Binding of *Salmonella*-Specific Antibody Facilitates Specific T Cell Responses via Augmentation of Bacterial Uptake and Induction of Apoptosis in Macrophages

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**Background.** Most antigens from intracellular bacteria or vaccines induce both humoral and cell-mediated immune responses, but interactions between these responses are not fully understood. This study aims to resolve how specific antibodies participate in the activation of specific T cells in protecting hosts against *Salmonella enterica* serotype Typhimurium (*S. typhimurium*) infection.

**Methods.** Mice were administered anti-*Salmonella* immunoglobulin G (IgG) 1 day before *Salmonella* infection, and survival rate was observed. For in vitro assay, *Salmonella* bacteria were treated with anti-*Salmonella* IgG or control IgG before infection of the RAW264.7 or HEP2 cells. After infection, cell-associated bacteria number, induction of apoptosis, and production of nitric oxide were examined. In addition, antigen presentation assays using *Salmonella*-primed T cells were performed.

**Results.** Treatment of *S. typhimurium* with anti-*Salmonella* IgG enhanced the macrophages’ uptake of bacteria and induced high-frequency apoptotic cell death. In vitro antigen presentation assay revealed that the extracellular vesicles isolated from apoptotic cells caused by infection with anti-*Salmonella* IgG–treated *S. typhimurium* facilitated the responses of *Salmonella*-specific T cells.

**Conclusion.** Our findings suggest that humoral immunity cooperates with cell-mediated immunity upon induction of apoptosis in host cells to establish protective immunity against *Salmonella* infection, even if it does not directly eliminate intracellular microorganisms.

Cell-mediated immunity protects the host against intracellular pathogens such as *Salmonella* species and *Mycobacterium tuberculosis* [1, 2]. However, most antigens from intracellular pathogens induce humoral immune responses, including the production of specific antibodies, as well as cell-mediated immune responses during the process of infection [3–6]. *Salmonella* causes a severe invasive disease in mice, and infection due to *Salmonella* species shares many features with infections due to other intracellular pathogens. Therefore, *Salmonella* has been used as an immunological research model for the development of new vaccines against intracellular pathogens, such as human *Salmonella typhi* and *M. tuberculosis* [1].

B cells and/or antibodies have been shown to protect against *Salmonella* infection in in vivo experiments using mouse models [3–6]. However, the underlying infection defense mechanism of the *Salmonella*-specific antibody is not understood. The contribution of antibodies to intracellular pathogen infection seems to depend on the susceptibility of the mouse strain, as well as the route of infection. Although the direct binding and neutralization of infectious agents are thought to
have only a limited effect on pathogen clearance and the establishment of protective immunity against intracellular pathogens, the treatment of pathogens with specific antibodies inhibits infectivity by a mechanism involving Fc receptor-mediated antigen uptake and activation of the antimicrobial mechanisms of macrophages [7–9]. Moore et al [10] reported that, for antibodies to induce an optimal T cell response, the antigen uptake by FcγR-mediated endocytosis must lead to a more rapid and greater degree of T cell activation. However, Fc receptors have been reported to be unnecessary for the induction of T cell–mediated immunity against Salmonella infection [11].

Recent studies have reported that infection-induced apoptosis results in specific T cell activation against Salmonella and M. tuberculosis [12, 13]. Schaible et al [13] showed that mycobacteria induce apoptosis in macrophages by causing the release of apoptotic vesicles that carry bacterial antigens on antigen-presenting cells. Yrlík et al [12] reported that the bystander dendritic cells present Salmonella antigen on major histocompatibility complex (MHC) class I and MHC class II molecules from apoptotic infected macrophages. Salmonella has an evolved mechanism that enables it to survive in phagosomal compartments and can induce apoptotic cell death in macrophages [14].

Taking these findings together, we hypothesized that the specific antibody might contribute to the activation of specific T cells via induction of apoptosis in host cell during the infection of intracellular pathogens. In the present study, we showed that binding of specific antibody to Salmonella enhances bacterial uptake by macrophages and induces apoptotic cell death at a high frequency. We also found that extracellular vesicles isolated from apoptotic cells induced by infection with anti-Salmonella immunoglobulin G (IgG)–treated Salmonella facilitated the response of Salmonella-immunized T cells.

