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Simian immunodeficiency virus–induced mucosal interleukin-17 deficiency promotes *Salmonella* dissemination from the gut

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Abstract

Salmonella typhimurium causes a localized enteric infection in immunocompetent individuals, whereas HIV-infected individuals develop a life-threatening bacteremia. Here we show that simian immunodeficiency virus (SIV) infection results in depletion of T helper type 17 (T_H17) cells in the ileal mucosa of rhesus macaques, thereby impairing mucosal barrier functions to *S. typhimurium* dissemination. In SIV-negative macaques, the gene expression profile induced by *S. typhimurium* in ligated ileal loops was dominated by T_H17 responses, including the expression of interleukin-17 (IL-17) and IL-22. T_H17 cells were markedly depleted in SIV-infected rhesus macaques, resulting in blunted T_H17 responses to *S. typhimurium* infection and increased bacterial dissemination. IL-17 receptor–deficient mice showed increased systemic dissemination of *S. typhimurium* from the gut, suggesting that IL-17 deficiency causes defects in mucosal barrier function. We conclude that SIV infection impairs the IL-17 axis, an arm of the mucosal immune response preventing systemic microbial dissemination from the gastrointestinal tract.

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AUTHORS CONTRIBUTIONS M.R. contributed to the experimental design, contributed partly to Figures 1, and 4b, performed experiments on the host response to *Salmonella* infection in Figures 2 and 6, contributed to bacteriological analysis in Figure 5b,c and collected tissue during the ligated ileal loop surgery. R.L.S. designed and performed ligated ileal loop surgery, histopathological analysis, analyzed data in Figure 3a,c and contributed to writing the article. D.E.V. performed multi-color flow cytometric experiments and analysis for Figures 3d and 4. S.E.W. performed cell isolation for experiments in Figures 3d and 4. R.P.W. performed bacteriologic analysis for experiments in Figure 5a and collected tissue during the ligated ileal loop surgery. I.G. performed mouse experiments, bacteriologic analysis for experiments in Figure 5b,c and analysis of host responses in Figure 6. S.S. performed tissue processing, SIV measurement and IL-17 fluorescent immunohistochemistry experiments, data analysis and preparation of Figure 3b and Supplementary Figure 2. T.A.P. performed experiments in Figure 6g. M.D.G. performed experiments and DNA microarray analysis and generated Figure 1 and Supplementary Figure 1. M.A.G. served as consultant for the presentation of NTS bacteremia in African subjects. J.K.K. served as collaborator on studies with *Il17ra*$^{-/-}$ mice and provided useful comments on the experimental design. S.D. designed and supervised the SIV infections of rhesus macaques, blood sample scheduling, macaque protocols, processing and cell isolations for flow cytometry and DNA microarray analyses. A.J.B. was responsible for the experimental design and supervision of mouse studies, ligated ileal loop experiments in rhesus macaques, macaque protocols and analysis of host responses to *Salmonella* infection. A.J.B. collected tissue during the ligated ileal loop surgery and was responsible for the final manuscript preparation. A.J.B. and S.D. provided financial support for the study and equally contributed to the experimental design, supervision and data interpretation.

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Although nontyphoidal *Salmonella* serotypes (NTS) are common agents causing acute food-borne disease worldwide, it is unusual to isolate them from the blood of adults, because in immunocompetent individuals these pathogens remain localized to the intestine and cause a self-limiting gastroenteritis[1]. However, in people with underlying immunosuppression, NTS may spread beyond the intestine and reach the bloodstream, a serious complication known as NTS bacteremia2. The rise in the number of people with AIDS in sub-Saharan Africa has led to a dramatic increase in the frequency of NTS bacteremia3. In marked contrast to the pre-AIDS era4, NTS is currently a leading cause of hospital admission of adults and among the most common bacterial blood isolates from hospitalized adults in sub-Saharan Africa5, the vast majority of whom are HIV positive[3]. NTS infection in HIV-positive African adults is associated with high acute mortality rates (47%)[6]. Although the occurrence of NTS bacteremia in HIV-positive people is well documented, there are no reports investigating the mechanisms by which HIV infection increases susceptibility.

Human infections with NTS are characterized by a rapidly developing acute inflammatory reaction in the terminal ileum and colon[1]. Initial inflammatory responses elicited by *S. typhimurium* in the intestine are important for the disease outcome, as they enable immunocompetent people to contain bacteria at the site of infection, thereby causing a localized gastroenteritis. The induction of this rapid inflammatory response can be studied by using *S. typhimurium* infection of bovine ligated ileal loops, where inflammation is initiated by bacterial invasion of the intestinal epithelium[7], resulting in translocation into the lamina propria within 1 to 2 h after infection[8]. Within the lamina propria, *S. typhimurium* is observed within mononuclear phagocytes or neutrophils[9]. Stimulation of mononuclear phagocytes with *S. typhimurium in vitro* triggers the induction of a proinflammatory gene expression profile[10]. However, host responses observed *in vitro* may not account for all changes in the gene expression profiles observed *in vivo*, because inflammatory responses may be amplified by paracrine mechanisms in tissue.

