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Roles for T and NK Cells in the Innate Immune Response to Shigella flexneri

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Shigella flexneri, an enteroinvasive Gram-negative bacterium, is responsible for the worldwide endemic form of bacillary dysentery. The host response to primary infection is characterized by the induction of an acute inflammation, which is accompanied by polymorphonuclear cell (PMN) infiltration, resulting in massive destruction of the colonic mucosa. However, PMN play a major role in the recovery from primary infection, by restricting the bacterial infection at the intestinal mucosa. In this study, we assessed the roles for T and NK cells in the control of primary S. flexneri infection, using an alymphoid mouse strain (Rag°γ°) devoid of B, T, and NK cells. Using the mouse pulmonary model of Shigella infection, we showed that alymphoid Rag°γ° mice were highly susceptible to S. flexneri infection in comparison with wild-type (wt) mice. Whereas PMN recruitment upon infection was similar, macrophage recruitment and production of proinflammatory cytokines were significantly decreased in Rag°γ° mice compared with wt mice. Upon selective engraftment of Rag°γ° mice with polyclonal αβ T cells, but not with αβ T cells from IFN-γ°, S. flexneri infection could be subsequently controlled. Rag° mice devoid of B and T cells but harboring NK cells could control infection. Local IFN-γ production by T and NK cells recruited to the lung was demonstrated in S. flexneri-infected wt mice. These data demonstrate that both αβ T cells and NK cells contribute to the early control of S. flexneri infection through amplification of an inflammatory response. This cellular lymphocyte redundancy assures IFN-γ production, which is central to innate immunity against Shigella infection. The Journal of Immunology, 2005, 175: 1735–1740.

Acute infectious colitis remains a major pediatric problem of worldwide concern, because it represents a significant public health burden among the larger group of diarrheal diseases with the highest mortality rate. Among the different causes of acute colitis of infectious origin, shigellosis (also called bacillary dysentery) is a highly infectious disease producing a spectrum of symptoms, from watery to severe dysentery with fever, abdominal cramps, bloody, mucopurulent diarrhea, and marked systemic toxicity. These symptoms largely result from bacterial invasion into the colonic and rectal mucosa. Shigella, the causative agent of the disease, is a Gram-negative enteroinvasive bacterium that has the capacity to invade, disrupt, and cause inflammatory destruction of the intestinal epithelial barrier. However, at the expense of causing tissue destruction, the local acute inflammatory response to Shigella limits the infection at the intestinal level, thus allowing recovery from primary infection (1).

Recent studies have shown that Shigella-induced inflammation occurs partly in response to epithelial cell activation by intracellular and extracellular bacterial products, and partly as a consequence of the induction of apoptosis of phagocytic cells, such as macrophages. Both mechanisms lead to the production of proinflammatory cytokines and chemokines, which are mediators of tissue damage (2). Within a few hours of Shigella invasion in humans, Shigella-induced IL-8 production recruits an influx of inflammatory cells to the mucosa, consisting predominantly of neutrophils but also monocytes (3). Neutrophils play a major role in the recovery from primary infection (4), by killing the bacteria through an original process, consisting in interfering with the escape of Shigella from the phagocytic vacuole (in contrast to what occurs in macrophages in which bacterial escape is highly efficient), thus facilitating the killing of bacteria by antimicrobial peptides and enzymes contained in neutrophil granules (5). Monocytes have also been shown to prevent phagosomal escape and to kill Shigella in vitro (6).

Lymphoid cells (particularly T cells) have also been observed in rectal mucosa of infected patients during the acute phase of shigellosis (7). T cell activation in peripheral blood during the course of the disease has also been reported (8). Whereas a role of specific T cell-mediated immunity to Shigella reinfecion has been reported (9), little data are available on the role of T and NK cells in the recovery of primary infection (10). Therefore, the purpose of this study was to analyze the contribution of these lymphoid cells in controlling bacterial infection in a mouse model of primary shigellosis. The murine pulmonary model of Shigella infection was used (11, 12), which mimics the acute intestinal inflammation observed in man. Mice infected intranasally (i.n.)3 with live virulent Shigella develop an acute bronchopneumonia characterized by a massive intra- and peribronchial PMN infiltrate, in addition to alveolitis. Shigella infections were performed in Rag°γ° mice, an alymphoid mouse strain that is deficient in both the RAG-2 (Rag) and the common cytokine receptor γ-chain (γc). Rag°γ° mice are therefore devoid of B, T, and NK cells (13). This mouse strain can be selectively reconstituted with specific lymphoid cell types, thus

