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Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens

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Infections by attaching and effacing (A/E) bacterial pathogens, such as *Escherichia coli* O157:H7, pose a serious threat to public health. Using a mouse A/E pathogen, *Citrobacter rodentium*, we show that interleukin-22 (IL-22) has a crucial role in the early phase of host defense against *C. rodentium*. Infection of IL-22 knockout mice results in increased intestinal epithelial damage, systemic bacterial burden and mortality. We also find that IL-23 is required for the early induction of IL-22 during *C. rodentium* infection, and adaptive immunity is not essential for the protective role of IL-22 in this model. Instead, IL-22 is required for the direct induction of the Reg family of antimicrobial proteins, including RegIIIβ and RegIIIγ, in colonic epithelial cells. Exogenous mouse or human RegIIIγ substantially improves survival of IL-22 knockout mice after *C. rodentium* infection. Together, our data identify a new innate immune function for IL-22 in regulating early defense mechanisms against A/E bacterial pathogens.

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Intestinal bacterial infection represents a major cause of mortality worldwide and continues to threaten global health[1]. A/E bacterial pathogens, such as enterohemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli* (EPEC), are responsible for diarrhea, morbidity and mortality, especially among infants and children in the developing world[2]. Infection by *E. coli* O157:H7, one of the EHEC strains, resulted in the hospitalization of many people, as well as three mortalities, in 2006 in the United States[3]. Much of our knowledge about how hosts control infection by A/E pathogens comes from experimental studies with *C. rodentium*, a naturally occurring pathogen in mice[4]. Like EHEC or EPEC in humans, *C. rodentium* attachment to mouse colonic epithelial cells results in effacement of brush border microvilli, termed an A/E lesion, as well as colonic mucosal hyperplasia[5]. Both intestinal epithelial cells and immune cells are crucial in host defense against A/E pathogens. The tight junctions of intestinal epithelial cells provide the first barrier preventing microbes from leaving the intestinal lumen[6]. Epithelial cells also secrete antimicrobial peptides to control pathogens in the gastrointestinal tract[7]. Studies of *C. rodentium* infection in immune-deficient mouse strains have established that $CD4^+$ T cells, B cells and *C. rodentium*–specific antibody responses are all essential components of adaptive immunity used for containing and eradicating the infection[8,9]. Many cytokines from lymphocytes, including interferon-γ and tumor necrosis factor-α, also have an important role in host defense during *C. rodentium* infection[10,11]. Both interferon-γ and tumor necrosis factor-α can enhance the innate immune response of epithelial cells and can also boost the proinflammatory phenotype of lymphocytes. However, it remains unclear what the most important pathways are that mediate the crosstalk between immune cells and colonic epithelial cells during *C. rodentium* infection.

IL-22 belongs to the IL-10 family of cytokines[12]. IL-22 signals through the IL-22 receptor (IL-22R) paired with IL-10Rβ (ref. 13). Whereas IL-10Rβ is ubiquitously expressed, IL-22R is expressed specifically on epithelial tissues[14] and is believed to mediate epithelial innate immunity[15]. IL-22 is produced by leukocytes, particularly T helper type 17 (T_H17) cells[16], and the role of IL-22 in host defense against bacterial infections is still largely unclear, despite the observation that IL-22 induces a marked antimicrobial response *in vitro* and is associated with some viral and bacterial infections *in vivo*[17–22]. *In vitro*, IL-22 production can be directly induced in $CD4^+$ T cells by IL-23 or IL-6, as well as in monocytes by IL-23 (ref. 16). Mice deficient in either the p19 (encoded by *Il23a*) or the p40 (encoded by *Il12b*) subunit of IL-23 lose their ability to control *C. rodentium* infection[23]. Therefore, we hypothesize that IL-22 may exert an important downstream host defensive function during *C. rodentium* infection.

RESULTS

IL-23 regulates IL-22 production during *C. rodentium* infection

During *C. rodentium* infection, IL-22 transcript and protein were induced in the colon of wild-type (WT) mice (**Fig. 1a**), as were cytokines that promote IL-22 production[16], including both the p19 and the p40 subunits of IL-23 (**Fig. 1b**) and IL-6 (**Fig. 1c**). All of these cytokines were rapidly induced, with peak expression around day 4 after inoculation. In contrast, IL-17 induction had slower kinetics, and its production reached a maximum at day 12 after inoculation (**Fig. 1d**).

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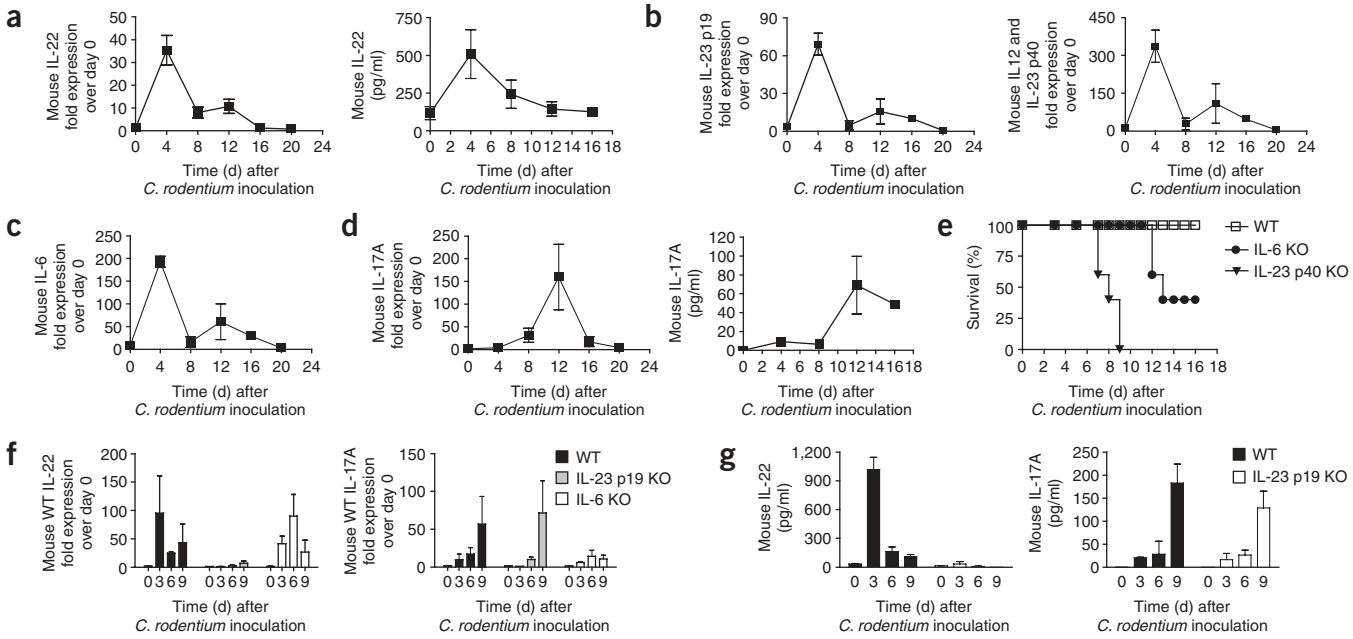