MATERIALS AND METHODS

Bacterial strains. The virulent S. typhimurium SR-11 (χ3306) [15], green fluorescent protein (GFP)–labeled virulent S. typhimurium (GFP-SH100), SR-11 with deficiency in the aroA gene (UF20) [16], SR-11 with deficiency in the invA gene (UF102) [17], and L. monocytogenes (10403s) were used in this study.

Immunization and subsequent challenge of virulent bacteria. To investigate the effect of specific B cells on protection against exponential-phase χ3306 infection, 7–8-week-old C57BL/6 female mice (Charles River) and C57BL/6 background X-linked immunodeficiency (XID) mice were infected with 5 × 10^3 colony-forming units (CFUs) of exponential-phase UF20 via intravenous injection. Forty days after UF20 infection, mice were infected with 1 × 10^3 CFUs (LD50 in C57BL/6 and XID mice: <10 CFUs) of χ3306 via intraperitoneal injection.

Forty days after intravenous injection of 5 × 10^3 CFUs of UF20, inactivated serum samples were collected from immunized mice, and the IgG fraction was purified using a protein G column (Pharmacia). To investigate the effect of inactivated serum samples or Salmonella–specific IgG on protection against χ3306 infection, naive mice were intravenously administered 50 μL inactivated serum samples or 1 mg per 200 μL phosphate-buffered saline Salmonella–specific IgG 1 day before intraperitoneal infection with 10 CFUs of χ3306 or 1 × 10^4 CFUs (LD50 in C57BL/6 mice: <1 × 10^5 CFUs) of L. monocytogenes. Inactive naive serum samples or IgG fractions from unimmunized mice were used as controls.

In vitro infection. RAW264.7 cells or HEp2 cells (1 × 10^5) were challenged with χ3306, GFP-virulent S. typhimurium, and L. monocytogenes or with UF102 at a multiplicity of infection (MOI) of 5 [18]. In some experiments, the Salmonella strain was treated with 10 μg/mL anti-Salmonella antibodies for 30 min at 37°C before being added to the cell culture. Thereafter, we also used the F(ab2)′ fraction of Salmonella–specific IgG for treatment.

At the indicated time points during infection, the viability of the infected RAW264.7 cells was measured by a WST-1 assay (Wako).

Measurement of nitric oxide and nitric oxide synthase. Infected RAW264.7 cells were sampled for further analysis with the Griess reaction by using cell culture supernatant [19]. At the indicated time points during infection, the supernatant was mixed with an equal volume of Griess reaction reagent. Absorbance was read at 550 nm using a plate reader (Molecular Devices).

At the indicated time points during infection, reverse-transcription polymerase chain reaction for the detection of inducible nitric oxide synthase (iNOS) messenger RNA from infected RAW264.7 cells was performed using the following primers: forward, 5′-TTTTCTCTTCAAAGTCAAATCCTACCA-3′; reverse, 3′-TGTGTCTGAGATGTGCTGAAAC-5′.

Detection of apoptosis. The in situ detection of fragmented DNA, a feature of apoptotic cells, was performed by the TdT-mediated dUTP Nick End Labeling (TUNEL) assay using the In situ Cell Death Detection Kit Fluorescein (Roche Diagnostics). TUNEL-positive cells were examined under a confocal laser microscope (Leica). Infected RAW264.7 cells were detected for further analysis with the activation of caspase-3, as described elsewhere [18]. Cells were lysed by a freeze-thaw cycle and centrifuged at 9000 g for 5 min at 4°C. The supernatant was mixed with caspase-3 fluorogenic substrate, Ac-DEVDA-MCA (Peptide Laboratory), and subsequently incubated at 37°C. Methylcoumarin acetate (MCA) fluorescence was quantified by
ultraviolet spectrofluorometry (excitation, 355 nm; emission, 460 nm).

Preparation of extracellular vesicles released by Salmonella-challenged peritoneal exudate cells. To prepare the extracellular vesicles as antigens, the culture supernatants obtained 20 h after infection were collected, as described elsewhere [13]. C57BL/6 mouse peritoneal exudate cells were challenged with \( \chi^{3306} \) at an MOI of 5, as described above. The culture supernatants were collected by consecutive centrifugations at 800 g for 10 min, 1800 g for 10 min, and 25,000 g for 10 min at 4°C. The final supernatants were precipitated by ultracentrifugation (100,000 g for 1 h at 4°C). The pellets were suspended in Dulbecco-modified Eagle medium (390 mg protein mL\(^{-1}\)). The prepared extracellular vesicles were free of live salmonellae, as verified by an LB-plate culture.