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3 Abbreviations used in this paper: i.n., intranasally(ly); γc, common cytokine receptor γ-chain; MPO, myeloperoxidase; wt, wild type; PMN, polymorphonuclear neutrophil; DC, dendritic cell.
allowing the analysis of the capacity of the engrafted cells to function in vivo (14). Shigella infections were also performed in Rag−/− mice that harbor NK cells but are devoid of B and T cells. Using this approach, we report here the role of T and NK cells, and the cytokines they produce, in the recovery of primary Shigella flexneri infection.

Materials and Methods

Bacterial strain and growth conditions

M90T-Sm, an invasive streptococcal isolate of S. flexneri serotype 5, is the virulent strain used in this study (15). Bacteria were grown in Luria broth at 37°C with aeration. For mouse infections, the bacteria were resuspended in a 0.9% NaCl solution. The concentration used for infection was calculated as follows: 1 OD at 600 nm corresponds to 5 × 10^7 bacteria/ml.

Mice

Rag−/− and Rag+/+ mice both on the C57BL/6 background (H-2b) have been previously described (13, 16). IFN-γ-deficient mice (IFN-γ−/−) were purchased from The Jackson Laboratory, and C57BL/6 mice from Janvier. All mice were housed at the Pasteur Institute (Paris, France) animal facility. All animal experiments described herein received prior approval from our local ethics committee.

Abs and reagents

FITC-, PE-, biotin-, and allophycocyanin-conjugated Abs were obtained from BD Pharmingen. Anti-CD19 and anti-DX5 microbeads and LS− magnetic separation columns were from Miltenyi Biotec. RPMI 1640, FCS, and antibiotics were from Invitrogen Life Technologies.

Infection of mice and determination of bacterial counts

Mice were inoculated i.n. with different doses of bacteria, ranging from 10^6 to 10^7 bacteria as indicated. At different time points postinfection, mice were sacrificed, and the lungs were removed and prepared in sterile 0.9% NaCl solution. Serial dilutions of the lung homogenates were plated on trypticase soy broth agar containing streptomycin to enumerate bacterial counts (CFU). The results are presented as the mean of eight mice per group, and three independent experiments.

Histopathological studies

At different time points postinfection, mice were anesthetized, their trachea were catheterized, and 4% formalin was injected into the bronchoalveolar space. Lungs were then removed and further fixed in 4% formalin before being embedding in paraffin. Classical H&E staining and immunohistochemistry was performed as previously described (12).

Determination of myeloperoxidase (MPO) activity

Lungs were perfused with sterile 0.9% NaCl solution and frozen at −20°C until use. Tissues were homogenized in ice-cold PBS for 30 s (Potter-Elvehjem glass homogenizer; Thomas). The extracts were centrifuged (10,000 × g for 10 min at 4°C), and the supernatants were discarded. The pellets were resuspended in PBS supplemented with hexadecyl trimethylammonium bromide (HTAB; 0.5%; Sigma-Aldrich) and EDTA (5 mM) and renormalized. After centrifugation, 100 μl of supernatant was placed in a test tube with 200 μl of PBS-HTAB-EDTA, 2 ml of HBSS, 100 μl of O-dianisidine dihydrochloride (4 mM), and 100 μl of H₂O₂ (0.4 mM). After 15 min of incubation with shaking at 37°C, the reaction was stopped by the addition of 100 μl of NaNO₂ (1%). MPO activity was expressed as the OD₄₅₀ value.

ELISA

ELISA for IL-1β, TNF-α, and IL-12p70 were performed according to manufacturer’s recommendations (DuoSet; R&D Systems). For measuring IL-6 concentrations, MP5-20F3 was used as capture mAb and MP5-32C11 manufacturer’s recommendations (Duoset; R&D Systems). For measuring IL-12p70, IFN-γ was measured according to manufacturer’s recommendations (Duoset; R&D Systems).

Flow cytometry

For surface Ab staining, cells were washed twice in PBS supplemented with 1% BSA (PBS-BSA), incubated on ice for 30 min with Abs, and subsequently washed twice in PBS-BSA before analysis. When appropriate, cells were incubated with biotin-conjugated Abs, washed three times, and then incubated for 30 min with the relevant streptavidin conjugate, and then washed three times before analysis. Samples were analyzed using a FACS calibrated flow cytometer running CellQuest software (BD Biosciences).