Like humans, rhesus macaques (*Macaca mulatta*) develop a localized gastroenteritis in response to infection with *S. typhimurium*[11]. SIV infection of rhesus macaques is an established model for studying human HIV disease[12]. By establishing the loop procedure in the rhesus macaque, we were able to determine how an underlying SIV infection changes host responses elicited by *S. typhimurium*.

RESULTS

T_H17 responses dominate the *Salmonella* gene expression profile

Four young adult healthy rhesus macaques (MK5, MK7, MK8 and MK9) underwent loop surgery. Loops of each macaque were inoculated by injecting either a *S. typhimurium* culture or sterile culture medium (mock infection) into the intestinal lumen, and individual loops of each treatment group were removed surgically at 2, 5 and 8 h after inoculation. We monitored the host response to *S. typhimurium* infection *in vivo* at defined early time points after infection and compared it to responses in mock-infected tissue collected from the same macaque at the same time point. *S. typhimurium* infection resulted in marked enteritis and fluid accumulation at 5 and 8 h after inoculation. To characterize mucosal responses to *S. typhimurium* infection, we performed gene expression profiling by comparing mRNA levels from *S. typhimurium*–infected or mock-infected loops (Fig. 1). The gene expression profile induced by *S. typhimurium* was dominated by responses linked to immunity and inflammation. The strongest up-regulation of gene expression was observed for IL-22, followed by IFN-γ and IL-26 (Fig. 1c and data not shown). IL-22 and IFN-γ synergize to induce inducible nitric oxide synthase (iNOS) expression[13], whose mRNA levels were markedly increased after *S. typhimurium* infection (Fig. 1c). Furthermore, *S. typhimurium* induced a marked upregulation of IL-17

expression and the expression of genes induced by IL-17, including those encoding lipocalin-2 (LCN2)14, CCL20 (MIP-3α)15, CXCL8 (IL-8)16 and CXCL10 (ref. 17). To verify changes in cytokine expression detected by gene expression profiling, we determined the mRNA levels of IL-17 and IL-17–regulated genes (those coding for MIP-3α, lipocalin-2 and IL-8) in samples from all four macaques by real-time PCR. Cytokines whose expression is not affected by IL-17, including interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), IL-6, IL-23 p19, IL-12 and IL-23 p40, and transforming growth factor-β (TGF-β) were analyzed for comparison (Fig. 2). These results show a marked increase in the mRNA levels of all proinflammatory cytokines after *S. typhimurium* infection.

All macaques used in this study were raised in a *Salmonella*-free colony and tested negative for *Salmonella* serotypes. Although there was no evidence of previous exposure of the macaques to this pathogen, mRNAs whose expression was upregulated most strongly at a very early time point (that is, at 5 h) after *S. typhimurium* infection included IL-22, IL-26 and IL-17 (Fig. 1c). The main cellular sources of these cytokines are T cells[18–20]. The importance of T cells in generating early inflammatory changes in response to *S. typhimurium* infection has not been apparent from previous studies[10,21]. Thus, the increased expression of these cytokines attracted our attention because T cell depletion has been linked to an inability of HIV-affected individuals to contain *S. typhimurium* at the site of infection (that is, the intestinal mucosa), resulting in the development of a life-threatening bacteremia[22]. Therefore, we wanted to determine whether the IL-17 responses elicited by *S. typhimurium* were altered by an underlying SIV infection.

Salmonella-induced T_H17 responses are blunted by SIV infection

Four young adult healthy rhesus macaques (MK3, MK4, MK6 and MK10) were infected with SIV (SIV mac251), and blood samples were collected to monitor the number of $CD4^+$ lymphocytes in the peripheral blood and the plasma viral load (Fig. 3). SIV-infected macaques underwent surgery between 6 and 10 weeks after inoculation. Compared to the SIV-negative macaques (see above), SIV-infected macaques had similar $CD8^+$ counts in the peripheral blood at the time of surgery, whereas the numbers of $CD4^+$ lymphocytes were lower. Loops of each macaque underwent *S. typhimurium* infection or mock infection as described above.

