ROLE OF ASC IN HELICOBACTER PYLORI INFECTION

Role of ASC in the Mouse Model of *Helicobacter pylori* Infection

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Abstract

Apoptosis associated speck-like protein containing a C-terminal caspase recruitment domain (ASC) is an adaptor molecule activating caspase-1 that stimulates pro-IL-1β and pro-IL-18, two proinflammatory cytokines with critical functions in host defense against a variety of pathogens. In the present study, we investigated the role of ASC in the host defense against *Helicobacter pylori* utilizing ASC-deficient mice. Mice were orally inoculated with *H. pylori*; bacterial load, degree of gastritis, and mucosal levels of inflammatory cytokines were analyzed and compared with those obtained from wild-type mice. We found more prominent *H. pylori* colonization in ASC-deficient mice, as revealed by colony-forming unit (CFU) counts. Both groups of mice developed gastritis; however, ASC-deficient mice showed significant attenuation of inflammation despite high *H. pylori* colonization. ELISA and quantitative RT-PCR analysis revealed complete suppression of IL-18 and substantial reduction of IFN-γ expression, respectively, in ASC-deficient mice without apparent upregulation of other cytokines including IL-10 and TNF-α. These results as a whole indicate that ASC exerts considerable influence on the host defense acting through IL-18 and subsequent IFN-γ production, which in turn contributes to continuous chronic inflammatory response and consequent reduction of *H. pylori* colonization.

Keywords: apoptosis associate speck-like protein containing a C-terminal caspase recruitment domain (ASC), IL-18, INF-γ, *Helicobacter pylori*, gastritis

Introduction

Apoptosis associated speck-like protein containing a C-terminal caspase recruitment domain (CARD) (ASC) is an adaptor molecule originally identified in an insoluble cytosolic fraction called the speck, found in cells undergoing apoptosis (Taniguchi and Sagara 2007). ASC is composed of an N-terminal pyrin domain (PYD) and a C-terminal caspase recruitment domain (Masumoto et al. 1999). Recent studies have shown that ASC specifically activates caspase-1 together with some intracellular nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) such as cryopyrin (Martinon et al. 2002). It has been proposed that ASC links those NLRs through PYD-PYD interactions, and recruits caspase-1 via CARD-CARD associations. Thus ASC mediates the formation of the endogenous multiprotein complex containing NOD-leucine-rich-repeats (LRR) (NOD-LRR) family proteins and caspase-1 called inflammasome, which promotes caspase-1 activation by molecular proximity (Martinon et al. 2002).

Caspase-1 is synthesized as an inactive zymogen that is activated by cleavage after aspartic residues to generate the enzymatically active heterodimer in response to proinflammatory stimuli and bacterial infections (Thornberry and Lazebnik 1998). Activated caspase-1 is essential for the processing and release of biologically active IL-1β and IL-18, two pro-inflammatory cytokines with critical roles in host defense against a variety of pathogens (Dinarello 1998). However, ASC is not required for the secretion of TNF-α and IL-6, which indicates a specific requirement for ASC in caspase-1 dependent secretion of IL-1β and IL-18.

Gene-targeted mice that lack ASC have revealed the *in vivo* role of ASC in several physiological and pathological settings (Yamamoto et al. 2004; Mariathasan et al. 2004). Among them, ASC-deficient mice showed extreme sensitivity to infection with *Francisella tularensis*

with markedly increased bacterial burdens and mortality compared to wild-type mice, which highlights the role of ASC in innate immune defense against certain intracellular bacterial pathogens (Mariathasan et al. 2005).