Intracellular IFN-γ staining

Anesthetized mice were exsanguinated via the abdominal aorta, and their lungs were perfused with 0.9% NaCl solution. Lungs were removed, cut into small pieces, and digested for 30 min at 37°C in RPMI 1640 medium containing 2 mg/ml collagenase and 1 mg/ml DNase 1 (Sigma-Aldrich). Cell suspensions were washed and resuspended in RPMI 1640 medium supplemented with 10% FCS, and were incubated in the presence of 20 ng/ml brefeldin A for 3 h at 37°C. Cells were subsequently washed in PBS supplemented with 3% FCS solution and surface stained with anti-NK1.1 and CD3 Abs before fixation in 2% paraformaldehyde for 10 min at room temperature. Intracellular staining was performed using PE-conjugated anti-IFN-γ or isotype-matched control Abs diluted in 0.5% saponin (BD Biosciences). Cells were washed twice in PBS-3% FCS before analysis.

Statistical analysis

Each point corresponds to the mean ± SD of the indicated number of experiments. Statistical significance between the individual groups was analyzed using the unpaired Student t test with a threshold of p < 0.05. A χ²-log rank analysis was used for survival curves.

Results

Rag−/− mice are highly susceptible to Shigella flexneri infection

Previous studies have assessed the roles for adaptive lymphocytes (αβ T cells, γδ T cells, and/or B cells) in the immune response to S. flexneri using mice genetically deficient in these lymphocyte subsets (10, 17). In contrast, S. flexneri infection studies using mice that are genetically deficient in NK cells have not been performed, although NK cells have been suggested to play a role in the antibacterial response (10). We, therefore, assessed the susceptibility of lymphoid Rag−/− mice (which are genetically deficient in B, T, and NK cells) (13) to S. flexneri infection. After i.n. administration of 10⁶ bacteria, lungs of both wt and Rag−/− mice were efficiently colonized by S. flexneri at 6 h postinfection (Fig. 1A). However, whereas wt mice cleared S. flexneri infection by day 4, and therefore survived the infection (Fig. 1B), Rag−/− mice were unable to control the infection (A) and died by day 9 (B). These results clearly indicated that lymphocytes play a crucial role in protecting mice from primary S. flexneri infection and complement previous studies attempting to identify a unique lymphocyte subset required for protection against this pathogen (10).

S. flexneri-induced inflammatory responses in Rag−/− mice

Next, we investigated whether the absence of lymphocytes in Rag−/− mice impacts on the inflammatory response induced upon S. flexneri infection. As shown in Fig. 2A, at day 4 postinfection, lungs of S. flexneri-infected wt mice exhibited an extensive inflammation in the peribronchial spaces with a diffuse alveolitis. In contrast, in infected Rag−/− mice at the same time point, overall inflammation was less extensive (Fig. 2B). We next quantified the recruitment of hemopoietic cells into the lung tissues upon infection using flow-cytometric analysis of collagenase-extracted tissues (Fig. 2C). In noninfected mice, the steady-state number of lung macrophages was significantly increased in Rag−/− mice compared with wt mice (Rag−/−, 0.60 ± 0.17 × 10⁶ cells, vs wt, 0.24 ± 0.02 × 10⁶ cells). At day 4 postinfection, a marked increase in the number of lung macrophages was observed in wt
mice (3.8 ± 1.9 × 10^6 cells; an increase of ~16-fold), whereas only a slight (2.5-fold) increase was observed in Rag°γc° mice (1.51 ± 0.4 × 10^6 cells). Analysis of T and NK cell recruitment in the lungs upon S. flexneri infection of wt mice revealed a 3.5-fold increase in the number of T cell in comparison with noninfected mice (infected, 1.3 ± 0.4 × 10^6 cells, vs noninfected, 0.37 ± 0.07 × 10^6 cells), whereas NK cells increased ~20-fold (infected, 1.6 ± 0.6 × 10^6 cells, vs noninfected, 0.09 ± 0.07 × 10^6; Fig. 2C). Because massive PMN recruitment is a feature of Shigella infection (3, 18), we quantitated the recruitment of these nonlymphoid cells indirectly by measuring the MPO activity from lung total extracts. We observed that both wt and Rag°γc° mice had similar basal MPO levels, which were markedly increased (~15-fold) upon infection with S. flexneri (Fig. 2D).