To identify mucosal responses elicited by *S. typhimurium* that were modified by an underlying SIV infection, gene expression profiling was performed with mRNA from *S. typhimurium*–infected and mock-infected loops collected from an SIV-infected macaque (MK6; Fig. 1d). The mRNA levels for genes involved in epithelial repair and maintenance were markedly upregulated in *S. typhimurium*–infected tissue from the SIV-negative macaque (MK5), and these responses were blunted by SIV infection (MK6) (Supplementary Fig. 1 online). Decreased expression levels of genes regulating epithelial barrier maintenance are also observed in people with HIV[23]. However, mRNA expression of genes linked to cell death and stress response were induced more strongly during *S. typhimurium* challenge of the SIV-infected macaque than the control macaque. Notably, mRNA expression of IL-22, IL-17 and genes regulated by IL-17 was blunted in the SIV-infected macaque compared to the SIV-negative macaque (Fig. 1c). To verify changes in mRNA levels detected by gene expression profiling, samples from all four SIV-infected macaques taken throughout the time course of *S. typhimurium* infection were analyzed by real-time PCR (Fig. 2). No significant differences between SIV-infected and SIV-negative macaques were observed for the *S. typhimurium*–mediated induction of IFN-γ, TNF-α, TGF-β, IL-6, IL-23 p19 or IL-12 and IL-23 p40 expression. In contrast, *S. typhimurium* challenge resulted in significantly ($P < 0.05$) higher mRNA levels of IL-17, IL-22, MIP-3α, LCN2 and IL-8 in SIV-negative compared to SIV-infected macaques. These data suggest that SIV-positive macaques develop a cytokine

deficiency during *S. typhimurium* infection because IL-17 responses, which dominate the gene expression profile elicited by this bacterial pathogen, are blunted in these macaques.

SIV-induced CD4$^+$ depletion reduces IL-17 production

IL-17 production detected by fluorescence microscopy of loop tissue suggested a cellular localization for IL-17 in mock-infected loops (Supplementary Fig. 2 online). Staining with antibody to Il-17 was markedly increased in *S. typhimurium*–infected loops of control macaques but produced a diffuse background signal, presumably because much of this cytokine is released during infection (Supplementary Fig. 2). To study the cellular sources of IL-17, we performed *in vitro* experiments with lymphocyte populations in which the release of IL-17 was prevented by treatment with brefeldin A.

Because T cells are a major source of IL-17, we investigated whether SIV-mediated T cell depletion affects IL-17 production in mucosal T cells. Memory CD4$^+$ T cell depletion in the intestinal mucosa occurs within the first few weeks after HIV infection in humans or SIV infection in rhesus macaques[24–28]. Lymphocyte populations isolated from the ileal mucosa of four SIV-infected macaques (MK3, MK4, MK6 and MK10) and six control macaques (MK1, MK2, MK5, MK7, MK8 and MK9) were analyzed for expression of surface markers (CD3, CD4, CD8 and CD95) and for their capacity to produce IL-17 and IFN-γ. To this end, one 10-cm segment of the ileum that had not been exposed to *S. typhimurium* was collected from SIV-negative and SIV-infected macaques, and lamina propria lymphocytes were isolated and analyzed by flow cytometry. The CD4$^+$ T cell population was markedly depleted in the intestinal mucosa of SIV-infected macaques ($P = 0.0005$), and this was accompanied by a relative increase in the fraction of CD8$^+$ T cells (Fig. 4a). For the eight macaques that underwent loop surgery (MK3–10), the severity of CD4$^+$ T cell depletion in the ileal mucosa (measured in tissue that had not been exposed to *S. typhimurium*) correlated directly with the magnitude of IL-17 mRNA expression induced in the same macaque 5 h after *in vivo* challenge of the loops with *S. typhimurium* (Fig. 4b).

We next investigated whether ileal T cell populations in SIV-negative macaques ($n = 6$) differed from those in SIV-infected macaques ($n = 4$) in their capacity to produce IL-17. To this end, lamina propria lymphocytes from each macaque (isolated from tissue that had not been exposed to *S. typhimurium*) were stimulated *ex vivo* with phorbol 12-myristate 13-acetate (PMA) and ionomycin in the presence of brefeldin A (to prevent cytokine secretion) and then analyzed by multicolor flow cytometry to assess IL-17 and IFN-γ production by T cells (Fig. 4c,d). The majority of lamina propria CD3$^+$ T cells showed high expression of CD95, indicative of a memory phenotype. The fraction of CD3$^+$ T cells that showed a T_H1 phenotype (that is, IFN-γ–producing CD4$^+$ T cells) was significantly ($P = 0.019$) smaller in SIV-infected macaques (1.9% on average) compared to control macaques (9.2% on average; Fig. 4d). This depletion of T_H1 cells did not, however, result in reduced IFN-γ expression during *S. typhimurium* infection (Fig. 2), presumably because these cells are not an important source of this cytokine at early times after infection. No significant differences between SIV-infected and SIV-negative macaques were observed in IL-17 production by CD8$^+$ T cells in response to PMA-ionomycin stimulation. In contrast, the average percentage of lamina propria CD3$^+$ T cells that was IL-17–producing and CD4$^+$ (T_H17 cells) was significantly ($P = 0.005$) lower in SIV-infected macaques (0.12% on average) compared to control macaques (3.7% on average; Fig. 4c). These data show that SIV infection significantly lowers the number of T_H17 cells in the intestinal mucosa, which is predominantly the result of the overall CD4$^+$ T cell depletion caused by the infection. The finding that SIV-mediated memory CD4$^+$ T cell depletion significantly ($P < 0.01$) lowers the number of T_H17 cells provides a plausible explanation for the blunted IL-17 response elicited by *S. typhimurium* in SIV-infected macaques (Fig. 2).