Helicobacter pylori is a gram negative microaerophilic bacterium that colonizes the gastric mucosa of about half the world's population (Marshall and Warren 1984; Graham 1997). *H. pylori* causes persistent gastritis characterized by neutrophilic and lymphoplasmacytic infiltration and induction of pro-inflammatory cytokines, and is directly linked to the development of peptic ulcer disease as well as gastric adenocarcinoma and gastric malignant lymphoma of mucosa-associated lymphoid tissue-type (Sipponen and Hyvarinen 1993; Huang et al. 1998; Kobayashi et al. 2004). Fortunately, only a small percentage of the population develops serious disease due to *H. pylori* infection (Cave 2001). Who develops such disease is determined largely by the inflammatory response to the infection, which is influenced by the virulence of the infecting strain, host genetic predisposition to disease, and environmental co-factors (Fox et al. 2000).

The initial migration into the gastric mucosa and subsequent activation of inflammatory cells are believed to depend on the production of pro-inflammatory cytokines (Yamaoka et al. 1997). Cytokines are important immune mediators in the host defense against microbial pathogens, including *H. pylori*. The cytokine response in the gastric mucosa of patients chronically infected with *H. pylori* is thought to be predominantly of the Th-1 type (Lindholm et al. 1998). In this regard, pro-inflammatory cytokines such as IL-1β, TNF-α, IFN-γ, and IL-18 have been implicated in the pathogenesis and immunity to *H. pylori* infection.

It remains unclear, however, whether ASC is involved in the host defense mechanism against *H. pylori* infection. In the present study, we utilized mice deficient in ASC to examine

the role of this adaptor molecule in host defense against *H. pylori* infection.

Materials and methods

Animals

The generation of mice deficient in ASC has been described previously (Yamamoto et al. 2004). The mice were backcrossed to C57BL/6 genetic background for over eight generations. Homozygotes for null allele of ASC were produced by crossing heterozygotes, and genotyping of offsprings was confirmed by PCR of genomic DNA. Wild-type littermates were used as controls. All mice were bred at the Animal Facility of Shinshu University under specific pathogen-free conditions, housed in plastic cages, and offered commercially available food pellets and water *ad libitum.*

Bacteria

Mouse-adapted *H. pylori* Sydney strain 1 (SS1), first developed by Lee et al. (Lee et al. 1997), was used in this study. The *H. pylori* was plated on 5% sheep blood agar plates (Becton Dickinson, Cockeys-ville, MD), and incubated at 37C for 4 days under microaerophilic conditions with high humidity. To prepare bacterial suspension, semi-confluent bacterial colonies on plates were scraped with a cotton swab, transferred into 50 ml of Brucella broth (Becton Dickinson) supplemented with 50 μg/ml cholesterol, and incubated at 37C overnight with agitation at 120 rpm under microaerophilic conditions with high humidity. After the incubation, 10 ml of culture was added to 50 ml of brain heart infusion broth (Eiken Chemical, Tokyo, Japan) supplemented with 0.5% yeast extract (Becton Dickinson) and 10% fetal bovine serum (HyClone, South Logan, UT), and incubated further 24 hr. After checking the morphology and mobility of bacteria by a polarizing microscope, the concentration of the bacterial suspension was adjusted at 1.0×10^8/ml by measuring the optical density at 650 nm with a spectrophotometer.

Experimental Infection and Sample Collection

Specific pathogen-free 8 week-old male mice deficient in ASC (n=72) and wild-type littermates (n=72) were subjected to *H. pylori* infection as described (Kobayashi et al. 2007). After overnight fasting, each animal was orogastrically inoculated three times every other day with 0.5 ml of the bacterial suspension by using a gastric intubation needle. Mice were then maintained on fasting further 4 hr after each inoculation, and housed as described above.

Eight mice from each group were sacrificed by cervical dislocation at 2, 10, and 16 weeks after the last inoculation. Each stomach was removed, cut along the greater curvature with separate sterile surgical blade to avoid cross-contamination, and briefly washed with sterile saline. Immediately after macroscopic observation, the stomach was longitudinally dissected into 3 pieces of tissue fragments so that each fragment contains gastric cardia, body, and antrum. For each stomach, one piece was used for quantitative bacterial cultures, and another piece was fixed in 10% neutral buffered formalin at 4C for 24 hr for histopathological examination. The remaining piece was immediately stored in RNA later (Ambion, Austin, TX), frozen in liquid nitrogen, and stored at -80C for measurement of cytokine mRNA expression levels.