Figure 1 Host defense against *C. rodentium* infection. (**a**) Time-course real-time RT-PCR analysis (left) and *ex vivo* colon culture ELISA (right) of IL-22 in WT mouse colons after *C. rodentium* infection. (**b**,**c**) Time-course real-time RT-PCR analysis of IL-23 p19 and p40 subunits and IL-6 in WT mouse colons after *C. rodentium* infection. (**d**) Time-course real-time RT-PCR analysis (left) and *ex vivo* colon culture ELISA (right) of IL-17 in WT mouse colons after *C. rodentium* infection. (**e**) Survival of C57BL/6 ($n = 5$), IL-12 and IL-23 p40 knockout (KO; $n = 5$) and IL-6 KO ($n = 5$) mice after *C. rodentium* infection. (**f**) Time-course real-time RT-PCR analysis and (**g**) *ex vivo* colon culture ELISA of IL-22 (left) and IL-17 (right) expression in C57BL/6, IL-23 p19 KO and IL-6 KO mouse colons after *C. rodentium* infection. All data are representative of at least two independent experiments.

To define the roles of IL-23 and IL-6 in regulating IL-22 production during *C. rodentium* infection, we first compared the survival rates of WT, IL-23 p19 knockout, IL-12 and IL-23 p40 knockout, and IL-6 knockout mice after *C. rodentium* infection. In multiple repeat experiments, we consistently observed that all IL-12 and IL-23 p40 knockout mice died before day 12 (**Fig. 1e** and data not shown), whereas all IL-23 p19 knockout mice died from around day 10 to day 14 after inoculation (**Supplementary Fig. 1a** online and data not shown)[23]. Notably, 60% mortality was also observed in the IL-6 knockout group around day 12 (**Fig. 1e**), indicating that IL-6 is required, to some extent, for complete control of *C. rodentium* infection. Next, we examined IL-22 and IL-17 expression in both IL-23 p19 knockout and IL-6 knockout mice (**Fig. 1f**,**g**). Whereas IL-17 expression was not altered in IL-23 p19 knockout mice[23], induction of IL-22 was diminished in IL-23 p19 knockout mice compared to WT mice. In IL-6 knockout mice, although the peak amount of IL-22 was comparable to that of WT mice, its induction was delayed (**Fig. 1f**). Furthermore, induction of IL-17 was substantially reduced in these mice, which is consistent with an essential role for IL-6 in IL-17 production. These data demonstrate that IL-23 is essential for IL-22 regulation in infectious diseases.

IL-22 is critical for host defense against *C. rodentium*

To directly test the role of IL-22 in this model, we inoculated IL-22 knockout mice[16] with *C. rodentium*. Whereas WT littermates transiently lost weight but were able to fully recover 6 d after infection, IL-22 knockout mice continued to lose weight after *C. rodentium* infection (**Fig. 2a**). In multiple repeat experiments, the mortality of IL-22 knockout mice ranged from 80% to 100% in the second week after infection (**Fig. 2a** and data not shown). On day 8 after inoculation, histological analysis of colons from *C. rodentium*–infected IL-22 knockout mice showed increased mucosal hyperplasia compared to WT mice (**Fig. 2b–e**), as well as increased submucosal inflammation (**Fig. 2b**). Furthermore, *C. rodentium* infection was predominantly superficial in WT mice, whereas large numbers of bacteria penetrated deeply into the colonic crypts of IL-22 knockout mice (**Fig. 2c**). There were also multiple foci of colonic mucosal ulceration and marked transmural inflammation in IL-22 knockout mice (**Fig. 2d**). These data suggest that IL-22 may be one of the key downstream effector cytokines that contribute to the biology of IL-23 in controlling bacterial infections.

Both IL-22 receptor chains, IL-22R and IL-10Rβ, are expressed in the gastrointestinal tract of WT C57BL/6 mice (**Fig. 2f**). Expression of both chains is higher in the duodenum, jejunum, ileum and colon than in the skin[16]. Whereas IL-10Rβ is expressed on both lymphocytes and epithelial cells[24], only colonic epithelial cells and subepithelial myofibroblasts, not peripheral lymphocytes, are reported to respond to IL-22 (refs. 17,25,26). FACS analysis with an IL-22R–specific antibody (**Supplementary Fig. 1b**) revealed that IL-22R was expressed by E-cadherin$^+$ primary mouse colonic epithelial cells, but not by CD45$^+$ intraepithelial lymphocytes (IELs) or lamina propria mononuclear cells (LPMCs; **Fig. 2g**). Similarly, primary human colonic epithelial cells also expressed IL-22R (**Fig. 2h**). These data suggest that colonic epithelial cells are directly targeted by IL-22.

IL-19, IL-20, IL-24 and IL-17 are dispensable in this model

Other IL-10–family cytokines, IL-19, IL-20 and IL-24, were also upregulated in the colons of WT mice during *C. rodentium* infection (**Fig. 3a**). Previous studies have shown that these cytokines have biological effects on epidermal keratinocytes similar to those induced by IL-22 (ref. 27). IL-19 signals through IL-20Rα and IL-20Rβ chains, whereas IL-20 and IL-24 can signal through two different receptor

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pairs, IL-20Rα–IL-20Rβ and IL-22R–IL-20Rβ (ref. 13). Expression of the IL-20Rα and IL-20Rβ chains was considerably lower in the gastrointestinal tract than in the skin of WT mice infected with *C. rodentium* (**Fig. 3b**). To determine the roles of these three cytokines during *C. rodentium* infection, we generated IL-20Rβ knockout mice (**Supplementary Fig. 2** online). The ear skin from these mice did not upregulate S100 family proteins when treated with recombinant IL-20, indicating a defect in IL-20 signaling *in vivo* (**Supplementary Fig. 2c**). IL-20Rβ knockout mice had survival rates comparable to WT mice after *C. rodentium* infection (**Fig. 3c**), indicating that IL-19, IL-20 and IL-24 are all dispensable for host defense against *C. rodentium*.

