Fcy Receptor Regulation of *Citrobacter rodentium* Infection

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*Citrobacter rodentium*, a murine model pathogen for enteropathogenic *Escherichia coli*, colonizes the colon utilizing attaching and effacing lesions to adhere specifically to the surfaces of intestinal epithelial cells and cause mucosal inflammation. CD4+ T cells, B cells, and immunoglobulin G (IgG), but not secretory IgA or IgM, play a critical role in eradicating this pathogen. Consistent with the importance of IgG in *C. rodentium* eradication, IgG transport by the neonatal Fc receptor for IgG within the intestinal epithelium also has a critical role in the regulation of *C. rodentium* infection. It remains to be determined, however, whether Fcy receptors (FcγRs), the receptors for the Fc portion of IgG, regulate this bacterial infection within mucosal tissues. Therefore, we investigated the roles of FcγRs during *C. rodentium* infection. Fc receptor common gamma chain (FcγR)-deficient mice were more susceptible to *C. rodentium*-induced colitis. This occurred through decreased efficiency of FcR-mediated endocytosis and maturation of dendritic cells and consequently T-cell activation of antigen-specific T cells. Moreover, in the absence of FcγRs, phagocytosis by macrophages was significantly diminished. Therefore, activating FcγRs play an important role in defending against *C. rodentium* infection, indicating that the critical role played by IgG in this infection is not mediated by IgG alone but is dependent upon this class of receptors.

Fcy receptors (FcγRs), the receptors for the Fc portion of immunoglobulin G (IgG), are essential for antibody-dependent immune responses (14). FcγRs are detected on many hematopoietic cells, including macrophages, neutrophils, dendritic cells (DCs), eosinophils, basophils, mast cells, and NK cells (13). Functionally, there are two types of Fc receptors: activating receptors and inhibitory receptors, which transmit their signals via immunoreceptor tyrosine-based activation motifs or immunoreceptor tyrosine-based inhibitory motifs, respectively (15). In the mouse, the FcγRs that associate with a Fc receptor common gamma chain (FcγR) homodimer that has immunoreceptor tyrosine-based activation motifs are Fcγ receptor I (FcγRI), FcγRII, and FcγRIV, which lead to activation of downstream-signaling pathways. On the other hand, FcγRIIB is a unique FcγR with an immunoreceptor tyrosine-based inhibitory motif domain that directs an inhibitory program. The coexpression of activating and inhibitory FcγRs regulate the immune response by establishing a threshold for immune cell activation. In many murine model systems, the expression of aberrant FcγRs can result in uncontrolled immune responses and the initiation of autoimmune disease (2, 8, 19). Mice deficient in Fcγγ (FcγRγ−/−), a subunit common to FcγRI, FcγRII, FcγRIV, FcεRI, FcεRII, exhibit genetic inactivation of all activating FcγRs. This results in abrogated or heavily impaired immune complex-mediated immune responses, such as antibody-dependent cell-mediated cytotoxicity, release of inflammatory mediators, cytokine release, and phagocytosis of immune complexes (12, 20).

Enteric bacteria, such as enteropathogenic *Escherichia coli*, evade many mechanisms of systemic host defense by restricting their colonization to the luminal surface of the gut epithelium. *Citrobacter rodentium*, a murine model pathogen for enteropathogenic *E. coli*, colonize the epithelium of the colon utilizing attaching and effacing lesions to adhere to the surfaces of intestinal epithelial cells and cause mucosal inflammation. A few hours after oral challenge with 106 to 107 CFU of *C. rodentium*, initial colonization is observed at the cecum with colonization of the distal colon detectable at 2 or 3 days after infection. *C. rodentium* is usually spontaneously eradicated by day 28 after oral administration in wild-type mice (10). From the infection experiments using immune cell-deficient mice, CD4+ T cells, B cells, and IgG, but not secretory IgA or IgM, have been shown to play a critical role in eradicating this pathogen (3, 9, 17). It is speculated that *C. rodentium*-specific IgGs produced by B cells after stimulation of CD4+ T cells are necessary to eradicate this pathogen.