Blood samples were collected by cardiac puncture using 1 ml tuberculin syringes. Sera were separated from blood clots by centrifugation at 3,000 rpm for 10 min, and stored at -80C until evaluated for cytokine protein levels.

All animal experiments were conducted according to the protocols approved by the Animal Experiment Committee of Shinshu University School of Medicine.

Assessment of *H. pylori* Infection

The presence of viable *H. pylori* in individual mice was determined by quantitative bacterial

culture as described previously (Chen et al. 1999). A piece of gastric tissue fragment was weighted, and then homogenized in 500 μl of sterile saline with disposable grinders and tubes. Homogenates at a dilution of 1:1, 1:10, and 1:100 with sterile saline were prepared, and 50 μl of each dilution were spread onto *H. pylori*-selective agar plates (NISSUI Pharmaceutical, Tokyo, Japan). The plates were incubated at 37C for 7 days under microaerophilic conditions with high humidity. Bacterial colonies were identified by the rapid urease test and Gram-staining for morphology. The number of colonies was counted, and the amount of viable *H. pylori* was expressed as colony-forming unit (CFU)/gram of stomach. For statistical analysis, the data were normalized by log transformation ($\log_{10}$CFU/gram of stomach).

Histopathological Evaluation

Formalin-fixed gastric tissues were embedded in paraffin, cut into 4 μm-thick sections, and stained with hematoxylin and eosin (HE). Additionally, tissue sections were immunostained with rabbit polyclonal anti-*H. pylori* antibody (DAKO, Kyoto, Japan) using an indirect immunoperoxidase method with peroxidase-labeled goat anti-rabbit Ig antibody (DAKO) as described previously (Kobayashi et al. 2007).

Gastritis-composing factors, e.g. i) *H. pylori* density, ii) polymorphonuclear (PMN) cell (neutrophil) infiltration, and iii) lymphoplasmacytic infiltration, were categorized into four grades (normal, 0; mild, 1; moderate, 2; marked, 3) using visual analog scale based upon the updated Sydney system (Dixon et al. 1996). All samples were blinded and grading was carried out.

Quantitative RT-PCR

Total RNA was extracted from gastric tissue samples using RNeasy Mini Kit (QIAGEN, Valencia,

CA) as manufacturer's instructions. Single-stranded cDNA was then synthesized using Prime-Script Reverse Transcriptase (Promega, Madison, WI) with random primers (Promega) following the manufacturer's instructions. Negative controls were obtained by omitting the reverse transcriptase.

Quantitative PCR was carried out using ABI Prism 7900HT sequence detection system (Applied Biosystems, Tokyo, Japan) with TaqMan® Gene Expression Assays (Applied Biosystems) containing the following mouse-specific primers and TaqMan probes; IL-10 (Mm00439616_m1), TNF-α (Mm00443258_m1), IFN-γ (Mm00801778_m1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Mm99999915_g1) as internal control. Each reaction was comprised of 1 x PCR Buffer (Applied Biosystems) with 200 μM of dNTP (Applied Biosystems), 1.25 μl of TaqMan® Gene Expression Assay, 2.5 μl of cDNA template, and 0.1 μl of AmpliTaq Gold® (Applied Biosystems) in a final volume of 25 μl. After initial denaturation at 50C for 2 min followed by 95C for 10 min, 50 cycles of amplification with denaturation at 95C for 15 sec and annealing-extension at 60C for 60 sec was performed as manufacturer's instructions. Each sample was assayed in duplicate, and Ct value was calculated using SDS 2.3 software (Applied Biosystems). Fold changes were determined using the Ct method, and the expression levels of cytokine genes were normalized to that of GAPDH gene, expressed as a ratio (cytokine/GAPDH x 10^5). Negative controls did not show significant amplifications.