IL-17 induction was maintained in IL-23–deficient mice but was significantly ($P < 0.05$ on day 9) diminished in IL-6 knockout mice (**Fig. 1f**). The IL-17 pathway is crucial for controlling infection by many extracellular bacteria, such as *Klebsiella pneumoniae*[28]. IL-17 signals through IL-17R and IL-17RC (refs. 29,30) and induces pro-inflammatory responses from many cell types, including epithelial cells[31]. IL-17RC knockout mice developed normally, but did not respond to either IL-17A or IL-17F stimulation (**Supplementary Fig. 3** online). After *C. rodentium* inoculation, both IL-17RC knockout mice and WT littermates survived infection without any significant weight loss (**Fig. 3d**) or any differences in colon histology (data not shown). These results indicate that the IL-17A and IL-17F pathways are not required for early host defense against *C. rodentium* infection.

IL-22 protects colonic epithelial integrity in early phase

Histological findings from IL-22 knockout mice suggested that deficiency in IL-22 signaling leads to compromised epithelial barrier function (**Fig. 2c,d** and **Supplementary Fig. 4** online). Consistently, we found that the bacterial burdens in the mesenteric lymph node, spleen and liver, but not in colon, of IL-22 knockout mice were increased compared to those in WT mice (**Fig. 4a**). There was also evidence of systemic bacterial spread in IL-22 knockout mice, such as multifocal hepatocellular necrosis with embolic microabscessation in

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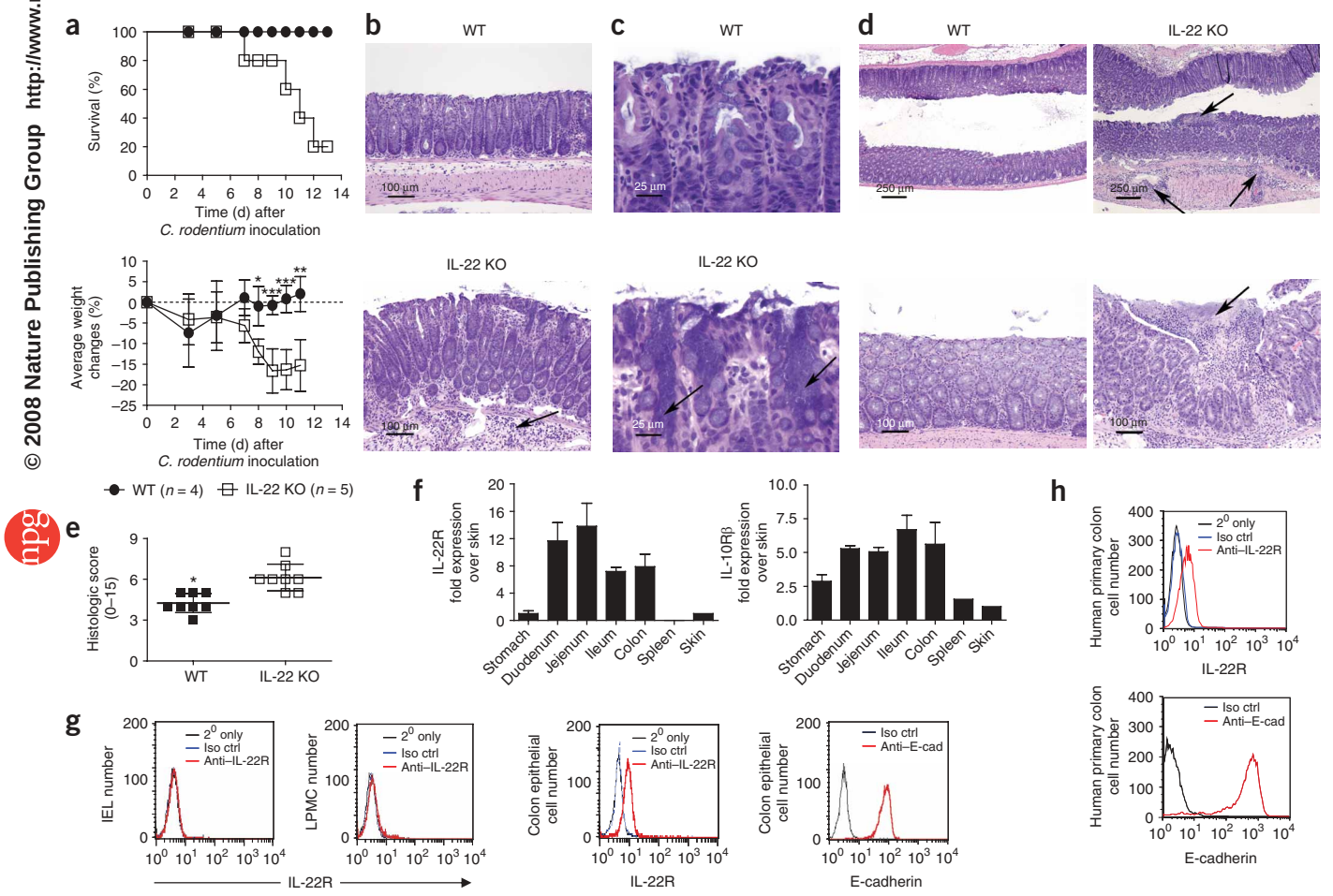

Figure 2 IL-22 deficiency renders mice susceptible to *C. rodentium* infection. (**a**–**e**) Six to seven–week–old IL-22 KO and WT littermate mice were orally inoculated with *C. rodentium* and were weighed at the indicated time points. (**a**) Survival rates (top) and average weight changes (bottom) for WT and IL-22 KO mice at the indicated time points. (**b**–**e**) Histological analysis of representative colons from IL-22 KO and WT mice 8 d after inoculation. H&E staining illustrates increased mucosal hyperplasia in IL-22 KO mice versus WT mice. Arrows indicate submucosal inflammation (**b**; scale bars, 100 μm), bacterial invasion deep into mucosal glands (**c**; scale bars, 25 μm) and focal ulceration and transmural inflammation (**d**; scale bars, 250 μm (top) and 100 μm (bottom)). (**e**) Histological scores for colons from WT and IL-22 KO mice. (**f**) Real-time RT-PCR analysis for IL-22 receptor subunits IL-22R (left) and IL-10Rβ (right) in uninfected WT mouse gastrointestinal tract. (**g**) FACS analysis of IL-22R expression on isolated mouse IELs, LPMCs and colonic epithelial cells. E-cadherin positive staining is shown in far right panel. (**h**) FACS analysis of IL-22R expression on primary human colonic epithelial cells. E-cadherin positive staining is shown in bottom panel. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. All data are representative of at least two independent experiments.

