of Education, Culture, Sports, Science and Technology of Japan (to J.K., N.S., and H.K); and the Program for Promotion of Basic and Applied Researches for Innovations in Bio-Oriented Industry (to J.K.). We also thank the Matthew J. Ryan Veterinary Hospital Pathology Lab, the National Institute of Diabetes and Digestive and Kidney Disease Center for the Molecular Studies in Digestive and Liver Disease Molecular Pathology and Imaging Core (grant P30DK50306), the Penn Microarray Facility, and the Abramson Cancer Center Flow Cytometry and Cell Sorting Resource Laboratory [partially supported by National Cancer Institute (NCI) Comprehensive Cancer Center Support grant #2-P30 CA016520] for technical advice and support. Several human tissue samples were provided by the Cooperative Human Tissue Network, which is funded by the NCI. The data presented in the paper are tabulated in the main paper and in the supplementary materials.

Supplementary Materials

www.sciencemag.org/cgi/content/full/336/6086/1321/DC1
Materials and Methods
Figs. S1 to S12
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28 November 2011; accepted 24 April 2012
10.1126/science.1222551

Regulated Virulence Controls the Ability of a Pathogen to Compete with the Gut Microbiota

Nobuhiko Kamada,[1] Yun-Gi Kim,[1] Ho Pan Sham,[2] Bruce A. Vallance,[2] José L. Puente,[3] Eric C. Martens,[4] Gabriel Núñez[1]*

The virulence mechanisms that allow pathogens to colonize the intestine remain unclear. Here, we show that germ-free animals are unable to eradicate *Citrobacter rodentium*, a model for human infections with attaching and effacing bacteria. Early in infection, virulence genes were expressed and required for pathogen growth in conventionally raised mice but not germ-free mice. Virulence gene expression was down-regulated during the late phase of infection, which led to relocation of the pathogen to the intestinal lumen where it was outcompeted by commensals. The ability of commensals to outcompete *C. rodentium* was determined, at least in part, by the capacity of the pathogen and commensals to grow on structurally similar carbohydrates. Thus, pathogen colonization is controlled by bacterial virulence and through competition with metabolically related commensals.

Enterohemorragic *Escherichia coli* (EHEC) and enteropathogenic *E. coli* (EPEC) are important causes of diarrhea and mortality worldwide (*1*, *2*). These Gram-negative bacteria attach to and colonize the intestinal tract by inducing attaching and effacing (AE) lesions on the intestinal epithelium (*1*, *2*). The genomes of AE pathogens harbor the locus of enterocyte