Cytokine Enzyme-Linked Immunosorbent Assay (ELISA)

Serum protein levels of IL-1β and IL-18 in ASC-deficient mice and wild-type littermates were measured using ELISA kits (R&D systems, Minneapolis, MN), based on the quantitative

immunometric sandwich enzyme immunoassay technique, according to the manufacturer's instructions. All sera were assayed in duplicate and results were reported as pg/ml.

Statistical Analysis

All values are expressed as means ± standard error of the mean (SEM). Log_{10}CFU/gram of stomach, grading of inflammation, and the cytokine mRNA expression levels were compared between ASC-deficient mice and wild-type littermates using Mann-Whitney *U*-test. Statistical significance was assumed for *p*-values less than 0.05.

Results

Increased *H. pylori* Colonization in ASC-Deficient Mice

To examine the effect of ASC deficiency on *H. pylori* colonization, we compared CFU/gram of stomach between ASC-deficient mice and wild-type littermates at 2, 10, and 16 weeks post-inoculation. The bacterial load of both groups of mice showed gradual decrease with time, and there is a tendency that ASC-deficient mice showed higher CFU values (Figure 1A). We found that the difference in $\log_{10}$CFU/gram of stomach between ASC-deficient mice and wild-type littermates was not statistically significant by 10 weeks post-inoculation; however, the difference became apparent at later time point. At 16 weeks post-inoculation, while wild-type mice showed substantial drop in bacterial loads, the degree of colonization in ASC-deficient mice was significantly higher with a mean value of $\log_{10}$CFU/gram of stomach 4.52 ± 0.08 vs. 3.87 ± 0.09 in wild-type mice (p=0.0043) (Figure 1A). Additionally, immunostaining for *H. pylori* showed numbers of bacteria on the surface mucosa and in the gastric proper glands in both ASC-deficient and wild-type mice (Figure 1B). Taken together, these findings indicate that the deficiency of ASC increases susceptibility to *H. pylori* colonization in the stomach, suggesting the protective role of ASC against *H. pylori* infection.

H. pylori-Induced Gastritis Is Attenuated in ASC-Deficient Mice

To evaluate the impact of ASC deficiency on the pathogenesis of *H. pylori*-induced gastritis, tissue sections obtained from the stomach of ASC-deficient and wild-type mice infected with *H. pylori* were examined histologically. We found that both ASC-deficient and wild-type mice developed some gastric inflammation at all the time points examined. At 2 and 10 weeks after infection, both groups of mice showed very mild neutrophilic and lymphoplasmacytic infiltrations, with a small number of eosinophils, in the lamina propria, particularly in the vicinity

of muscularis mucosae (Figure 2, A-D). It is noted that neutrophilic infiltrations were almost always conspicuous in fundic gland area and minimal in pyloric gland area in both ASC-deficient and wild-type mice, consistent with the previous report that neutrophilic infiltrations are site dependent in mice (Lee et al. 1990). There were no statistical differences in the scores of inflammatory cell infiltrate between the two groups at 10 weeks after infection (Figure 3A and B). However, at 16 weeks after infection, wild-type mice showed moderate corpus gastritis. The inflammation was characterized by a chronic inflammatory cell infiltrate composed mostly of lymphocytes, which formed discrete foci in the deeper portion of mucosa and submucosa. Mild neutrophilic infiltration was also present in the gastric corpus (Figure 2E). In contrast to wild-type mice, the degree of inflammation in ASC-deficient mice was significantly attenuated (Figure 2F). The score for activity in ASC-deficient mice was significantly lower than that in wild-type mice (p=0.0427) (Figure 3B). Scores for mononuclear cell infiltration were also lower in ASC-deficient mice compared to wild-type mice, although the difference was not statistically significant (Figure 3A). It is noted that neither atrophy nor intestinal metaplasia was observed in any mice examined during the 16 weeks time period. Taken together, these data demonstrate that the deficiency of ASC in mice leads to less severe inflammatory response against *H. pylori* infection, suggesting that ASC contributes to host resistance against *H. pylori* infection by increasing gastric inflammatory response.