Antigen presentation assay. The prepared extracellular vesicles were free of live salmonellae, as verified by culture. T cells (5 × 10\(^5\)) were purified from the spleens of either Salmonella-immunized or naive mice by using an anti-CD3 (eBioscience), anti-CD4 (BD Pharmingen), and anti-CD8 (eBioscience) magnetic cell sorting system. CD3\(^+\), CD4\(^+\), and CD8\(^+\) cells (5 × 10\(^5\)) were then cocultured with dendritic cells (1 × 10\(^3\)) from C57BL/6 mouse spleens in each well of a 96-well flat-bottom microtiter plate. The proliferation assay was performed using a 5-Bromo-2′-deoxyuridine cell proliferation assay kit (Roche Molecular Biochemicals).

Flow cytometric analysis of intracellular cytokine production. T cells (2 × 10\(^5\)) were purified from spleens of C57BL/6 mice by using anti-Thy1.2 (eBioscience) and a magnetic cell sorting system. Purified T cells were cocultured with dendritic cells (1 × 10\(^3\)) in the presence or absence of extracellular vesicles from apoptotic cells for 3 days. Six hours before the cells were harvested, GolgiStop (BD Pharmingen) was added to block the intracellular transport processes. The cells were harvested, washed, and stained with anti-CD4, anti-CD8, anti-mouse interferon \( \gamma \) (IFN-\( \gamma \)) (eBioscience), and anti-mouse interleukin 4 (IL-4) (eBioscience). The fluorescence intensity of blast T cells was analyzed on the EPICS flow cytometer (Beckman Coulter).

Statistical analysis. Statistical analysis was performed using the Student \( t \) test to compare the 2 groups. Data are presented as the mean ± standard deviation for 3–5 samples per group.
RESULTS

Contribution of Salmonella-specific antibody to protection against S. typhimurium infection in mice. To determine the role of specific antibodies in protection against S. typhimurium infection, C57BL/6 and XID mice from the same genetic background were immunized by intravenous injection of UF20 (aroA) followed by an intraperitoneal challenge with χ3306. Both naive and immunized XID mice and naive wild-type control mice died within 8 days of challenge with the virulent S. typhimurium strain, whereas immunized wild-type control mice survived (Figure 1A). Naive mice were administered heat-inactivated Salmonella-immunized mouse serum 1 day before challenge with χ3306. The administration of immune serum from wild-type mice, but not from XID mice, to naive mice conferred a protective effect against the challenge with the χ3306, even in the naive XID mice (Figure 1B). In regard to the production of Salmonella-specific IgG in serum, immunized XID mice produced lower levels than immunized wild-type mice (data not shown). This protective effect was also observed when the IgG fraction from immune serum samples was administered to naive mice (Figure 1C). The Salmonella-specific IgG had no effect on infection with other intracellular bacteria, such as L. monocytogenes (Figure 1D). Therefore, anti-Salmonella IgG demonstrated a specific protective effect against Salmonella infection in vivo.

Increased uptake of bacteria in macrophages by the binding of Salmonella-specific IgG. We performed in vitro infection assays using the mouse macrophage-like cell line RAW264.7 and human epithelial-like cell line HEp2 to examine the molecular mechanism underlying the protective effect of anti-Salmonella IgG against infection. As shown in Figures 2A and 2B, the number of bacteria that were associated with and the number within RAW264.7 cells both increased as a result of treatment with anti-Salmonella IgG (P<.005). Similar results were obtained when peritoneal macrophages were used instead of RAW264.7 cells (data not shown). Although the association of bacteria with HEp2 cells, which do not demonstrate phagocytosis, was increased by pretreatment of χ3306 with specific IgG, no intracellular uptake of bacteria was observed (Figures 2C and 2D). To distinguish between phagocytosis and infection, we used invasion-deficient S. typhimurium strain UF102 (invA-). Experiments using the invasion-deficient S. typhimurium strain UF102 indicated that treatment with specific IgG enhanced the adhesion of bacteria to both RAW264.7 and
HEp2 cells, but only the former showed an increase in the number of intracellular bacteria (Figures 2A–2D).