IL-17 deficiency accelerates *Salmonella* dissemination

At the end of the experimental procedure (8 h after inoculation of loops), samples of the draining mesenteric lymph nodes of rhesus macaques were processed for quantification of *S. typhimurium*. SIV-infected macaques had significantly higher numbers (330-fold increase, $P = 0.019$) of *S. typhimurium* in the mesenteric lymph node than SIV-negative macaques (Fig. 5a). These data provide a noteworthy illustration of the magnitude by which SIV-induced defects in mucosal barrier function increase the ability of *S. typhimurium* to disseminate within its host.

To determine whether there is a causal link between the defect in IL-17 mRNA expression (Fig. 2) and increased bacterial dissemination (Fig. 5a), we used a well-established mouse model of *S. typhimurium* infection. Whereas mice normally develop few inflammatory changes in the intestinal mucosa during *S. typhimurium* infection, pretreatment with streptomycin drastically exacerbates neutrophil influx and inflammation in the cecal mucosa[29]. By using this model, we have previously shown that Il-17 mRNA expression is increased 150-fold in the cecal mucosa of streptomycin–pretreated mice by 48 h after *S. typhimurium* infection[30]. To assess the importance of IL-17 in controlling *S. typhimurium* infection, we pretreated two groups of six IL-17 receptor–deficient mice (*Il17ra*$^{-/-}$ mice) bred on a C57BL/6 background with streptomycin and inoculated them with *S. typhimurium* or sterile LB broth. As a control, two groups of C57BL/6 mice were treated identically. Organs were collected 48 h after infection (Fig. 5b,c). A significantly increased ability of *S. typhimurium* to disseminate to the mesenteric lymph nodes (40-fold, $P = 0.001$) and the spleens (42-fold, $P = 0.009$) was detected in *Il17ra*$^{-/-}$ mice compared to C57BL/6 mice (Fig. 5c). These data provide direct evidence that the IL-17–IL-17RA axis limits bacterial translocation from the intestinal mucosa to systemic sites of infection.

We further investigated the host response elicited by *S. typhimurium* in *Il17ra*$^{-/-}$ mice by measuring cytokine responses in the cecal mucosa by real-time PCR. *S. typhimurium* infection resulted in similar IFN-γ and IL-23 p19 mRNA levels in C57BL/6 mice as in *Il17ra*$^{-/-}$ mice (Fig. 6). A compensatory increase in Il-17 mRNA levels was observed during *S. typhimurium* infection of *Il17ra*$^{-/-}$ mice. Compared to infection of *Il17ra*$^{-/-}$ mice, *S. typhimurium* infection of C57BL/6 mice elicited significantly increased mRNA levels of lipocalin-2 (eightfold, $P = 0.003$). These data suggest a cause-and-effect relationship between blunted Il-17 responses in SIV-positive macaques and the inability of these macaques to increase lipocalin-2 expression in response to *S. typhimurium* infection (Fig. 2). *S. typhimurium* elicited significantly higher mRNA levels of the keratinocyte-derived chemokine (Kc; threefold, $P = 0.016$), a murine neutrophil chemoattractant related to CXCL1, in C57BL/6 mice than in *Il17ra*$^{-/-}$ mice (Fig. 6). Increased expression of Kc mRNA in the cecal mucosa was accompanied by an increased neutrophil influx detected in histological sections from the cecal mucosa of C57BL/6 mice compared to *Il17ra*$^{-/-}$ mice. In summary, our data show that IL-17 is required for the full induction of responses that lead to neutrophil influx and the production of antimicrobials in the cecal mucosa during *S. typhimurium* infection.

DISCUSSION

To our knowledge, this study is the first to investigate the gene expression profile elicited by *S. typhimurium* in the ileal mucosa *in vivo*. We found that in contrast to gene expression profiling performed with macrophages or epithelial cells[10,21], *in vivo* gene expression profiling revealed that T cell products, including IL-17 and IL-22, are among the genes whose expression is upregulated most strongly during *S. typhimurium* infection. Studies of a mouse model of *Klebsiella pneumoniae* lung infection show that bacterial stimulation of pattern recognition receptors on mononuclear cells results in IL-23 production, which in turn triggers the production of IL-17 by T cells[31,32]. This paracrine IL-23–mediated mechanism also triggers

the release of IL-22 and IL-17 from T cells in other models of inflammation[33,34]. These studies suggest that T_H17 cells amplify signals (for example, IL-23) generated by mononuclear cells after encounter of invasive bacteria in the intestinal mucosa. The existence of such a paracrine amplification loop would explain why IL-17 responses were among the most prominent changes in gene expression observed in the ileal mucosa during *S. typhimurium* infection in this study.