ASC Deficiency Resulted in Complete Suppression of IL-18 and Substantial Reduction of INF-γ Expression

We further investigated the host response elicited by *H. pylori* in ASC-deficient mice by measuring cytokine responses including IL-10, TNF-α, IFN-γ, IL-1β, and IL-18, in the gastric

mucosa and in the serum at 16 weeks after infection by quantitative RT-PCR and ELISA, and compared with data obtained from wild-type littermates. ASC-deficient mice had significantly (p=0.0286) lower levels of mRNA for the Th1 cytokine IFN-γ than did wild-type mice, whereas IL-10 and TNF-α mRNA expression levels did not differ significantly between the two groups (Figure 4A).

We next measured the serum concentration of IL-1β and IL-18 by ELISA in both ASC-deficient and wild-type mice. As expected, we found that ASC deficiency completely abolished IL-18 secretion (Figure 4B). However, we did not detect measurable serum IL-1β following infection with *H. pylori* in both groups of mice (data not shown).

Discussion

To our knowledge, the present study is the first to analyze the role of ASC in the host defense mechanism against *H. pylori* infection. We found that ASC-deficient mice were more susceptible to *H. pylori* infection, and the inflammatory lesions, corresponding to a mild to moderate gastritis, were significantly attenuated in ASC-deficient mice at 16 weeks post-inoculation, compared to wild-type mice. This was supported by the finding that ASC-deficient mice had lower IFN-γ expression levels in the stomach than wild-type mice did.

However, the expression levels of TNF-α and IL-10 in both groups did not differ significantly. The significant decreased local IFN-γ mRNA in ASC-deficient mice was likely to relate to the deficiency of IL-18, a potent inducer of IFN-γ (Okamura et al. 1995). This is consistent with the requirement of ASC for caspase-1 activation leading to IL-18 secretion *in vivo*.

Although colonization showed wide inter-individual variation at 16 weeks post-infection, the level of colonization was substantially higher in ASC-deficient mice compared to wild-type mice. The relatively low *H. pylori* colonization in wild-type littermates was histologically associated with a strong inflammatory cell infiltrate as compared with ASC-deficient mice. This is consistent with the previous report that IFN-γ-deficient mice showed higher gastric colonization by *H. pylori* than wild-type mice in C57BL/6 background (Sawai et al. 1999). Thus, the lower levels of gastric IFN-γ expression, observed in the present study, may provide an explanation for higher colonization of *H. pylori* in ASC-deficient mice. Also supportive of our findings is the observation that despite a chronic 15 months infection with *H. pylori*, the IFN-γ-deficient mice have minimal gastritis, compared with severe inflammation in the stomach of C57BL/6 mice (Censini et al. 1996). Furthermore, IFN-γ levels measured by RT-PCR have been recognized as a useful predictor of degree of gastritis in C57BL/6 mice infected with *H. pylori*

(Goto et al. 1999).

The hallmark of gastric inflammatory response to *H. pylori* is its capacity to persist for decades (Everhart 2000; Blaser et al. 1998). This is in contrast to inflammatory reactions induced by other mucosal pathogens, such as *Salmonella* spp., that either resolves within days or progress to eliminate the host (Zhang et al. 2003; Gordon et al. 2001). Previous study in ASC-deficient mice has focused on the role of ASC in the innate immune response against *F. tularensis* infection, which causes a plague-like disease in humans after exposure to as few as 10 cells (Mariathasan et al. 2004). Important differences have arisen, which may be due to the site of infection and/or to the persistent nature of *H. pylori* infection. Mariathasan et al. reported that *F. tularensis*-infected ASC-deficient mice showed markedly increased bacterial burdens and mortality as compared with wild-type mice, demonstrating a key role for ASC in innate defense against infection by this pathogen (Mariathasan et al. 2004). In contrast, in our hand, the severity of gastritis induced by *H. pylori* infection was clearly attenuated in ASC-deficient mice compared to wild-type littermates in spite of heavy colonization at 16 weeks post-inoculation.