The removal of the Fc portion of IgG and addition of FcγR-blocking mAb reduced the uptake of Salmonella (P = .01; Figure 2F). In addition, the Salmonella-specific IgG had no effect on infection due to other intracellular bacteria, such as L. monocytogenes (Figure 2G). The induction of bacterial uptake by macrophages might be an effect of specific IgG during infection with live bacteria. Experiments using GFP-labeled S. typhimurium indicated that treatment with anti-Salmonella IgG increased the proportion of infected macrophages as well as the number of bacteria within a single cell (Figure 2E). Taken together, these findings indicate that the specific IgG-mediated enhancement of the bacterial uptake by macrophages is dependent on phagocytosis and the host cells’ interactions with FcγR.

Increased frequency of apoptotic cell death due to the increased uptake of bacteria. Although macrophages are an important site of residence for Salmonella, lysosomal enzymes and radical production by nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase in these immune cells have been shown to eliminate or suppress bacterial replication. Therefore, we examined whether specific IgG-mediated enhancement of bacterial uptake induces an increase in the antibacterial activity of phagocytic cells. Unexpectedly, the levels of iNOS messenger RNA expression and nitric oxide production in RAW264.7 cells after specific IgG–treated Salmonella infection were comparable to those observed after the production in RAW264.7 cells after specific IgG–treated levels of iNOS messenger RNA expression and nitric oxide the antibacterial activity of phagocytic cells. Unexpectedly, the ated enhancement of bacterial uptake induces an increase in

No activation of macrophages Salmonella typhimurium treated with specific immunoglobulin G (IgG). Inducible nitric oxide synthase (iNOS) messenger RNA expression in infected RAW264.7 cells were detected by reverse-transcription polymerase chain reaction. RAW264.7 cells were infected with antibody-treated χ3306 (A). Cells were harvested at 90 (upper) and 120 min (lower) after infection, and RNA was prepared. Nitric oxide production was detected from infected RAW264.7 cells treated with anti-Salmonella IgG (filled), control IgG (open), or none (shaded) (B). The culture supernatant was harvested 8 h after infection.

Figure 3. No activation of macrophages Salmonella typhimurium treated with specific immunoglobulin G (IgG). Inducible nitric oxide synthase (iNOS) messenger RNA expression in infected RAW264.7 cells were detected by reverse-transcription polymerase chain reaction. RAW264.7 cells were infected with antibody-treated χ3306 (A). Cells were harvested at 90 (upper) and 120 min (lower) after infection, and RNA was prepared. Nitric oxide production was detected from infected RAW264.7 cells treated with anti-Salmonella IgG (filled), control IgG (open), or none (shaded) (B). The culture supernatant was harvested 8 h after infection.
Figure 4. A, Apoptotic cell death of macrophages due to *Salmonella typhimurium* treated with specific immunoglobulin G (IgG). RAW264.7 cells were easy to separate from the culture dish after infection of *S*. *typhimurium* pretreated with specific IgG. Photography of *Salmonella*-specific IgG–treated *S*. *typhimurium*-infected RAW264.7 cells (left) and control IgG–treated (right) at 48 h after infection are shown. B, The survival rate of infected RAW264.7 cells. RAW264.7 cells were infected with specific IgG–treated *S*. *typhimurium*, and viability was measured by the WST-1 assay after infection. Noninfected RAW264.7 cells at each time point were used as controls and served as the baseline value of each infected RAW264.7 cell. C, Activation of caspase-3 in infected RAW264.7 cells. Cells were harvested after 10 h of culturing and lysed. The caspase-3 was measured using fluorogenic substrates. Panels B and C show RAW264.7 cells treated with anti-*Salmonella* IgG (filled) and control IgG (open). D, Confocal laser scanning microscopic images of infected RAW264.7 cells in detection of apoptosis (upper) and merging of upper panel and light microscopy (lower) by a Terminal dUTP Nick End Labeling assay at 20 h. The results of the 3 experiments were similar, and the results of a representative experiment are shown here.

from the spleens of either *Salmonella* vaccine strain–infected or naive mice. Purified CD3+ T cells were cultured with splenic dendritic cells in the presence of various concentrations of extracellular vesicles from apoptotic peritoneal macrophages (data not shown). The extracellular vesicles isolated from apoptotic cells provoked by infection with anti-*Salmonella* IgG–treated *Salmonella* thus induced the proliferation of *Salmonella*-primed T cells. In contrast, fragments of apoptotic cells induced by treatment with actinomycin D did not affect immune T cell proliferation (*P* < .001; Figure 5A). Apoptotic fragments from RAW264.7 cells induced immune T cell proliferation to the same extent (data not shown). We attempted to examine the
specific T cell responses using nonspecific IgG–treated $\chi_{3306}$-infected macrophages. Because treatment with nonspecific IgG does not induce the bacterial uptake or apoptosis in $\chi_{3306}$-infected macrophages (Figures 2 and 4), apoptotic vesicles could not be prepared from nonspecific IgG–treated, $\chi_{3306}$-infected macrophages.