Here we show that SIV infection selectively blunts IL-17 responses elicited by *S. typhimurium* in rhesus macaques. This is probably caused by a general depletion of $CD4^+$ T cells in the ileal mucosa, as indicated by the similar depletion of both T_H1 and T_H17 populations. Our data specify a concrete immune defect that impairs the ability of SIV-infected macaques to orchestrate a normal mucosal inflammatory response to *S. typhimurium* infection. In our study, an underlying SIV infection markedly increased the ability of *S. typhimurium* to disseminate to the mesenteric lymph node of rhesus macaques. Experiments with *Il17ra*$^{-/-}$ mice demonstrated that defective IL-17 responses accelerate bacterial translocation to mesenteric lymph node and spleen. These data suggested that an important function of IL-17 is to orchestrate immune responses that limit bacterial dissemination from the intestinal mucosa. SIV selectively impaired this arm of the immune response, which provides an attractive explanation as to why people with HIV are at an increased risk of developing NTS bacteremia[35–37]. This SIV-mediated defect in mucosal barrier function may also help explain the presence of lipopolysaccharide in the circulation of people with HIV[38].

It has been proposed that one function of T_H17 cells is to regulate innate immune responses by producing cytokines[20]. IL-22 and IL-17 target epithelial cells and modulate their expression of antimicrobial peptides[18,39], migration and cell turnover in the intestinal epithelium40,41; they also modulate intestinal proinflammatory responses40,42. Among the cytokines whose expression is induced by IL-17 in epithelial cells are neutrophil chemoattractants such as CXCL8 (IL-8)[16]. Compared to wild-type mice, *Il17ra*$^{-/-}$ mice showed reduced mRNA levels of the neutrophil chemoattractant Kc and reduced neutrophil recruitment into the cecal mucosa during *S. typhimurium* infection. Finally, IL-17 induces the production of granulocyte colony–stimulating factor[43], which, in turn, is required for neutrophil functionality[44]. Neutrophils from people with HIV show reduced antimicrobial activity[45], and this defect can be restored by treatment with granulocyte colony–stimulating factor[46,47]. It is currently not clear which of the responses controlled by IL-17 is most important for limiting *S. typhimurium* dissemination from the intestinal mucosa. The fact that neutropenia is associated with NTS bacteremia in other clinical settings[48] points to a reduced neutrophil recruitment, reduced neutrophil functionality, or both as possible causes for a defect in mucosal barrier function in people with HIV. NTS bacteremia is commonly found in HIV-infected individuals when $CD4^+$ T cell counts in the blood fall below 200/μl[5], which suggests a contribution of systemic defects, possibly because depletion of T_H17 cells at extraintestinal sites may also impair neutrophil functionality in regional lymph nodes or in the circulation. Further work is necessary to precisely pinpoint the defect in mucosal barrier function caused by IL-17 deficiency.

METHODS

Animal experiments

All experiments were approved by the Institutional Animal Care and Use Committee at the University of California, Davis. We used eight healthy, *Salmonella*-free, male rhesus macaques ranging from 2 to 4 years of age (MK3–10) for ligated ileal loop surgery. We isolated lamina propria T cells from all macaques undergoing loop surgery and two additional macaques (MK1 and MK2). We inoculated four macaques (MK3, MK4, MK6 and MK10) intravenously with previously titrated frozen stocks of SIV mac251. We determined plasma viral loads as previously described[49]. Macaques were pre-anesthetized with ketamine (10 mg/kg; Parke-

Davis), followed by placement of an endotracheal tube and maintenance of the anesthesia with isofluorane. When needed, we kept the macaques under a positive-pressure respirator. We performed a laparotomy, exposing the ileum and ligating 13 loops with an average of 4 cm in length, leaving 1-cm spacer loops in between. We inoculated the loops by intralumenal injections of 1 ml of either sterile LB broth or a logarithmically grown culture containing 1×10^9 colony-forming units (CFU) of wild-type *S. typhimurium* (IR715)[30]. We collected loops at 2, 5, or 8 h after inoculation.

We streptomycin-pretreated and orally infected groups of eight female C57BL/6 mice or six female *Il17ra*$^{-/-}$ mice (B6.IL-17R mice, Taconic), aged 8–10 weeks, with *S. typhimurium* as described previously[30]. At 48 h after infection, we killed the mice and collected the organs for mRNA isolation and bacteriology. A veterinary pathologist performed blinded histopathological evaluation of H&E-stained sections from the cecum.

Bacteriology

We collected two 6-mm biopsy punches from each loop. At the end of the surgery, we collected two biopsy punches (6-mm) from the mesenteric lymph node. We incubated biopsy punches for 1 h in PBS containing 50 mg/l of gentamicin, homogenized them, serially diluted the homogenate and plated on LB agar plates containing the appropriate antibiotics.