The neonatal Fc receptor for IgG, FcRn, is a critical molecule that is involved in the transport of IgG and its protection from catabolism. FcRn is structurally related to major histocompatibility complex (MHC) class I molecules and consists of a heterodimer composed of a glycosylated heavy chain in noncovalent association with β2-microglobulin (16). FcRn is known to have two cellular functions. One is the bidirectional transport of IgG across epithelial cells, and the other is the protection of IgG from catabolism by escape from lysosomal degradation. It has been shown that human FcRn is the vehicle by which IgG is transported across the intestinal epithelium...
and can in turn also recycle the IgG together with its cognate antigen as an immune complex back across the intestinal epithelial barrier into the lamina propria for processing by DCs and presentation to CD4+ T cells (23).

IgG transport by FcRn may regulate immune responses to luminal pathogens. Specifically, it has been shown that the transport of IgG and antigen-IgG complexes by FcRn plays a role in the immune defense against C. rodentium infection (24). It has been speculated that the effects of the anti-bacterial IgG antibodies that are transported by FcRn are derived from the direct protection against bacterial invasion into lamina propria from the epithelium and indirectly by affecting antigen presentation to antigen-specific T cells, which leads to the activation and proliferation of antigen-specific CD4+ T cells that assist in the killing of invading bacteria or to the differentiation of immature B cells into plasma cells for the production of bacterial antigen-specific IgGs. Beyond FcRn, it is not known whether the protective effects of IgG in C. rodentium infection are also dependent upon FcγRs. It remains to be determined whether and how FcγRs regulate this bacterial infection of mucosal tissues. Therefore, in this report, we have investigated the roles of classical FcγRs during C. rodentium infection. Our results indicate that the elimination of activating FcγRs for IgG, through deletion of FcγRIIB, and inhibitory FcγRs for IgG, through deletion of FcγRIIb, affect the susceptibility to C. rodentium infection.

**MATERIALS AND METHODS**

**Animals.** FcγRIIB−/− mice (20), and FcγRIIb−/− mice (21) were used. All mice were backcrossed more than six generations onto C57BL/6 mice. For ovalbumin (OVA) studies, OT-II mice were used (Jackson Laboratory, Bar Harbor, ME) (1). All mice were housed and bred in the Animal Unit of the Kobe University School of Medicine in a specific-pathogen-free facility under an approved experimental protocol.

**Antibodies.** CD11c-biotin (clone N418), MHC class II-PE (MHC class II conjugated to phycoerythrin) (clone M5/114.15.2), CD86 conjugated to PE (clone M5/114.15.2), CD11b-PE (clone M1/70) were purchased from BD Bioscience (San Jose, CA). Alexa Fluor 488-conjugated goat anti-rat IgG (heavy and light chains) was purchased from Molecular Probes (Eugene, OR). Rabbit anti-OVA polyclonal antibody was purchased from Rockland (Gilbertsville, PA). Polyclonal anti-C. rodentium sera was obtained from wild-type mice 6 weeks after C. rodentium infection as described previously (3).

**Protocol for induction of colitis by C. rodentium infection.** C. rodentium strain DB100 (catalog no. 51459; ATCC) was kindly provided by C. Sasakawa, Tokyo University, Japan. Establishment of C. rodentium producing OVA and GFP. Constitutively OVA-expressing C. rodentium (OVA-C. rodentium) was created by electroporating the plasmid pUT-mini-Tn5 Km vector, including the chicken OVA construct (25, 26) under the control of the two gal operon promoters into C. rodentium strain DB100. C. rodentium producing green fluorescent protein (GFP) (GFP-C. rodentium) was kindly provided by C. Sasakawa, Tokyo University, Japan.

**Adoptive transfer with CFSE-labeled OVA-specific OT-II CD4+ T cells and antigen challenge with C. rodentium.** Three-week-old FcγRIIB−/− mice and wild-type mice were orally inoculated with OVA-C. rodentium. The mice received 500 μg of antigen in PBS subcutaneously on day 2 after bacterial inoculation and received carboxyfluorescein succinimidyl ester (CFSE)-labeled CD4+ OT-II cells on day 3. On day 5, mesenteric lymph node (MLN) cells were subjected to flow cytometric analysis for the evaluation of CFSE intensity of the CD4+ T cells gated on TCR Vα2+ cells. MLN cells were also stained on day 5 for CD69 expression using an anti-CD69 antibody (clone H1.2F3; BD Bioscience, San Jose, CA), and phagocytic activity was measured as fluorescence intensity per CD11b-positive cells by flow cytometry.