To further characterize the inflammatory response induced in S. flexneri-infected Rag°γc° mice, the production of proinflammatory cytokines was analyzed in lung homogenates at the early time points postinfection. As shown in Fig. 3, production of TNF-α, IL-1β, IL-6, and IL-12p70 were clearly up-regulated in wt mice by day 2 after infection with S. flexneri and sustained until day 4. In contrast, the induction of these cytokines in infected Rag°γc° mice was limited in terms of amplitude (TNF-α, IL-6, and IL-12p70) and duration (IL-1β). Taken together, these results demonstrate that lymphocytes are required for establishing and maintaining a proper inflammatory response to S. flexneri. The relatively normal recruitment of PMN and their associated MPO activity in infected Rag°γc° mice contrasts with the lymphocyte-dependent recruitment of macrophages.

Rag°γc° mice engrafted with αβ T cells from wt, but not from IFN-γ° mice control S. flexneri infection

To dissect the role for different lymphocyte subsets in the early immune response to S. flexneri, we selectively reconstituted Rag°γc° mice with polyclonal αβ T lymphocytes purified from lymph nodes of congenic C57BL/6 mice as previously described (14). These reconstituted mice (at 6 wk post-T cell transfer) harbor a mixture of naive and activated/memory CD4^+ and CD8^+ T cells, similar to their wt counterparts, but do not harbor γδ T cells, NK-T cells, NK cells, or B cells (14). αβ T-reconstituted Rag°γc° mice were inoculated i.n. with 10^6 S. flexneri and the lung-bacterial load was measured at day 4 postinfection. Whereas Rag°γc° mice did not control the infection, αβ T cell-reconstituted Rag°γc° mice were able to limit the bacterial multiplication similar to wt mice (Fig. 4A). Accordingly, the reconstituted Rag°γc° mice survived the infection (Fig. 4B). These data demonstrated that polyclonal αβ T cells were sufficient to control S. flexneri infection in the absence of γδ T cells, NK cells, and NK-T cells.

IFN-γ plays an important role in the amplification of the early innate inflammatory response (19). To assess the role for αβ T cell-derived IFN-γ in the response to S. flexneri, Rag°γc° mice were reconstituted with αβ T cells from IFN-γ° mice. When αβ T (IFN-γ°) cell-reconstituted mice were subsequently infected with
NK cells can produce IFN-\(\gamma\) and TNF-\(\alpha\) in the lungs of wild-type (wt) mice after intracellular pathogens (20, 21). Because we observed a significant influx of NK cells into the lungs of wt mice after infection (day 0), and at days 2 and 4 postinfection with \(S.\) flexneri \((10^7\) i.n.) in wt and \(\gamma_c\) mice. Each dot represents a mouse, and the mean value is indicated by the black line. *: \(p < 0.05\). Only statistical analysis comparing wt and \(\gamma_c\) at days 2 and 4, respectively, are indicated. Statistical analysis comparing the cytokine production before and after infection in wt or \(\gamma_c\) mice gives \(p < 0.05\) for IL-1\(\beta\), IL-6, and TNF-\(\alpha\) upon infection in wt mice at day 2 and day 4, and for IL-12 at day 4. Upon infection in \(\gamma_c\), \(p < 0.05\) only for the production of IL-1\(\beta\) at days 2 and 4, and TNF-\(\alpha\) at day 2.

\(S.\) flexneri, they failed to control the infection and exhibited a lung-bacterial load similar to that observed in non-reconstituted \(\gamma_c\) mice (Fig. 4A). These \(\alpha\beta\) T (IFN-\(\gamma\)) cell-reconstituted \(\gamma_c\) mice succumbed to the infection at about day 12 (Fig. 4B), similar to non-reconstituted \(\gamma_c\) mice. Altogether, these results demonstrated that T cell-derived IFN-\(\gamma\) can provide an activation signal (presumably to macrophages), which can result in bacterial clearance and subsequent protection of mice from primary \(S.\) flexneri infection.