RNA analysis

We collected two 6-mm biopsy punches from each loop for RNA isolation, which provided comparable tissue samples across different areas of the intestinal mucosa (gut-associated lymphoid tissue in rhesus macaques was composed of randomly distributed individual lymphoid follicles). We performed RNA extraction from intestinal biopsy punches from macaques or organs collected from mice as previously described[30].

We monitored gene expression profiles in ileal tissues with newly developed rhesus macaque genome–specific high-density oligonucleotide microarrays (Affymetrix). The arrays contained probe sets representing over 20,000 rhesus macaque genes. To minimize the occurrence of false positives in the array data, we used a minimum twofold difference in mRNA levels (*P* value <0.05; 95% confidence) between control and experimental samples as a criterion for identifying a change in gene expression. We determined statistical confidence through analysis of at least 11 independent 25-nucleotide oligonucleotide probes for each gene in each sample. We performed real-time PCR as described previously[30] with primers listed in Supplementary Table 1 online.

Fluorescence microscopy

We stained rhesus ileal sections with rabbit polyclonal antibody to human IL-17 (Santa Cruz Biotechnology) and FITC-conjugated antibody to rabbit IgG. We mounted sections with Slow Fade with DAPI (Invitrogen) and captured images by confocal laser microscopy using LSM 5 and PASCAL software (Zeiss)[23].

Cell isolation, stimulation and flow cytometry

For each macaque, we collected one 10-cm segment of the terminal ileum at surgery. We incubated the tissue in 2.5 mM EDTA, washed it twice with PBS and digested with collagenase type IV (Sigma). After filtering the cell suspension over glass wool, we washed the cells and incubated them with or without 50 ng/ml PMA and 1,000 ng/ml ionomycin in the presence of 0.01 mg/ml brefeldin A for 6 h. We blocked the cells with 2% human γ-globulin (Sigma) and then stained with Amcyan live amine dye (Invitrogen). We used antibodies to CD3 (allophycocyanin (APC)-Cy7–labeled; SP34.2), CD4 (Pacific Blue–labeled; OKT4), CD8

(APC-Cy5.5-labeled; 3B5), γδ T cell receptor (phycoerythrin-labeled; 5A6.E9) and CD95 (phycoerythrin-Cy5–labeled; DX2), followed by intracellular staining with FITC-labeled antibody to IL-17 (eBio64CAP17) and APC-labeled antibody to IFN-γ (4SB3) using 2 × Perm/Fix solution (BD). We performed multicolor immunophenotyping on a modified LSRII with a minimum of 500,000 events collected.

Statistical analyses

We analyzed microarray data using model-based algorithms (dChip, http://biosun1.harvard.edu/complab/dchip) and *t*-tests. We performed biological analysis of microarray data with the Affymetrix NetAFFX web interface and the DAVID (http://david.abcc.ncifcrf.gov/) annotation tool. Statistically over-represented ($P < 0.05$) biological processes within sub-clusters were identified with EASE (http://david.abcc.ncifcrf.gov/). Fold changes in mRNA levels measured by real-time PCR and CFU numbers underwent logarithmic transformation, and percentage values underwent angular transformation before ANOVA followed by either Student's *t*-test or the Student-Newman-Keuls test.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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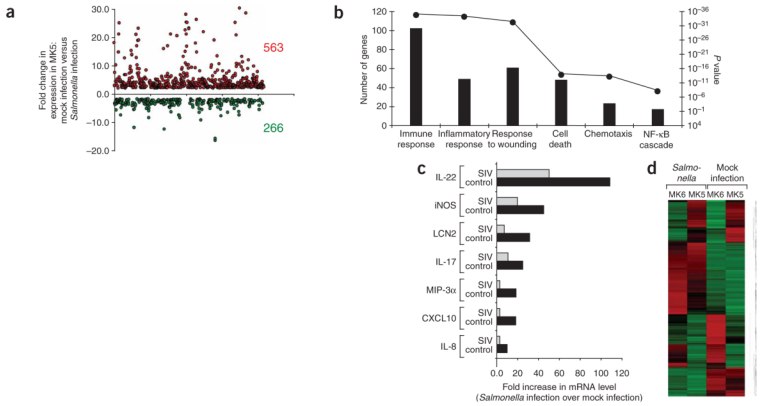

Figure 1.