**Establishment of C. rodentium producing OVA and GFP.** C. rodentium producing OVA and GFP was established by transfecting the plasmid pUT-mini-Tn5 Km vector, including the chicken OVA construct (25, 26) under the control of the two gal operon promoters into C. rodentium strain DB100. C. rodentium producing green fluorescent protein (GFP) (GFP-C. rodentium) was kindly provided by C. Sasakawa, Tokyo University, Japan.

**Preparation of peritoneal macrophages and evaluation of phagocytosis in FcγR-deficient macrophages.** Wild-type, FcγRIIB−/−, and FcγRIIb−/− mice were injected with 1 ml of 3% thioglycollate into the peritoneal cavity. The peritoneal cells were harvested by peritoneal lavage with 10 ml cold sterile saline twice from each mouse. Macrophages from the peritoneal cavities of wild-type, FcγRIIB−/−, and FcγRIIb−/− mice and GFP-C. rodentium were incubated for 30, 45, and 60 min with anti-C. rodentium IgG or control IgG and stained with TO-PRO-3 and FITC-conjugated goat anti-rat IgG (heavy and light chains) was purchased from Molecular Probes (Eugene, OR). Rabbit anti-OVA polyclonal antibody was purchased from Rockland (Gilbertsville, PA). Polyclonal anti-C. rodentium sera was obtained from wild-type mice 6 weeks after C. rodentium infection as described previously (3).

**Preparation of bone marrow-derived DCs.** Bone marrow-derived DCs were prepared as described previously (7). Cells were plated at a concentration of 1 × 10^6 cells/well in each well (24 wells). The cells were plated in 1 ml RPMI 1640 supplemented with 10% fetal calf serum, 0.1 U/ml penicillin, 0.1 μg/ml strepto-

**RESULTS**

**Susceptibility to C. rodentium in the absence of FcR gamma chain.** To evaluate the roles of IgG Fc receptors in mucosal infection, we used the Citrobacter rodentium model in a C57BL/6 mouse background (6). Previous studies have shown that C57BL/6 and BALB/c strains exhibit differing sensitivities to C. rodentium infection. C57BL/6 mice exhibit greater clinical evidence of disease and a fecal burden of bacteria compared with BALB/c mice. Three-week-old FcγRIIB−/− mice (C57BL/6) and control mice (FcγRIIB−/−, mice, C57BL/6) were orally inoculated with 5 × 10^9 or 5 × 10^10 CFU of C. rodentium. A dose of 5 × 10^9 CFU/mouse was lethal in 75% of FcγRIIB−/− mice on day 16 and 100% lethal on day 21. In contrast, the same dose was lethal in 12.5% of control mice on day 16 and 50% lethal on day 21. Even a dose of 5 × 10^8 CFU of C. rodentium was lethal in 12.5% of FcγRIIB−/− mice on day 21 after infection (Fig. 1A). These studies indicate that FcγRIIB−/− mice are more susceptible to C. rodentium infection.
To assess the role of the FcγR chain in the adaptive immune response during *C. rodentium* infection in detail, we used a dose of 5 × 10⁸ CFU/mouse in the following studies. FcγR−/− mice exhibited more body weight loss (Fig. 1B) and higher bacterial concentrations in the feces at 14 and 21 days after infection (Fig. 1C) than littermate matched FcγR+/− mice. FcγR−/− mice exhibited a significantly greater increase of IgG levels on day 21 in sera and on days 14 and 21 in feces in mature DCs display a higher level of uptake in comparison to control mice. This increased production of IgG by FcγR−/− mice was associated with an approximately 50% survival on day 21 in response to a challenge with 5 × 10⁸ CFU of *C. rodentium* in striking comparison to FcγR+/− mice in which this dose was uniformly lethal (Fig. 1A). Consistent with this, macroscopic and microscopic injury was greater in FcγR−/− mice than in FcγR+/− mice. The colons of FcγR−/− mice were shorter and edematous compared with FcγR+/− mice (data not shown). FcγR−/− mice exhibited greater histologic injury in comparison to FcγR+/− mice on days 14 and 21 after infection (Fig. 1F, G, and H). The submucosa and lamina propria of infected FcγR−/− mice contained a greater number of polymorphonuclear leukocytes infiltrating the tissues as defined by Ly-6G expression (Fig. 1H), coincident with crypt hyperplasia 14 and 21 days after infection. In contrast, on day 21 after infection, there was no inflammation in FcγR+/− mice. These results indicate that FcγR−/− mice are more susceptible to *C. rodentium* colitis than FcγR+/− mice.