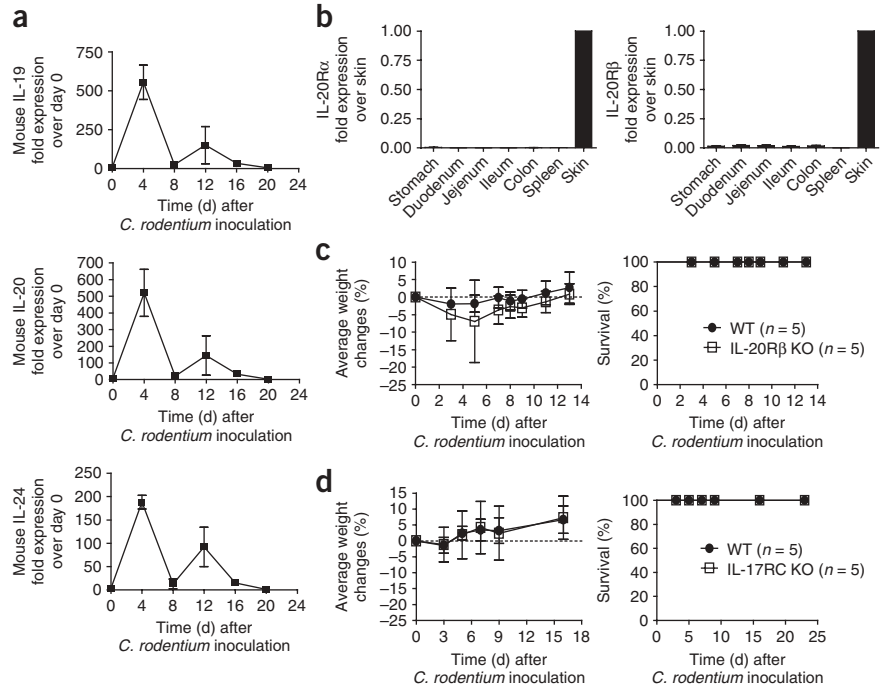

Figure 3 IL-19, IL-20, IL-24 and IL-17 are all dispensable during *C. rodentium* infection. (**a**) C57BL/6 mice were orally inoculated with *C. rodentium*. Colons were collected at the indicated time points and isolated RNA was used for real-time RT-PCR analysis of mouse IL-19 (top), IL-20 (center) and IL-24 (bottom) expression. (**b**) Real-time RT-PCR analysis for the common receptor subunits for IL-19, IL-20 and IL-24, IL-20Rα (left) and IL-20Rβ (right), in uninfected WT mouse gastrointestinal tract. (**c**,**d**) Infection of IL-20Rβ KO and IL-17RC KO mice with *C. rodentium*. Average weight changes (left) and survival rates (right) at the indicated time points. All data are representative of two independent experiments.

their livers (**Fig. 4b**). Together, these data support the hypothesis that colonic epithelial integrity is compromised in IL-22 knockout mice during *C. rodentium* infection.

The early window of IL-22 upregulation (**Fig. 1a**) could be crucial for the host to mount a sufficient response preventing lethality caused by *C. rodentium* infection. To test this hypothesis, IL-22–specific neutralizing antibody[16] was administered starting at either day 0 or day 8 after inoculation of *C. rodentium*. As expected, WT mice who received IL-22–specific antibody at the same time as the bacterial inoculation continued to lose weight, and all became moribund or died 12 d after inoculation, whereas all isotype control antibody–treated mice survived (**Fig. 4c**). In contrast, mice who received IL-22–specific antibody starting 8 d after inoculation had an outcome similar to that of the isotype control monoclonal antibody (mAb)–treated mice, with full recovery from infection.

Similarly to IL-22 knockout mice, WT mice treated with IL-22–specific antibody on day 0 developed more severe bloody diarrhea and had an increased incidence of rectal prolapse compared to WT mice treated with an isotype control mAb 8 d after inoculation (data not