To determine the capacity of apoptotic fragments to stimulate antigen-specific CD4$^+$ and CD8$^+$ T cells, purified CD4$^+$ or CD8$^+$ T cells were used as responding cells. As shown in Figure 5B, immune CD4$^+$ T cells but not CD8$^+$ T cells proliferated in response to the apoptotic fragments in the presence of dendritic cells ($P<.001$). These results indicated that the activation of immune CD4$^+$ T cells was induced by specific antibody–mediated Salmonella-induced apoptotic fragments.

**Induction of Salmonella-specific Th1 T cell and CD8$^+$ T cell responses via apoptotic cell death in infected macrophages.** We utilized an intracellular cytokine analysis to determine whether Salmonella pretreated with specific IgG predominantly induced Th1 responses or Th2 responses. The analysis demonstrated a greater increase in the population of IFN-$\gamma$–positive CD4$^+$ T cells ($P<.05$), compared with IL-4–positive CD4$^+$ T cells ($P>0.1$) (Figure 6). These findings suggest that the apoptotic fragments induced by infection with *Salmonella* pretreated with specific IgG predominantly induced Th1 responses.

Although purified CD8$^+$ T cells did not proliferate in response to the apoptotic fragments (Figure 5B), the number of IFN-$\gamma$–positive immune CD8$^+$ T cells increased in cultures of whole splenic T cells as a result of the presence of apoptotic fragments (Figure 6B). In addition, immune CD4$^+$ T cells and CD8$^+$ T cells produced greater amounts of IFN-$\gamma$ than did naive T cells when IFN-$\gamma$ was measured with an enzyme-linked immunosorbent assay, using supernatant from the Figure 5B experiment (data not shown). Our findings suggest that the apoptotic fragments induced by infection with *Salmonella* pretreated with specific IgG also induced responses from both CD4$^+$ T cells and CD8$^+$ T cells [3].

**DISCUSSION**

Here, we describe the mechanism underlying the role of a specific antibody in protection against *S. typhimurium* in mice. Specific IgG rapidly induced apoptotic cell death by excessive uptake of bacteria into macrophages (Figure 4). As shown in

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**Figure 5.** Enhanced activation of specific T cell responses due to apoptosis in macrophages induced by infection with specific immunoglobulin G (IgG)–treated *Salmonella*. A, Purified CD3$^+$ T cells of UF20-immunized mice and naive dendritic cells cocultured with stimulation of extracellular vesicles prepared from culture supernatant of IgG-treated $\chi_{3306}$-infected macrophages or control vesicles prepared from actinomycin D-treated macrophages. B, Purified CD4$^+$ or CD8$^+$ T cells of UF20-immunized mice and naive dendritic cells cocultured with stimulation of extracellular vesicles prepared from culture supernatant of IgG-treated $\chi_{3306}$-infected macrophages. These results were measured at day 3. The panels show T cells from UF20-immunized mice (filled) and naive mice (open).

**Figure 6.** Enhanced activation of Th1 and CD4$^+$ T cell responses due to apoptosis in macrophages induced by infection with specific immunoglobulin G (IgG)–treated *Salmonella*, as shown by fluorescence-activated cell sorting. A, Detection of interferon $\gamma$ (IFN-$\gamma$)– or interleukin 4–producing CD4$^+$ T cells. B, Detection of IFN-$\gamma$–producing CD8$^+$ T cells. Purified Thy1.2$^+$ T cells of UF20-immunized mice and naive dendritic cells were cocultured with stimulation of extracellular vesicles. Extracellular vesicles were prepared as described in Figure 5B. The results were measured at day 3. The panels show T cells from UF20-immunized mice (filled) and naive mice (open).