**Decreased uptake of FITC-conjugated C. rodentium via FcγR in FcγR−/− DCs.** Since the above studies showed that activating FcγRs are necessary for protection against *C. rodentium* colitis, we then evaluated the function of FcγR-deficient DCs. At first, we evaluated FcγR-mediated endocytosis by DCs. To do so, bone marrow-derived DCs from wild-type, FcγR−/−, and FcRIIB−/− mice were incubated for 1 h with FITC-conjugated *C. rodentium* with anti-C. rodentium IgG or control IgG. The uptake of FITC-conjugated *C. rodentium* in the context of control IgG was comparable in each type of DC. In contrast, there was a remarkable decrease in FITC-conjugated *C. rodentium* uptake by FcγR−/− DCs with anti-C. rodentium IgG compared with either wild-type or FcRIIB−/− DCs. This decrease in the FITC signal was especially evident in the MHC class II low fraction of DCs which are indicative of the immature subset of DCs (Fig. 2A and B). These observations are consistent with previous observations that unstimulated or immature DCs display a higher level of uptake in comparison to mature DCs (18). These studies indicate that uptake of immune complexes in relationship to *C. rodentium* infection is directly regulated by FcγR.

**Incorporation of C. rodentium-IgG complexes effectively induces DC maturation and production of proinflammatory cytokines.** We next investigated the maturation of FcγR-deficient DCs after taking up bacterial antigens with IgG. Bone marrow-derived DCs from wild-type, FcγR−/−, and FcRIIB−/− mice were incubated for 24 h with FITC-conjugated *C. rodentium* and anti-C. rodentium IgG or control IgG, and the expression levels of various surface markers (CD86 and MHC class II) indicative of activation on DCs were assessed by flow cytometry. Exposure of all types of DCs examined to *C. rodentium* with control IgG was observed to cause a slight up-regulation of CD86 and MHC class II expression presumed to result from direct stimulation caused by the bacterium alone. In contrast, exposure of the wild-type and FcRIIB−/− DCs to *C. rodentium* and anti-*C. rodentium* IgG, but not FcγR−/− DCs to the same conditions, induced a further increase in CD86 and MHC class II expression beyond that caused by the bacterium with the control IgG. Consistent with these observations, the supernatants of the culture medium collected from these various types of incubations revealed that *C. rodentium*-specific IgG stimulated increased production of TNF-α by wild-type and FcRIIB−/− DCs, but not FcγR−/− DCs, in comparison to that induced by control IgG complexes of *C. rodentium* (Fig. 3B). These results indicate that FcγR expressed on DCs can induce maturation and activation of production of proinflammatory cytokines after taking up immune complexes of *C. rodentium*.

**C. rodentium-IgG complexes effectively induce antigen-specific T-cell activation in MLNs.** Next we evaluated antigen-specific T-cell activation in the mucosal lymphoid tissues and their regulation by Fcγ receptors. To examine whether FcγR plays a role in infection-induced acquired immune responses, a genetically engineered *C. rodentium* strain was created that constitutively expressed OVA (OVA-*C. rodentium*). Immunoblot analysis confirmed the expression of OVA in the cell sonicates of OVA-*C. rodentium* but not control *C. rodentium* (Fig. 4A). To evaluate the antigen-specific T-cell responses, FcγR−/− and FcγR+/− mice were infected with OVA-*C. rodentium* at 3 weeks of age. Anti-*C. rodentium* IgG or control IgG was injected on day 2 after infection. CFSE-labeled OT-II cells were transferred intravenously on day 3 after infection. Mononuclear cells were collected from the MLNs, and the cells derived from these nodes were examined by flow cytometry (Fig. 4B). Injection of *C. rodentium*-specific IgG dramatically increased the number of OVA-specific CD4+ T cells (actual total number of OVA-specific CD4+ T cells in MLN: FcγR+/− mice with anti-*C. rodentium* IgG: FcγR−/− mice with anti-*C. rodentium* IgG = 9.6 × 10⁴ ± 0.5 × 10⁵; 2.8 × 10⁴ ±
as demonstrated by an enhancement of cell divisions within the MLNs of FcRγ−/− mice in comparison to FcRγ-deficient mice (Fig. 4C). In contrast, even in the presence of C. rodentium-specific IgG, no significant increase of OVA-specific CD4+ T cells was detected within the MLNs of FcRγ−/− mice. The same results were obtained by assessing the levels of CD69 expression on OT-II cells by flow cytometry wherein OT-II cells expressed higher levels of CD69 when adoptively transferred into FcRγ−/− mice in comparison to FcRγ−/− mice (Fig. 4E). As a control, C. rodentium without OVA expression did not enhance the proliferation of OT-II cells (Fig. 4D). These results reveal that FcRγ enhances the antigen-specific T-cell responses in the mucosa-associated lymphoid tissues in response to C. rodentium.