\(\gamma_c\) mice control early \(S.\) flexneri infection

NK cells can produce IFN-\(\gamma\) following infection by viruses or intracellular pathogens (20, 21). Because we observed a significant influx of NK cells into the lungs of wt mice after \(S.\) flexneri infection (Fig. 2C), we assessed whether NK cells could participate in the early immune response to this pathogen. \(\gamma_c\) mice (which lack B and T cells but have NK cells) (16) were infected i.n. with \(S.\) flexneri, and the bacterial burden in the lung was measured at day 4 postinfection. \(\gamma_c\) mice controlled the infection in a similar fashion to wt mice (Fig. 4A), and survived the infection (B). The results obtained with \(\gamma_c\) mice clearly contrast with that of \(\gamma_c\) (Figs. 1 and 4), and shows that NK cells in the absence of other lymphocytes can participate in protecting mice after primary \(Shigella\) infection.

Local production of IFN-\(\gamma\) by \(\alpha\beta\) T cells and NK cells in wt mice following \(S.\) flexneri infection

We next assessed whether \(\alpha\beta\) T cells and NK cells recruited into the lung after \(S.\) flexneri infection could contribute to the early innate protection by production of IFN-\(\gamma\). Although IFN-\(\gamma\) production by macrophages and APC (including dendritic cells (DC)) upon recognition and activation at the site of infection, these cells undergo apoptosis in response to \(S.\) flexneri (2). Because APC are essential in directing lymphocytes (including B, T, and/or NK cells) toward the generation of cytotoxic cells and for Ab production, \(S.\) flexneri-induced apoptosis could impact on the generation of adaptive immunity. Still, the cellular and molecular mechanisms that coordinate innate and adaptive immunity in response to \(S.\) flexneri are poorly understood.

In this report, we demonstrate that early inflammatory responses to \(S.\) flexneri have lymphocyte-dependent and -independent aspects. By comparing the recruitment kinetics and characteristics of
the inflammatory cells in lymphocyte-competent (wt) and allopred (Rag\(^{2}\)\(\gamma\)\(^{c}\)) mice, we found that 1) lymphocytes were not required for PMN infiltration or induction of MPO activity (which appears constitutive in PMN); 2) increases in macrophage numbers following S. flexneri infection require lymphocytes; and 3) induction and maintenance of inflammatory cytokine production (TNF-\(\alpha\), IL-1\(\beta\), IL-6, and IL-12p70) depends on the presence of lymphocytes. To our knowledge, this is the first report demonstrating the essential role for lymphocytes in Shigella-induced inflammation.

The observed dependence on lymphocytes for macrophage recruitment and production of inflammatory cytokines (TNF-\(\alpha\), IL-1\(\beta\), IL-6, and IL-12p70) supports the notion of a lymphocyte macrophage “loop” whereby cell:cell interactions or elaborated soluble products are required for sustained macrophage activation. This “classical” macrophage-dependent phase of inflammation is essential for protection against several pathogens (22). The reasons why macrophages accumulation is reduced in S. flexneri-infected Rag\(^{2}\)\(\gamma\)\(^{c}\) mice is not known, but several hypotheses can be forwarded. It is possible that macrophage recruitment is similar in both wt and Rag\(^{2}\)\(\gamma\)\(^{c}\)-infected mice, but that the rate of Shigella-induced macrophage apoptosis is greater in the latter, because these mice fail to clear the infection. Given that macrophage cell numbers increase ~15-fold in the lungs of wt mice, this would mean that ~80% of the macrophages are induced to undergo programmed cell death in Rag\(^{2}\)\(\gamma\)\(^{c}\)-infected mice. Because only activated macrophages are exquisitely sensitive to Shigella-induced apoptosis (2), this would imply that macrophage activation is lymphocyte independent. An alternative explanation is that lymphocytes act to induce and amplify macrophage recruitment at the site of infection. Activated macrophages would also elaborate soluble factors (especially C-C and C-X-C chemokines), which could act to further recruit monocytes into the inflamed tissue.