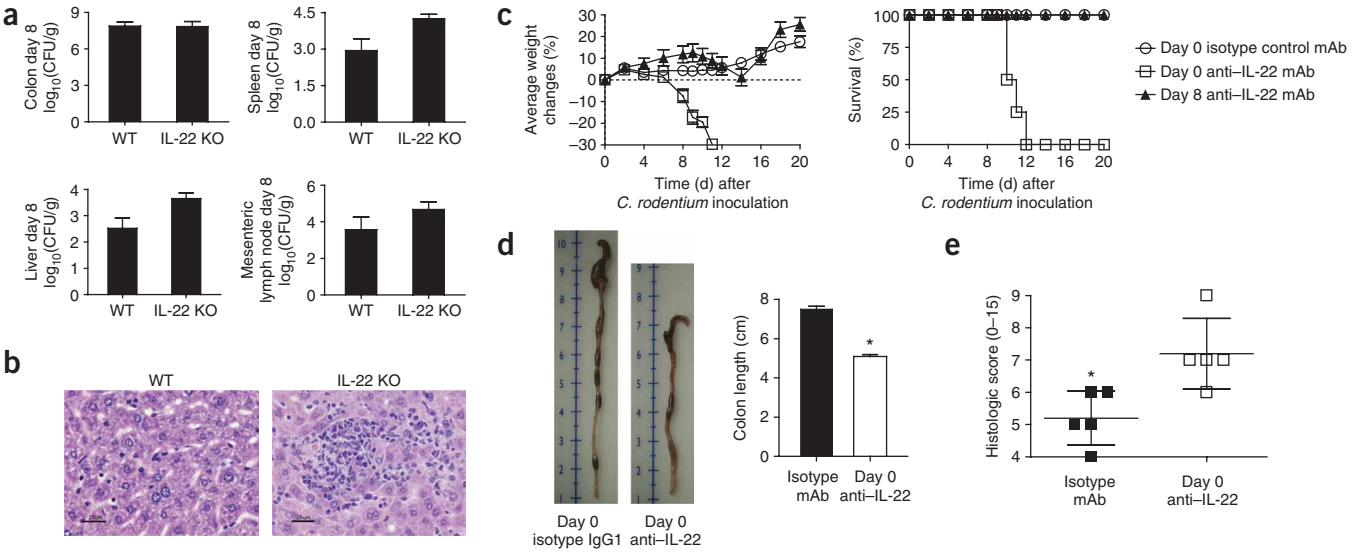

Figure 4 Crucial role of IL-22 in early host defense for maintenance of colonic epithelial cell integrity against *C. rodentium*. IL-22 KO and WT littermate mice were orally inoculated with *C. rodentium*. (**a**) Log_{10} CFU of *C. rodentium* in colon (top left), spleen (top right), liver (bottom left) and mesenteric lymph node (bottom right) 8 d after inoculation. (**b**) Histological analysis of H&E-stained livers (8 d after inoculation) from infected WT (left) and IL-22 KO (right) mice. Right panel shows a hepatic embolic microabscess in an IL-22 KO mouse. Representative images are shown; Scale bars, 25 μm. (**c**–**e**) WT C57BL/6 mice also received 150 μg of isotype control IgG1 mAb or IL-22–specific neutralizing mAb intraperitoneally every other day starting on day 0 or day 8 after *C. rodentium* inoculation. (**c**) Average weight changes (left) and survival rates (right) of the mice at the indicated time points. (**d**,**e**) On day 10, colons from day 0 mAb treated groups were photographed (**d**, left) and individual colon length was measured (**d**, right) and histologically scored (**e**). * $P < 0.05$. All data are representative of two independent experiments.

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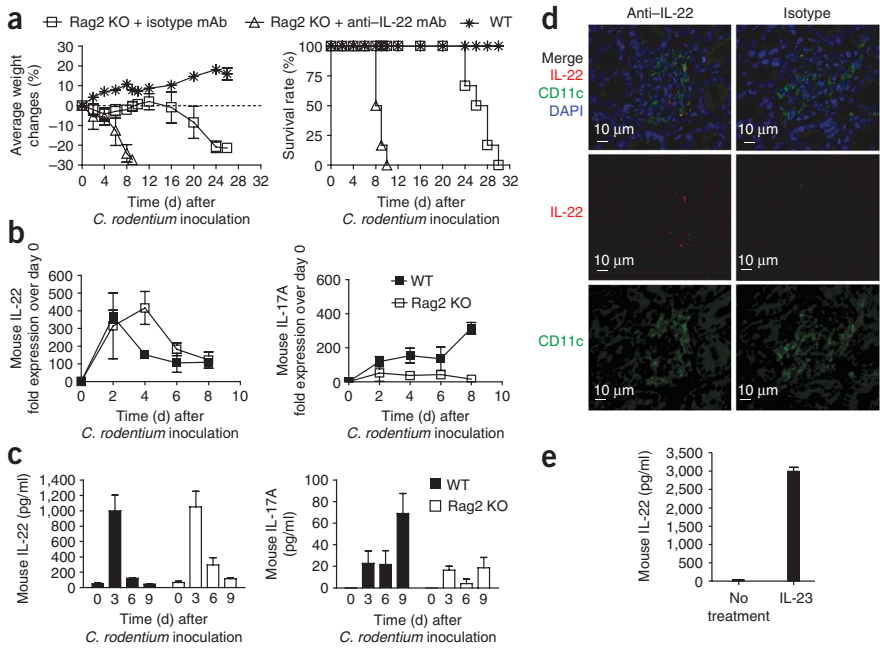

Figure 5 IL-22, produced by DCs, is crucial for innate immune responses against *C. rodentium* infection. Rag2 KO and WT BALB/c mice were orally inoculated with *C. rodentium*. (**a**) Mice received 150 μg of isotype control IgG1 mAb or IL-22–specific neutralizing mAb intraperitoneally every other day, starting at the same day as bacterial inoculation, and were weighed (left) and evaluated for survival (right) at the indicated time points. (**b**,**c**) Time-course real-time RT-PCR analysis (**b**) and *ex vivo* colon culture ELISA (**c**) of IL-22 (left) and IL-17 (right) expression in colons of WT BALB/c and Rag2 KO mice after *C. rodentium* infection. (**d**) Immunohistochemical staining for IL-22 (red), CD11c (green) and DAPI (blue) in day 4 colons from *C. rodentium*–infected Rag2 KO mice. Scale bars, 10 μm. (**e**) IL-23 directly induces IL-22 production, as measured by ELISA, from isolated mouse CD11c$^+$ DCs *in vitro*. All data are representative of two independent experiments.

shown). Colons from mice treated with IL-22–specific antibody on day 0 were thickened and shortened compared to those of isotype control–treated mice 10 d after inoculation (**Fig. 4d**). Cecums from IL-22–specific antibody–treated mice were smaller than those of isotype control–treated mice. Similar histological and pathologic findings were seen in colons from IL-22–specific antibody–treated mice and IL-22 knockout mice (**Fig. 4e**). Therefore, IL-22 is crucial in the early stage of *C. rodentium* infection but has only a minimal role during the later phase of host defense, when bacteria are being eradicated.

IL-22 is crucial in innate immunity against *C. rodentium*

To further critically examine the functions of IL-22 in innate versus adaptive immunity during *C. rodentium* infection, we infected recombination activating gene-2–deficient (Rag2 knockout) mice. Rag2 knockout mice gradually lost weight and eventually became moribund or died around day 30, owing to their lack of T and B cells and thus their inability to mount antibody responses to *C. rodentium* (**Fig. 5a**)[9,32]. In contrast to IL-23 p19 knockout or IL-22 knockout mice, none of the Rag2 knockout mice lost more than 10% of their body weight or died during the first 2 weeks of infection. However, all Rag2 knockout mice treated with IL-22–specific antibody lost weight very rapidly and became moribund or died around day 10 (**Fig. 5a**). These data suggest that the IL-22 pathway is still active in Rag2 knockout mice and is essential for protecting mice from death during the early phase of *C. rodentium* infection in the absence of adaptive immunity.