Macrophage phagocytosis of opsonized bacteria is regulated by specific IgG via FcRγ chain in response to C. rodentium. Antigen-specific T-cell activation via FcRγ induces differentiation of B cells to produce antigen-specific IgG. In turn, antigen-specific IgG can stimulate phagocytosis by macrophages via FcRγRs. Therefore, peritoneal macrophages from wild-type, FcRγ−/−, and FcRIIB−/− mice were collected after peritoneal injection with thioglycolate and then cultured with GFP-C. rodentium for 45 min with anti-C. rodentium IgG or control IgG. The number of C. rodentium bacteria that were incorporated into macrophages was increased in wild-type and FcRIIB−/− mice treated with anti-C. rodentium IgG, but not in FcRγ−/− macro-
phages treated in the same manner (Fig. 5A and B). The same results were obtained by measurement of phagocytic activity as defined by flow cytometry (Fig. 5C). These results indicate that FcR

Therefore, to evaluate the phagocytic activity of each type of FcγR-deficient macrophage in detail, macrophages and GFP-rodentium were incubated for 30, 45, and 60 min with anti-C. rodentium IgG. Consistent with the previous results as described above, only a few bacteria that were opsonized with specific IgG could be detected in FcRγ−/− macrophages even at 60 min after incubation (Fig. 6). Interestingly, the GFP signals were higher in the FcRIIB−/− macrophages than in the wild-type macrophages 60 min after incubation, which is consistent with the loss of this inhibitory receptor (Fig. 6). These results indicate that phagocytosis of immune complexes by macrophages are regulated in the opposite manner by activating and inhibitory FcγRs in response to C. rodentium infection.

DISCUSSION

In this work, we examined the roles of FcγRs in mucosal colonic inflammation induced by C. rodentium infection. IgG,