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Figure 2, internalization of live bacteria occurs within 90 min of infection. When we consider that antibody-dependent cell-mediated cytotoxicity or antibody-dependent macrophage-mediated cytotoxicity occurs at the cell surface by recognition of the antibody-bound target, we prefer the idea that apoptotic cell death is induced by excessive uptake of bacteria. However, the significance of pathogen-induced apoptosis still remains controversial [14]. By inducing macrophage apoptosis, Salmonella can escape from activated macrophages and reinfect neighboring cells. As activated macrophages are more sensitive to Salmonella-induced apoptosis than nonactivated macrophages, Salmonella-induced apoptosis seems to eliminate the host’s effector cells [26, 27]. However, pathogen-induced apoptosis in macrophages has been shown to induce the release of apoptotic vesicles that carry pathogen-derived antigens to uninfected antigen-presenting cells, such as dendritic cells. These antigen-presenting cells present epitopes from bacterial antigens through MHC-I, MHC-II, and CD1b to bacteria-sensitized donor T cells [13, 28]. Our findings suggest that the apoptotic fragments induced by infection with Salmonella pre-treated with specific IgG predominantly induced Th1 responses (Figure 6A). It is possible that IFN-γ produced by Th1 cells activates macrophages.

CD8+ T cells are also activated through apoptosis induced by infection with M. tuberculosis or S. typhimurium [12, 13, 29, 30]. In the present study, not only CD4+ T cells but also CD8+ T cells were activated. Although purified CD8+ T cells did not proliferate in response to the apoptotic fragments (Figure 5B), apoptotic fragments increased the number of IFN-γ-positive immune CD8+ T cells in cultures of whole splenic T cells (Figure 6B). These findings might reflect that the apoptotic fragments contain a large quantity of antigens with the different epitopes. Because the quantity or quality of antigen in which MHC-I is used might be different, CD8+ T cells did not proliferate in response to the apoptotic fragments. It is suggested that specific IgG activates specific CD8+ T cells by inducing apoptotic cell death through increased bacterial uptake, and host defense could be augmented because CD8+ T cells expose the bacteria by killing the infected cell. Thus far, we have found that 28 kDa and 39 kDa bacterial antigens were detected mainly by Western blotting using anti-Salmonella IgG (data not shown). We are examining whether these antigens are involved in the activation of specific T cells.

It seems likely that specific IgG plays a role in both primary and secondary immune responses to Salmonella infection. On the basis of the results shown in Figure 5, it can be assumed that the transferred IgG in Figures 1B and 1D exercises its synergistic function with the specific T cells generated by the Salmonella challenge [3]. The binding of specific antibodies to Salmonella has been shown to enhance bacterial uptake by macrophages [9, 31]. Salmonella infection also induces apoptosis of host cells, and the engulfment of released apoptotic vesicles by uninfected dendritic cells facilitates the activation of the bacteria-specific T cell responses [12, 29]. This study is the first, to our knowledge, to report that these independent observations are closely linked to the promotion of optimal specific-memory T cell responses in protective immunity.

In primary infection, the phagocytosis by macrophages seems to be the major effector for the clearance of pathogens during the early stage. In the late stages of primary and secondary infections, the generated specific antibody, macrophages, dendritic cells, and generated specific T cells (or memory T cells) cooperate on the clearance of pathogens. An improved understanding of the relative contributions and relationships between the effectors of humoral and T cell–mediated immunity for the clearance of pathogens and the establishment of protective immunity will facilitate the development of more efficacious vaccines. The results of the present study provide an immunological basis for designing improved vaccines against intracellular microbial pathogens. Although many vaccines target B cells, it has been commonly assumed that humoral immunity has little effect against many intracellular pathogens [32, 33]. Our findings suggest that vaccines containing both T cell and B cell epitopes may induce better protective immune responses than do those vaccines containing only a single epitope. These findings suggest that specific antibodies cooperate with specific T cell responses upon the induction of apoptosis in host cells to establish protective immunity against Salmonella infection.

In the case of intracellular pathogens against which T cell–mediated immunity provides host protection, specific IgG can promote strong and optimal cell-mediated immunity even if it does not directly eliminate the pathogen. The strategies for vaccine design that consider the functions of specific antibodies, as described in this article, may also be utilized in regard to infection due to other bacteria that induce apoptosis in phagocytes, including M. tuberculosis [34–36], Yersinia pseudotuberculosis [37], and Shigella flexneri [38]. We suggest that the development of a vaccine targeting B cells could therefore lead to a new therapeutic approach against bacterial infection.

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