IL-22 production in Rag2 knockout mice was comparable to that of WT mice after *C. rodentium* infection (**Fig. 5b**,**c**). In contrast, induction of IL-17A was reduced in Rag2 knockout mice (**Fig. 5b**,**c**). Significant reduction of IL-23 and IL-6 induction was not evident in Rag2 knockout mice (**Supplementary Fig. 5a** online). T and B cells, therefore, are not the sources of IL-22 in this model. Immunohistochemical staining with an IL-22–specific antibody (**Supplementary Fig. 5b**) detected IL-22–positive cells in the colons of WT mice infected with *C. rodentium*, but not in uninfected colons or colons from infected IL-22 knockout mice. To reveal the potential cellular sources of IL-22, we examined IL-22 expression in dendritic cells (CD11c$^+$), macrophage (F4/80$^+$), neutrophils (Gr-1$^+$), and NK cells (DX5$^+$). IL-22–positive cells primarily colocalized with CD11c$^+$ cell clusters in the colons of Rag2 knockout mice (**Fig. 5d**), but not with F4/80$^+$, Gr-1$^+$ or DX5$^+$ cells (data not shown). In addition, IL-23 induced IL-22 production directly from CD11c$^+$ dendritic cells (DCs) *in vitro* (**Fig. 5e**). Taken together, our data demonstrate that DCs may be one of the sources of IL-22 production during *C. rodentium* infection and that IL-23 can directly promote IL-22 production from DCs. We cannot exclude, however, other innate immune cells, especially natural killer cells, as additional sources of IL-22 production in this model.

Reg family proteins mediate mechanisms downstream of IL-22

IL-22 treatment of *ex vivo* colonic tissues from uninfected WT mice induced the upregulation of genes encoding many antimicrobial proteins, including S100A8, S100A9, RegIIIβ, RegIIIγ, haptoglobin, SAA3 and lactotransferrin, as revealed by microarray analysis (**Fig. 6a**, **Supplementary Fig. 6** and **Supplementary Table 1** online). The induction of these genes was confirmed by real-time RT-PCR (**Fig. 6b** and data not shown). During *C. rodentium* infection, however, only S100A8, S100A9, RegIIIβ and RegIIIγ mRNAs were differentially expressed in IL-22 knockout mice compared to WT mice (**Fig. 6c**). All other genes were either not induced or had similar expression levels in colons from WT and IL-22 knockout mice (data not shown). We also found no differences in the expression of defensins, which are important in host epithelial defense[33], between WT and IL-22 knockout mice (data not shown). Of note, the upregulation of RegIIIβ and RegIIIγ observed in WT mice was completely abolished in IL-22 knockout mice (**Fig. 6c**), as well as in IL-23 p19 knockout mice (**Supplementary Fig. 7a** online), after *C. rodentium* inoculation. RegIIIβ and RegIIIγ belong to a family of secreted C-type lectin proteins. We found that other family members, including RegI, RegII, RegIIIα and RegIIIδ (**Supplementary Fig. 7b**), but not RegIV (data not shown), were also upregulated in *C. rodentium*–infected colons, and their induction was completely abolished in IL-22 knockout mice. *In vitro*, RegIIIγ kills Gram-positive bacteria, but not Gram-negative bacteria[34] such as *C. rodentium* (data not shown). To our surprise, exogenous mouse RegIIIγ fusion protein significantly ($P < 0.05$) protected IL-22 knockout mice from weight

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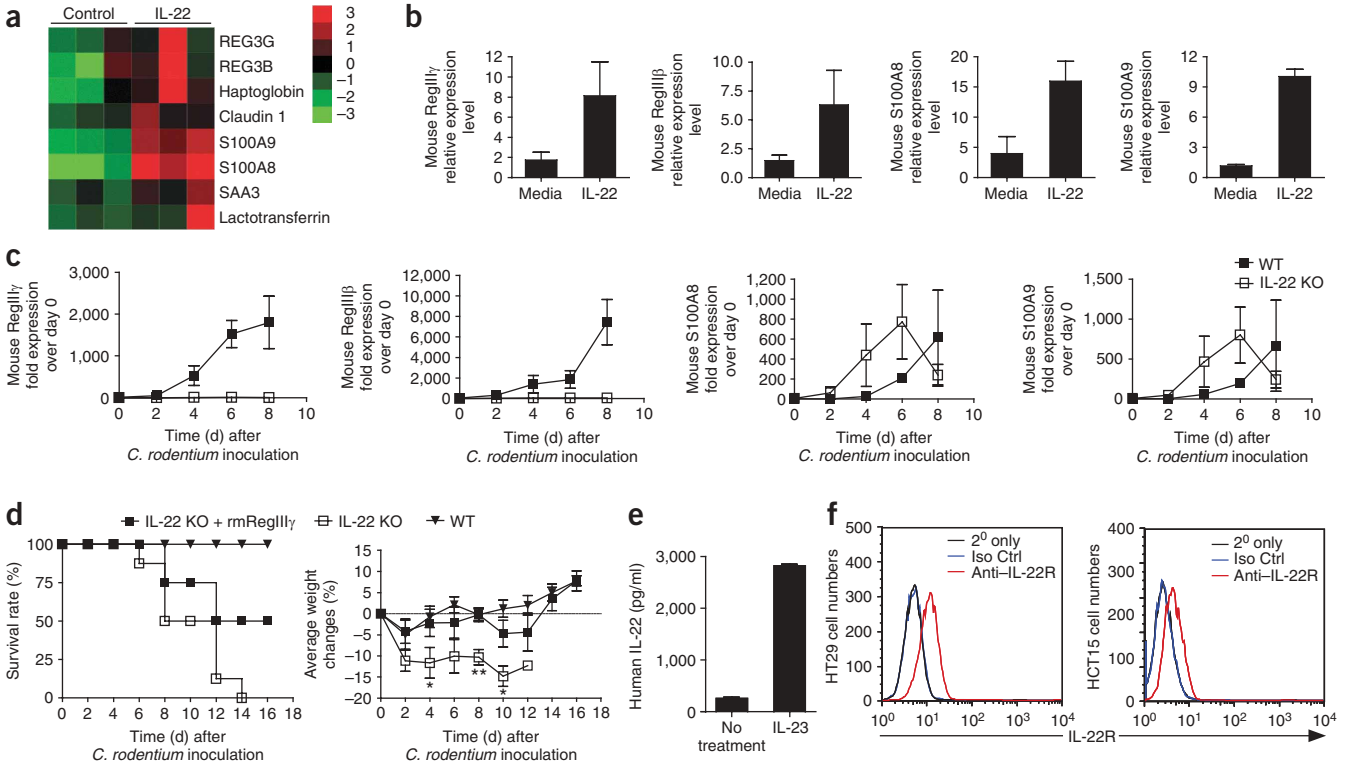