FIG. 3. C. rodentium-IgG complexes incorporation effectively induced DC maturation via FcγR. (A) Bone marrow-derived DCs from FcγR-deficient mice were incubated for 24 h in the absence (white) or presence of C. rodentium and control IgG (CR+control IgG) (gray) or C. rodentium and anti-C. rodentium IgG complexes (CR+anti-CR IgG) (black). Cells from wild-type (WT), FcRγ−/− (γ−−), and FcRIIB−/− (IIB−/−) mice are shown. DCs were stained with anti-CD86 or anti-MHC class II after gating on CD11c-positive cells and analyzed by flow cytometry. Results are from one representative experiment of three independent experiments. (B) Cytokine production of TNF-α was measured by an ELISA. The mean plus standard deviation (SD) are shown for each group (three mice per group). This experiment was evaluated by ANOVA. Values that are significantly different (P < 0.05) are indicated by brackets and two asterisks.
FIG. 4. *C. rodentium*-IgG complexes effectively induce T-cell activation. (A) Establishment of a genetically engineered *C. rodentium* strain that constitutively produces OVA. The immunoblot confirms the expression of OVA by *C. rodentium* CR, *C. rodentium* strain that constitutively produces OVA. (B) Summary of the experimental protocol with the inoculation of OVA-*C. rodentium*, injection of anti- *C. rodentium* IgG or control IgG, and the adoptive transfer of CD4+ OVA-specific T cells from OT-II mice. (C) The number of OVA-specific T cells in the MLNs in FcRγ+/- mice and control mice increased in the presence of anti-*C. rodentium* IgG or control IgG (three mice per group). Arrows indicate increasing rounds of cell division, anti-CR Ab i.v. OVA-CR p.o., anti-*C. rodentium* antibody given intravenously and OVA-CR given per os (orally). (D) Evaluation of CFSE-labeled OT-II cells in mice receiving *C. rodentium* without OVA expression. (E) Evaluation of CD69 expression on OT-II cells after in vivo stimulation with OVA-*C. rodentium* together with anti-*C. rodentium* IgG. Results are representative of two independent experiments.
but not secretory IgA or IgM, has been shown to play a critical role in prevention against infection by the attaching and effacing pathogen \textit{C. rodentium}. This model is therefore appropriate to evaluate the function of IgG and its relationship to FcR in defending against bacterial infection within mucosal tissues. In these studies, we show that deletion of the FcR chain causes decreased efficiency of various aspects of DC and macrophage function relevant to antimicrobial immunity, including endocytosis, phagocytosis, and antigen-presenting cell activation, leading to a less mature phenotype of the latter types of cells. Consistent with this, in the absence of FcR expression, there is less stimulation of antigen-specific T cells and thus adaptive immunity. This stimulation of T cells is associated with increased B-cell production of bacterium-specific IgGs. The results of these studies thus support an important role of FcR in coordinating innate and adaptive immune responses to invasive bacteria (Fig. 7). The susceptibility to \textit{C. rodentium} infection in FcR mice is thus explained by a broad decrease in the efficacy of the immune response associated with defense against this mucosal pathogen. Moreover, the effects

![FIG. 5. Macrophages phagocytose opsonized bacteria via the FcR gamma chain. (A) Macrophages from the peritoneal cavities of wild-type (WT), FcR\textsuperscript{-/-}, or FcRIIB\textsuperscript{-/-} mice and GFP-expressing \textit{C. rodentium} (white) were incubated for 45 min with control IgG or anti-\textit{C. rodentium} IgG and stained for nuclei and actin (phalloidin) (gray). Samples were examined by confocal microscopy. CR\textsuperscript{-/-} anti-CR IgG, \textit{C. rodentium} plus anti-\textit{C. rodentium} IgG. Magnification, \(*630\). (B) The number of \textit{C. rodentium} bacteria in each macrophage was counted by confocal microscopy. The mean plus standard deviation (error bar) is shown for each group (\(n = 100\)). This experiment was evaluated by ANOVA. Values that are significantly different (\(P < 0.05\)) are indicated by brackets and two asterisks. (C) Flow cytometry analysis of phagocytic activity in macrophages. Results are representative of three independent experiments. GFP-CR, GFP-expressing \textit{C. rodentium}.](fig5.png)
of FcRγ shown here are likely related to its effects on Fcγ receptors rather than Fcα receptors, since neither IgA or IgM has any role to play in protection against *C. rodentium* infection (3, 9, 17).

We examined the uptake of bacterium-IgG complexes in various FcγR-deficient DCs. Many studies have shown that the FcRγ chain has a critical role in the early endocytosis of antigen-IgG complexes (5). As shown with other bacterial infections and model antigens, such as OVA, we now show that the FcRγ chain also plays an important role in endocytosis of *C. rodentium*. We also examined the effect of FcRγ on the maturation of DCs in the context of *C. rodentium* infection. Yada et al. have previously shown that the maturation of FcRγ−/− DCs is delayed compared with wild-type or FcRIIB−/− DCs using OVA as an immune complex (22). We expected that bacterium-IgG complexes would cause a different effect because bacterial lipopolysaccharide, CpG, and other Toll receptor ligands would cause significant maturation of DCs. However, our results indicate that IgG Fc receptors, such as FcRγ, elicit unique and significant signals to DCs beyond that which is delivered by Toll-like receptor-derived signals and are important to mediating protection against *C. rodentium* infection.

The role of FcRIIB in bacterial infection is not well-known. Some studies have shown that FcRIIB−/− mice exhibit less inflammation than wild-type mice do during bacterial infection (4, 5). In preliminary studies, FcRIIB−/− mice exhibited less inflammation of the distal colon during *C. rodentium* infection (data not shown). Consistent with this, we show here that macrophages from FcRIIB−/− mice displayed increased phagocytic function in comparison to wild-type macrophages which we speculate is one mechanism by which FcRIIB−/− mice exhibit less inflammation during *C. rodentium* infection compared to that observed with wild mice. This point certainly deserves examination in future studies but suggests that FcRIIB−/− mice clear *C. rodentium* infection more effectively.