We confirm here previous studies demonstrating an essential role for IFN-\(\gamma\) in the early protection against S. flexneri (10, 23). IFN-\(\gamma\)-treated activated macrophages have increased bactericidal activity against S. flexneri (10). However, the cellular source of IFN-\(\gamma\) following S. flexneri infection has not been clearly identified. “Naturally” activated T cells (NK-T, \(\gamma\)\(\delta\) T cells) as well as NK cells are sources of “rapid” IFN-\(\gamma\) production in vivo (24), although DC also appear capable of IFN-\(\gamma\) secretion upon activation in vitro and following Listeria monocytogenes infection in vivo (25). Concerning the latter, we found that allopred Rag\(^{2}\)\(\gamma\)\(^{c}\) mice were as susceptible as IFN-\(\gamma\)-mice to S. flexneri infection, suggesting that DC-derived IFN-\(\gamma\) was not sufficient to protect mice in this context. Using several different experimental approaches, we provide evidence for cellular redundancy in the production of IFN-\(\gamma\) after S. flexneri infection. We found that both \(\alpha\)\(\beta\) T cells and NK cells were induced to secrete IFN-\(\gamma\) in vivo in the lungs of S. flexneri-infected wt mice. Mice harboring only NK cells (Rag\(^{2}\) mice) or harboring only \(\alpha\)\(\beta\) T cells (\(\alpha\)\(\beta\) T-reconstituted Rag\(^{2}\)\(\gamma\)\(^{c}\) mice) were able to control S. flexneri and survive the infection. Thus, multiple potential sources of IFN-\(\gamma\) can operate in the early innate immune response against S. flexneri.

Our selective reconstitution model provides a powerful system for dissecting the functionality and intercellular signals for immune cells in pathogen responses. NK cells have been implicated in the control of macrophage numbers in response to Shigella infection. This control is mediated through direct lysis of macrophages, and by the increased production of IL-12p70 by NK cells (26). Recent studies have highlighted the role of NK cells in the control of Salmonella typhimurium infection (27). NK cells have been shown to infiltrate infected organs in response to Salmonella infection and to control bacterial numbers by direct lysis, as well as by producing IFN-\(\gamma\) in vivo (28). The role of NK cells in controlling S. flexneri infection is less clear, as S. flexneri is extracellular in the intestinal lumen and intracellular in the gut lining. However, we found that NK cells are required for the control of S. flexneri infection in vivo. This is consistent with a previous report showing that NK cells are required for the control of S. flexneri infection in vivo (29). These findings suggest that NK cells have a role in the control of S. flexneri infection in vivo, and that this role is mediated through direct lysis of infected cells and the production of IFN-\(\gamma\).
in providing early IFN-γ production in the context of several infection models, including L. monocytogenes, bacillus Calmette-Guérin, lymphocytic choriomeningitis virus, and murine CMV (21, 26–28). Interestingly, in contrast to our results suggesting a redundant role for αβ T and NK cells in IFN-γ production, in Bordetella pertussis or Chlamydia trachomatis infection, NK cells play a nonredundant role in disease protection by producing early IFN-γ, which limits the infection and directs the induction of Th1 cells (29, 30). With regard to infection by S. flexneri, previous studies have suggested that NK cells may play an important role because partially NK-depleted wt mice are more susceptible (a 10-fold increase in the LD50) to S. flexneri infection (10). In this study, we demonstrate that NK-deficient mice can resolve a S. flexneri infection, provided that alternative sources of IFN-γ (such as αβ T cells) are present.

The ability of αβ T cells to produce IFN-γ at an early time point (4 days) after primary S. flexneri infection was somewhat unexpected. We consider it unlikely that naive T cells at the sites of the draining lymph nodes are activated and differentiate into IFN-γ-producing effectors that reach the lung within this short period of time. Another possibility (that we favor) is that these αβ T cells represent previously activated/memory T cells, which are known to recirculate through tissues and have the capacity to produce IFN-γ after infection by virtue of their ability to respond to locally produced cytokines including IL-12 and/or IL-23 (31). The possible “cross-protection” provided by activated/memory T cells (which may have been initially primed under an entirely different context) could thereby produce “recall” IFN-γ following stimulation in an appropriate cytokine context, and therefore represent an example of bystander activation. Because selective reconstitution or Ragγ- mice by polyclonal αβ T cells involves that transfer of αβ T cells with different activation phenotypes (LN harbor mostly naive αβ T cells, but also some activated and memory αβ T cells), the resultant αβ T-reconstituted Ragγ- mice harbor αβ T cells with all of these activation states and could therefore have the capacity to participate via bystander activation following S. flexneri infection.

No data are available concerning a role for NK cells upon Shigella infection in humans, but infiltration and expansion of T cells in rectal mucosa of Shigella-infected patients have been reported (7, 32). In addition, local production of IFN-γ is associated with the convalescent stage of shigellosis, when bacteria are no longer detectable in the feces of infected patients (33). Therefore, the results presented in this study are consistent with the hypothesis that IFN-γ-producing lymphocytes play a major role in the recovery of primary Shigella infection in human.

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