Figure 6 Reg family proteins mediate important host defense mechanisms downstream of IL-22. (**a**,**b**) *Ex vivo* cultures of C57BL/6 mouse colon were treated with 10 ng/ml of IL-22 for 24 h, RNA was isolated and used for microarray analysis (**a**) and real-time RT-PCR analysis (**b**). (**c**) IL-22 KO mice and WT littermates were orally inoculated with *C. rodentium*, and real-time RT-PCR was performed for RegIIIγ (far left), RegIIIβ (middle left), S100A8 (middle right) and S100A9 (far right) expression on RNA isolated from individual mouse colons collected at the indicated time points. (**d**) IL-22 KO mice and WT littermates were orally inoculated with *C. rodentium*. One group of IL-22 KO mice also received recombinant mouse RegIIIγ-Ig fusion protein (rmRegIIIγ). Mice were monitored for survival (left) and weighed (right) at the indicated time points. $^{*}P < 0.05$ and $^{**}P < 0.01$. (**e**) IL-23 directly induces IL-22 production from isolated human DCs, as measured by ELISA. (**f**) IL-22R expression by FACS on HT29 (left) and HCT15 (right) human colonic epithelial cell lines. (**g**) Western blotting shows that IL-22 can induce STAT3 activation in human colon cell lines. P-STAT3, phosphorylated (activated) STAT3. (**h**) Real-time RT-PCR analysis for REGIIIβ (left) and REGIIIγ (right) expression in human colonic epithelial cell lines treated with IL-22. All data are representative of two independent experiments.

loss upon *C. rodentium* infection, and approximately 50% of fusion protein–treated mice survived infection, whereas 100% of control-treated IL-22 knockout mice became moribund or died (**Fig. 6d**). These data support the hypothesis that Reg family proteins, such as RegIIIγ, mediate essential *in vivo* functions downstream of IL-22 upon *C. rodentium* infection.

Finally, we also validated the presence of the IL-23, IL-22 and Reg pathways in a human system. Human IL-23 induces IL-22 production from human DCs (**Fig. 6e**). Primary human colonic epithelial cells (**Fig. 2h**) and the human colonic epithelial cell lines HT29 and HCT15 express IL-22R (**Fig. 6f**). IL-22 induced STAT3 activation and REGIIIβ and REGIIIγ expression in these colonic epithelial cell lines (**Fig. 6g,h**). Importantly, human REGIIIγ fusion protein, like mouse RegIIIγ fusion protein, also lowered the mortality of IL-22 knockout mice to 40% after *C. rodentium* infection (**Supplementary Fig. 7c**). In conclusion, our data imply that the IL-22 pathway may have an essential role in controlling bacterial infections, particularly A/E bacterial infections, in the human gastrointestinal tract.

DISCUSSION

In this study, we examined the functions of IL-17A, IL-17F and several IL-10 family cytokines, including IL-19, IL-20, IL-22 and IL-24, during host defense against *C. rodentium* infection. Although all of these cytokines are quickly induced in the infected colons, only IL-22 is indispensable for host survival during the early phase of infection. IL-23 induces IL-22 production after A/E bacterial infection, which is consistent with our previous *in vitro* results[16]. Although IL-23 has very diverse proinflammatory functions[35], our data clearly establish that induction of IL-22 by IL-23 is crucial for the host to completely resolve *C. rodentium* infection. Notably, whereas IL-6 is required for IL-17 production, it is not required for maximal IL-22 induction in the colon after *C. rodentium* infection.

We did not expect to find that the majority of the IL-22 pathway is still intact in Rag2 knockout mice. Adaptive immunity does still have a role during the early phase of infection, as Rag2 knockout mice lost more weight than did WT mice. Rag2 knockout mice treated with IL-22–neutralizing antibody showed earlier mortality than did similarly treated WT mice (**Figs. 4c** and **5a**). Comparable production of

IL-22 in WT and Rag2 knockout mice excludes T cells, especially T_H17 cells, as a major source of colonic IL-22 during the early phase of *C. rodentium* infection. Our preliminary data suggest that DCs may be a source of IL-22 production; however, other innate immune cells, such as colonic natural killer cells, cannot be excluded as potential sources of IL-22 production during infection. Nonetheless, IL-23 is indispensable for promoting IL-22 production from these innate immune cells.

IL-22 knockout mice have compromised epithelial integrity during *C. rodentium* infection, and IL-22R is specifically expressed on colonic epithelial cells. These data suggest that IL-22 may directly target colonic epithelial cells to induce antimicrobial responses or to prevent colon cells from bacterial invasion and damage. The observation that IL-22 is indispensable for induction of Reg family proteins in colonic epithelial cells further supports this hypothesis. RegIIIβ and RegIIIγ expression increase substantially in response to bacterial colonization, as well as to other inflammatory stimuli in mice[36–38]. A recent study showed that RegIIIγ could directly bind and kill Gram-positive bacteria through its interaction with microbial peptidoglycan carbohydrate *in vitro*[34], and RegIIIβ is able to induce aggregation of *E. coli in vitro*[39]. Currently, we do not have any evidence that either RegIIIβ or RegIIIγ directly kills *C. rodentium*, a Gram-negative bacterium, *in vitro* or *in vivo*. Providing exogenous mouse or human RegIIIγ protein alone, however, is sufficient to markedly reduce the mortality and weight loss of IL-22 knockout mice after infection. Further mechanistic studies will help to explain these different results. Rather than directly killing bacteria in the intestinal lumen, RegIIIβ or RegIIIγ may prevent the invasion of *C. rodentium* deep into colonic crypts, as no differences in bacterial burden were observed in the colons of IL-22 knockout mice versus WT mice (**Fig. 4a**). Alternatively, the RegIIIβ or RegIIIγ proteins may act as autocrine growth factors that have a function in epithelial repair and protection in the setting of intestinal inflammation[37,40,41]. In addition, IL-22 and its downstream factors may also protect intestinal integrity through the regulation of tight junctions and the permeability of colonic epithelial cells[42].

In conclusion, our data clearly show the essential role of IL-22, produced by innate immune cells, during early host defense against A/E bacterial infection. Because IL-22R is broadly expressed in many other types of epithelial cells[14], it is also probably important for protecting many other epithelia from infection by a variety of bacteria. The IL-22 pathway is also active in humans; therefore, IL-22 and its downstream products, including RegIIIβ and RegIIIγ, may be beneficial for the treatment of certain infectious diseases in humans.