In summary, we show that the activating FcγR, FcRγ, plays a significant role in protection against *C. rodentium* infection. FcγR−/− mice are susceptible to *C. rodentium* infection by the broad decrease in the efficacy of antigen-presenting cell function, including early endocytosis and maturation of dendritic cells, antigenic activation of T cells, and phagocytosis of *C. rodentium* by macrophages when bacterium-specific IgG exists.
an important role in defending against a mucosal pathogen, Citrobacter rodentium. FcyR regulates a variety of important cellular processes within DCs and macrophages that enhance their microbiocidal activity and augmentation of both innate and adaptive immune pathways in response to this mucosal pathogen. As such, it can be predicted that the known protective effect of IgG in C. rodentium infection is mediated in large part through the properties of this activating FcyR.

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REFERENCES

1. Barnden, M. J., J. Allison, W. R. Heath, and F. R. Carbone. 1998. Defective TCR expression in transgenic mice constructed using cDNA-based alpha-and beta-chain genes under the control of heterologous regulatory elements. Immunol. Cell Biol. 76:34–40.

2. Bolland, S., and J. V. Ravetch. 2000. Spontaneous autoimmune disease in FcRiIB-deficient mice results from strain-specific epistasis. Immunity 13:277–285.

3. Bry, L., and M. B. Brenner. 2004. Critical role of T cell-dependent serum antibody, but not the gut-associated lymphoid tissue, for surviving acute mucosal infection with Citrobacter rodentium, an attaching and effacing pathogen. J. Immunol. 172:433–441.

4. Clatworthy, M. R., and K. G. Smith. 2004. FcgammaRIIB balances efficient pathogen clearance and the cytokine-mediated consequences of sepsis. J. Exp. Med. 199:717–723.

5. Gjerstsson, I., S. Kleinau, and A. Tarkowski. 2002. The impact of Fcgamma receptors on Staphylococcus aureus infection. Microb. Pathog. 33:145–152.

6. Higgins, L. M., G. Frankel, G. Douce, G. Dougan, and T. T. MacDonald. 1999. Citrobacter rodentium infection in mice elicits a mucosal Th1 cytokine response and lesions similar to those in murine inflammatory bowel disease. Infect. Immun. 67:3031–3039.

7. Inaba, K., M. Inaba, N. Romani, H. Aya, M. Deguchi, S. Ikehara, S. Maratsus, and M. R. Steenman. 1992. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. J. Exp. Med. 176:1693–1702.

8. Jean-Fasciay, A., S. J. de Kimpe, S. M. Hellwig, P. L. van Lent, F. M. Holhuis, H. H. van Oijl, C. Sedillik, S. A. da Silveira, J. Gerber, Y. F. de Jong, R. Rozenaal, L. A. Aarden, W. R. van den Berg, T. Saito, D. Messer, S. Amigorena, S. Izui, G. J. van Ommen, M. van Vught, J. G. van de Winkel, and J. S. Verbeek. 2002. FcgammaRI (CD64) contributes substantially to severity of arthritis, hypersensitivity responses, and protection from bacterial infection. Immunity 16:391–402.

9. Maasser, C., M. P. Housley, M. Imura, J. R. Smith, B. A. Vallance, R. B. Finlay, J. R. Schreiber, N. M. Variki, M. F. Kagnoff, and L. Eckmann. 2004. Clearance of Citrobacter rodentium requires B cells but not secretory immunglobulin A (IgA) or IgM antibodies. Infect. Immun. 72:3315–3324.

10. Mundy, R., T. T. MacDonald, G. Dougan, G. Frankel, and S. Wiles. 2005. Citrobacter rodentium of mice and man. Cell. Microbiol. 7:1697–1706.

11. Neurath, M. F., B. Weigmann, S. Finotto, J. Glickman, E. Nieuwenhuis, H. Iijima, A. Mizoguchi, E. Mizoguchi, J. Mulder, P. R. Galle, A. Bhan, F. Austenbach, B. M. Sulidjan, S. R. S. Blumberg, and R. S. Blumberg. 2002. The transcription factor T-bet regulates mucosal T cell activation in experimental colitis and Crohn’s disease. J. Exp. Med. 195:1129–1143.