Gene expression profiling of the host response to *S. typhimurium* infection. (**a,b**) Host response elicited 5 h after *S. typhimurium* infection in a healthy young adult macaque (MK5). (**a**) The graph shows the fold changes in gene expression for genes that were significantly ($P < 0.05$) upregulated (563) or downregulated (266) during *S. typhimurium* infection compared to mock-infected tissue. (**b**) Genes with significantly ($P < 0.05$) altered expression were subjected to hierarchical clustering, followed by functional and statistical analysis of the genes in each subcluster. The number of genes in each category (bars) and the corresponding *P* values for each biological process (filled circles) are indicated. (**c,d**) Comparison of host responses elicited 5 h after *S. typhimurium* infection in an SIV-infected macaque (MK6) compared to a control macaque (MK5). (**c**) IL-17 responses elicited 5 h after *S. typhimurium* inoculation of loops in an SIV-infected macaque (MK6) compared to an SIV-negative control macaque (MK5). (**d**) A heat diagram of changes in gene expression detected in *S. typhimurium*–infected tissue compared to mock-infected control tissue. Relative increase (red) or decrease (green) of mRNA level is shown.

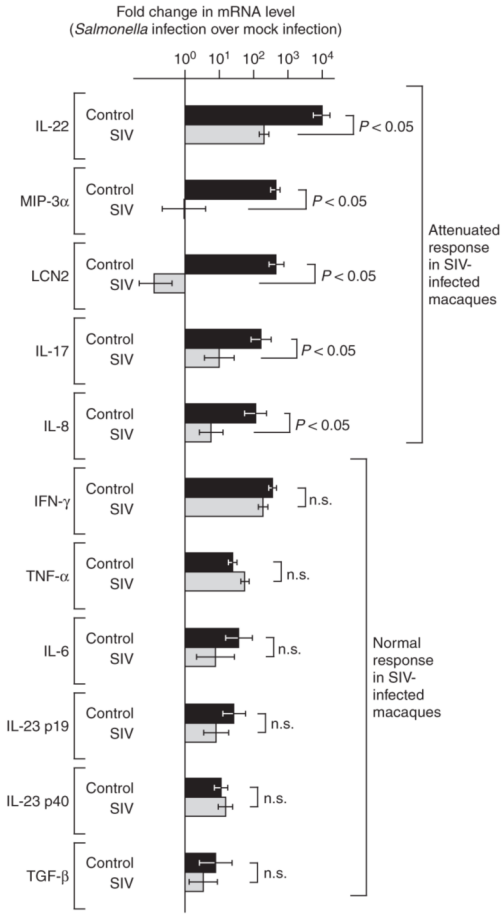

Figure 2.

Cytokine expression elicited by *S. typhimurium* in SIV-infected ($n = 4$) and SIV-negative control macaques ($n = 4$) 5 h after infection. Fold changes in gene expression observed in a *S. typhimurium*–infected loop compared to a mock-infected loop from the same macaque were determined. Data are shown as geometric means of fold-increases ± s.e.m. Statistically significant ($P < 0.05$) differences are indicated. n.s., not significant.

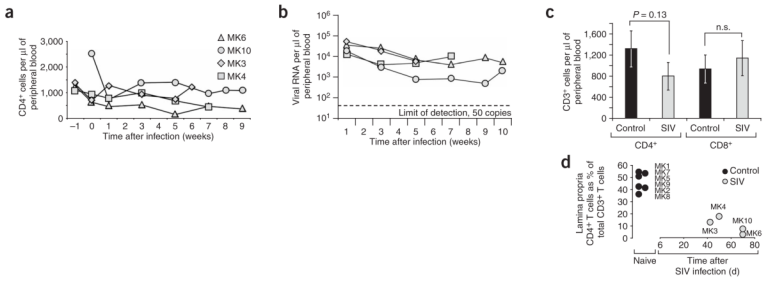

Figure 3.

Kinetics of lymphocyte depletion and viral replication in SIV-infected macaques compared to naive controls. (**a**) $CD4^+$ T cell counts in peripheral blood of SIV-infected macaques (MK3, MK4, MK6 and MK10) over the time course of the experiment. (**b**) Viral loads in peripheral blood of SIV-infected macaques over the time course of the experiment. A dashed line indicates the limit of detection. (**c**) $CD4^+$ and $CD8^+$ T cell counts in peripheral blood of naive controls compared to SIV-infected macaques at the time of surgery. (**d**) Fraction of $CD4^+$ T cells in the ileal lamina propria of naive controls compared to SIV-infected macaques at the time of surgery.

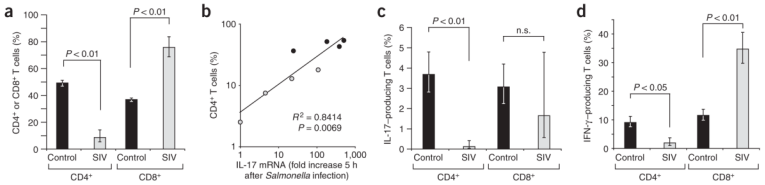

Figure 4.