METHODS

Mice. We purchased C57BL/6, IL-12 p40 knockout, and IL-6 knockout mice from the Jackson Laboratory and BALB/c and Rag2 knockout mice from Taconic. We generated IL-22 knockout and IL-23 p19 knockout mice as described before[16,43]. IL-17RC knockout and IL-20Rβ knockout mice were generated by Lexicon Pharmaceuticals as described (**Supplementary Figs. 2** and **3** and **Supplementary Methods** online). All animal experiments were approved by the Genentech Institutional Animal Care and Use Committee.

Bacterial strain and infection of mice. We fasted mice for 8 h before oral inoculation with 2×10^9 *C. rodentium* strain DBS100 (ATCC 51459; American Type Culture Collection) in a total volume of 200 μl per mouse. We allowed the mice access to food after the inoculation. We prepared bacteria by shaking at 37 °C overnight in LB broth. We assessed the concentration of bacteria by measuring absorbance at optical density 600 and serially diluted and plated each inoculation culture to confirm the colony-forming units (CFUs) administered.

Tissue collection, histology and colony-forming unit counts. We dissected the colon to the anus from the mice and used the terminal 0.5-cm piece for CFU analysis. We fixed proximal segments in 10% neutral buffered formalin and stained paraffin-embedded tissue sections with H&E to evaluate tissue pathology (**Supplementary Methods**). We weighed and homogenized spleen, liver, mesenteric lymph node and colon. We serially diluted homogenates and plated them in triplicate in MacConkey agar (Remel). *C. rodentium* colonies were identified as pink colonies. We counted colonies after 24 h of incubation at 37 °C.

RNA isolation and real-time reverse transcriptase PCR. We isolated cell and tissue RNA with the RNeasy Mini Kit (Qiagen). We conducted real-time RT-PCR on an ABI 7500 Real-Time PCR system (Applied Biosystems) with primers and probes (described in **Supplementary Methods**) and TaqMan one-step RT-PCR master mix (Applied Biosystems). Samples were normalized to the control housekeeping gene RPL-19 and reported according to the $\Delta\Delta C_T$ method: $\Delta\Delta C_T = \Delta C_{T\ \text{sample}} - \Delta C_{T\ \text{reference}}$.

Dendritic cell isolation and stimulation. We isolated DCs (90–97% purity) with MACS microbeads (Miltenyi Biotec) and cultured them as described (**Supplementary Methods**).

Colon culture enzyme-linked immunosorbent assay. We removed and cultured mouse colons (**Supplementary Methods**)at 37 °C for 24 h. We then collected supernatants for IL-17 and IL-22 ELISA as previously described[16].

Isolation of intraepithelial lymphocytes, lamina propria mononuclear cells and epithelial cells from mouse colon. We isolated IELs, LPMCs and colonic epithelial cells as described[44], with some modifications (**Supplementary Methods**).

FACS analysis. We determined the expression of IL-22R on mouse colonic epithelial cells by FACS analysis of purified primary colonic epithelial cells stained with antibodies as described (**Supplementary Methods**).

Cell culture. We cultured primary human colonic epithelial cells (Celprogen) according to the manufacturer's instructions. HCT15 and HT29 human colonic epithelial cell lines (ATCC) were grown in DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin and GlutaMAX (Invitrogen). We performed western blotting on these cultured cell lines as described (**Supplementary Methods**).

Immunohistochemistry. We harvested colons and fixed them in 10% formalin for 2 h at 23 °C. After a quick wash in PBS, we transferred colons to 30% sucrose and incubated at 4 °C overnight. We froze colons in optimal cutting-temperature medium (Tissue-Tek) and cut 6-μm sections. After rehydration in PBS, we fixed the sections in 4% paraformaldehyde for 10 min and then washed them in PBS. We blocked sections for 1 h in MOM Mouse Ig Blocking Reagent (Vector Laboratories) with 5 μg/ml antibody to CD16/32 and 5 μg/ml mouse IgG1 (BD Biosciences). After blocking, we incubated the sections with Alexa 555–conjugated, IL-22–specific (Genentech mIgG1, clone 8E11) antibody or isotype control mouse IgG1 diluted to 5 μg/ml in 2% BSA, 0.5% Tween-20 and PBS. We carried out antibody incubation for 45 min and an additional blocking step with 2% BSA and 5 μg/ml hamster IgG1 (BD Biosciences) for 30 min. We carried out CD11c-specific antibody staining with clone N418 conjugated to Alexa 488 for 45 min (eBioscience). We mounted slides with Prolong Gold with DAPI (Invitrogen) and acquired images with an Olympus BX61 microscope and Slidebook software (Olympus).

Blockade of mouse interleukin-22 *in vivo*. We intraperitoneally injected IL-22–specific mAb (Genentech Clone 8E11, isotype mouse IgG1)[16] before (day 0) or 8 d after (day 8) *C. rodentium* infection at a dose of 150 μg per mouse every other day. Certain control groups also received isotype control IgG1 mAb.

Reconstitution of RegIIIγ fusion protein *in vivo*. We intraperitoneally injected mouse RegIIIγ-Ig or human REGIIIγ-cFlag fusion protein (**Supplementary Methods**) on 2, 4, 6, 8 or 10 d after *C. rodentium* infection at a dose of 150 μg per mouse each time. Control groups received PBS or isotype control immunoglobulin.

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Microarray analysis. We performed microarray data analysis as described in the **Supplementary Methods** and all expression array data have been deposited in the Gene Expression Omnibus (GEO).

Statistics. We calculated statistical significance by one- or two-way ANOVA or paired *t*-test with Prism software (GraphPad). We considered all P values ≤ 0.05 significant. Unless otherwise specified, all studies for which data are presented are representative of at least two independent experiments.

Accession codes. GEO accession number for microarray data, GSE10010.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

Y.Z. performed the majority of the experimental work. P.A.V. characterized IL-20Rβ knockout mice, developed IHC staining for IL-22 and contributed partly to **Figures 2**, **3**, **4**, **5** and **6**. D.M.D. contributed to the majority of the histological analysis. Y.H. characterized the IL-17RC knockout mice and partly contributed to **Figure 3**. S.M.S. characterized human colon cell lines. Q.G. partly contributed to **Figure 4**. Z.M. and A.R.A. performed all of the microarray and bioinformatic analyses, respectively, and contributed partly to **Figure 6**. N.G. and F.J.d.S. provided and characterized p19 knockout mice and contributed partly to **Figure 1**. W.O. characterized IL-22R expression on various cells. W.O. and Y.Z. devised and planned the project. The manuscript was written by W.O., Y.Z., D.M.D. and P.A.V.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturemedicine/.

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