12. Nimmerjahn, F., and J. V. Ravetch. 2006. Fcgamma receptors: old friends and new family members. Immunity 24:19–28.

13. Qin, W. Q., D. de Bruin, B. H. Brownstein, R. Pearse, and J. V. Ravetch. 1990. Organization of the human and mouse low-affinity Fc gamma R genes: duplication and recombination. Science 248:732–735.

14. Ravetch, J. V., and J. P. Kinet. 1991. Fc receptors. Annu. Rev. Immunol. 9:457–492.

15. Ravetch, J. V., and L. L. Lanier. 2000. Immune inhibitory receptors. Science 290:84–89.

16. Simister, N. E., and K. E. Mostow. 1989. An Fc receptor structurally related to MHC class I antigens. Nature 337:184–187.

17. Simmons, C. P., S. Clare, M. Ghaem-Maghami, T. K. Uren, J. Rankin, A. Huett, R. Goldin, D. J. Lewis, T. T. MacDonald, R. A. Strugnell, G. Frankel, and G. Dougan. 2003. Central role for B lymphocytes and CD4+ T cells in immunity to infection by the attaching and effacing pathogen Citrobacter rodentium. Infect. Immun. 71:5077–5086.

18. Steinman, R. M., D. Hawiger, K. Liu, L. Bonifaz, D. Bonnyay, K. Mahnke, T. Iyoda, J. Ravetch, M. Dhodapkar, K. Inaba, and M. Nussenzweig. 2003. Dendritic cell function in vivo during the steady state: a role in peripheral tolerance. Ann. N. Y. Acad. Sci. 987:15–25.

19. Sylvestre, D. L., and J. V. Ravetch. 1994. Fc receptors initiate the Arthus reaction: redefining the inflammatory cascade. Science 265:1095–1098.

20. Takai, T., M. Li, D. Sylvestre, R. Clynes, and J. V. Ravetch. 1994. Fc gamma chain deletion results in plasminogen activator cell defects. Cell 76:519–529.

21. Takai, T., M. Ono, M. Hikida, H. Ohmori, and J. V. Ravetch. 1996. Augmented humoral and anaphylactic responses in Fc gamma RI-deficient mice. Nature 379:346–349.

22. Yada, A., S. Ebihara, K. Matsumura, S. Endo, T. Maeda, A. Nakamura, K. Akiyama, S. Aiba, and T. Takai. 2003. Accelerated antigen presentation and elicitation of humoral response in vivo by FcgammaRIIB- and FcgammaRIII-mediated immune complex uptake. Cell. Immunol. 225:21–32.

23. Yoshida, M., S. M. Claypool, J. S. Wagner, E. Mizoguchi, A. Mizoguchi, D. C. Roopenian, W. I. Lencer, and R. S. Blumberg. 2004. Human neonatal Fc receptor mediates transport of IgG into luminal secretions for delivery of antigens to mucosal dendritic cells. Immunity 20:769–783.

24. Yoshida, M., K. Kobayashi, T. T. Kuo, L. Bray, N. J. Glickman, S. M. Claypool, A. Kaser, T. Nagaiishi, D. E. Higgins, E. Mizoguchi, Y. Watawaki, D. C. Roopenian, A. Mizoguchi, W. I. Lencer, and R. S. Blumberg. 2006. Neonatal Fc receptor for IgG regulates mucosal immune responses to luminal bacteria. J. Clin. Invest. 116:2142–2151.

25. Yoshida, M., Y. Shirai, T. Watanabe, M. Yamori, Y. Iwakura, T. Chiba, T. Kitai, and Y. Watawaki. 2002. Differential localization of colitogenic Th1 and Th2 cells monospecific to a microflora-associated antigen in mice. Gastrenterology 123:1949–1961.

26. Yoshida, M., Y. Watawaki, Y. Kobayashi, T. Itoh, K. Murakami, A. Mizoguchi, T. Usui, T. Chiba, and T. Kitai. 1999. Cloning and characterization of a novel membrane-associated antigenic protein of Helicobacter pylori. Infect. Immun. 67:286–293.

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