Analysis of lamina propria T lymphocytes. (**a**) T cells in the ileal lamina propria of SIV-infected macaques ($n = 4$) compared to naive controls ($n = 6$). The *y*-axis indicates the numbers of $CD4^+$ or $CD8^+$ T cells as percentage of the total number of lamina propria $CD3^+$ Tcells. (**b**) Correlation between the fraction of $CD4^+CD8^-$ T cells and the fold increases in IL-17 expression elicited in the ileal mucosa 5 h after *S. typhimurium* infection of SIV-infected macaques ($n = 4$) or SIV-negative macaques ($n = 4$). The y-axis indicates the numbers of $CD4^+$ T cells as percentage of the total number of lamina propria $CD3^+$ Tcells. (**c,d**) Cytokine expression by $CD3^+$ lamina propria T cells from SIV-infected macaques ($n = 4$) or naive controls ($n = 6$) in response to PMA-ionomycin stimulation. (**c**) The *y*-axis indicates the numbers of IL-17–producing $CD4^+$ or $CD8^+$ T cells as percentage of the total number of lamina propria $CD3^+$ Tcells. (**d**) The *y*-axis indicates the numbers of IFN-γ–producing $CD4^+$ or $CD8^+$ T cells as percentage of the total number of lamina propria $CD3^+$ T cells. Bars represent geometric means ± s.e.m. Statistically significant ($P < 0.05$) differences are indicated.

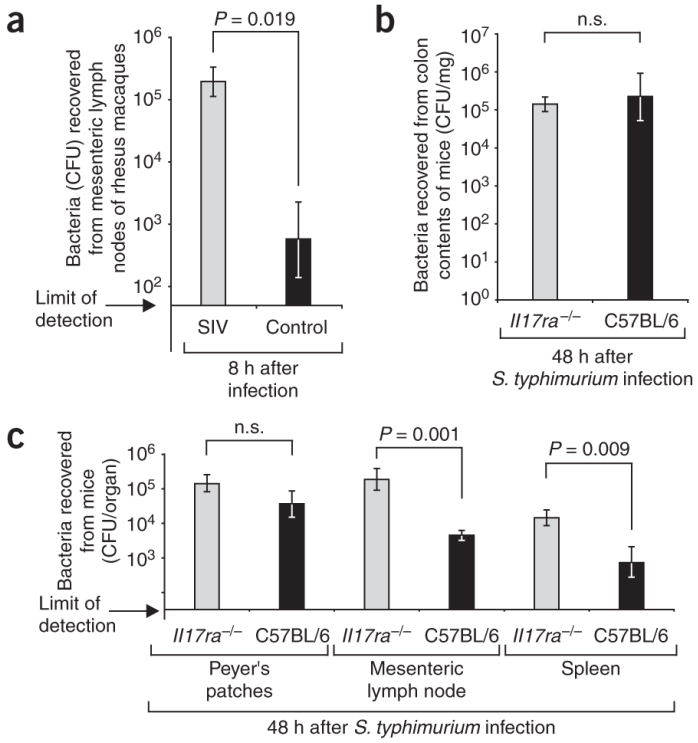

Figure 5.

Bacterial translocation from the intestinal mucosa to internal organs in rhesus macaques and mice. (**a**) Recovery of *S. typhimurium* from the mesenteric lymph nodes of macaques 8 h after infection of ligated ileal loops. Data represent geometric means from control macaques or SIV-infected macaques ± s.e.m. (**b,c**) Recovery of *S. typhimurium* from the cecum (**b**) or internal organs (**c**) of C57BL/6 mice or *Il17ra*$^{-/-}$ mice 48 h after infection. Data represent geometric means ± s.e.m. Statistically significant ($P < 0.05$) differences are indicated.

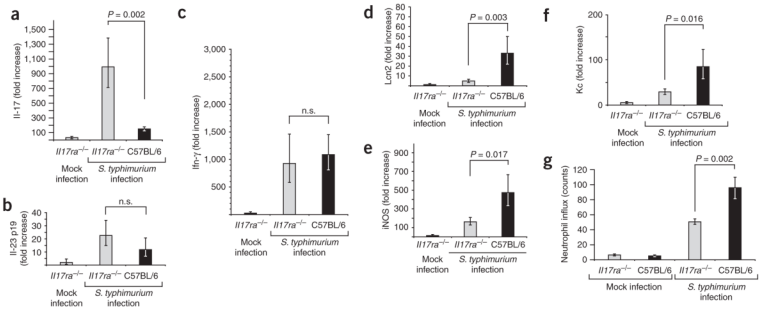

Figure 6.

Host responses elicited 48 h after *S. typhimurium* infection in the cecal mucosa of C57BL/6 mice or*Il17ra*$^{-/-}$ mice. (**a**–**f**) Data are expressed as fold changes of mRNA levels over mRNA levels detected in mock-infected C57BL/6 mice. Data represent geometric means ± s.e.m. Statistically significant ($P < 0.05$) differences are indicated. (**g**) Numbers of neutrophils per microscopic field (*y*-axis) were determined by a veterinary pathologist during a blinded examination of slides from the cecal mucosa. Data represent means ± s.e.m. Statistically significant ($P < 0.05$) differences are indicated.