Our experiment specifies a concrete immune defect that impairs the ability of ASC-deficient mice to orchestrate a normal mucosal inflammatory response to *H. pylori* infection. This may occur via more than one mechanism. However, our available data so far indicate that ASC is critical for production of IL-18 and subsequent IFN-γ secretion in response to *H. pylori* infection, contributing to continuous chronic inflammatory response and consequent reduction of *H. pylori* colonization.

H. pylori-infected ASC-deficient mice showed a significantly lower score in PMN cell infiltration at 16 weeks after infection. This data is relevant not only to the less inflammation observed but also to the level of colonization, because neutrophils are involved primarily in the

host response against *H. pylori* infection. We also found that ASC deficiency completely abolished IL-18 secretion and dramatically reduced IFN-γ expression levels during *H. pylori* infection as measured by ELISA and quantitative RT-PCR, respectively. Taken together, these data suggest that the increased susceptibility and less inflammation observed in ASC-deficient mice could be due to a reduced inflammatory environment, e.g. lack of IL-18, less IFN-γ production and PMN cell infiltration.

The association between chronic inflammation and cancer is now well established (Coussens and Werb 2002). This association has recently received renewed interest with the recognition that microbial pathogens can be responsible for the chronic inflammation observed in many cancers, particularly those originating in the gastrointestinal system (Macarthur et al. 2004). A prime example is *H. pylori*, which infects more than 50% of the world's population and is known to be responsible for inducing chronic gastritis that progresses to atrophy, intestinal metaplasia, dysplasia, and gastric cancer (Correa 1995).

H. pylori infection in both humans and C57BL/6 mice is associated with Th1-like immune response (Mohammadi et al. 1996). Several studies have provided direct experimental evidence that Th1-type immune response is responsible for the progression of gastric preneoplastic lesions (Fox et al. 2000). In C57BL/6 mice, chronic infection with *H. pylori* is associated with considerable gastric inflammation and corpus atrophy, which is considered a premalignant lesion (Fox et al. 2000). However, deletion of the gene encoding the Th1 cytokine IFN-γ protects mice from gastric atrophy induced by infection with *H. pylori*. Here, we show that deficiency of ASC in mice completely abolished the secretion of IL-18, and drastically reduced IFN-γ expression; this was histologically associated with a weak inflammatory response against *H. pylori* infection. These findings suggest that ASC-deficient mice may avoid *H. pylori*-

induced gastric cancer.

There is mounting evidence that ASC mediates the clustering and activation of caspase-1 and subsequent maturation of proinflammatory cytokines IL-1β and IL-18 (Stehlik et al. 2003; Martinon et al. 2002). These cytokines promote cell-mediated immune responses and are capable of generating anti-tumor responses. For example, IL-18 has been shown to stimulate macrophages to elicit a potent cytotoxic response against glioma cells (Kikuchi et al. 2000). On the other hand, loss of ASC expression by methylation in breast and other cancers has been indicated to correlate with carcinogenesis, suggesting a tumor suppressor role in those cell types (McConnell and Vertino 2004). Based on these reports, ASC-deficient mice chronically infected with *H. pylori* may have an increased risk for developing gastric cancer. It is of great interest whether ASC-deficient mice with *H. pylori* infection represent a useful tool for investigating how ASC is involved in carcinogenesis.

In conclusion, the present study identified a new mechanism that may contribute to *H. pylori* pathogenesis. Loss of ASC protein abolishes production of IL-18 and significantly reduced IFN-γ expression in response to *H. pylori* infection, contributing to attenuated inflammatory response and increased *H. pylori* colonization. Further studies are necessary to clarify the role of ASC in gastric carcinogenesis during persistent *H. pylori* infection.

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Figure Legends

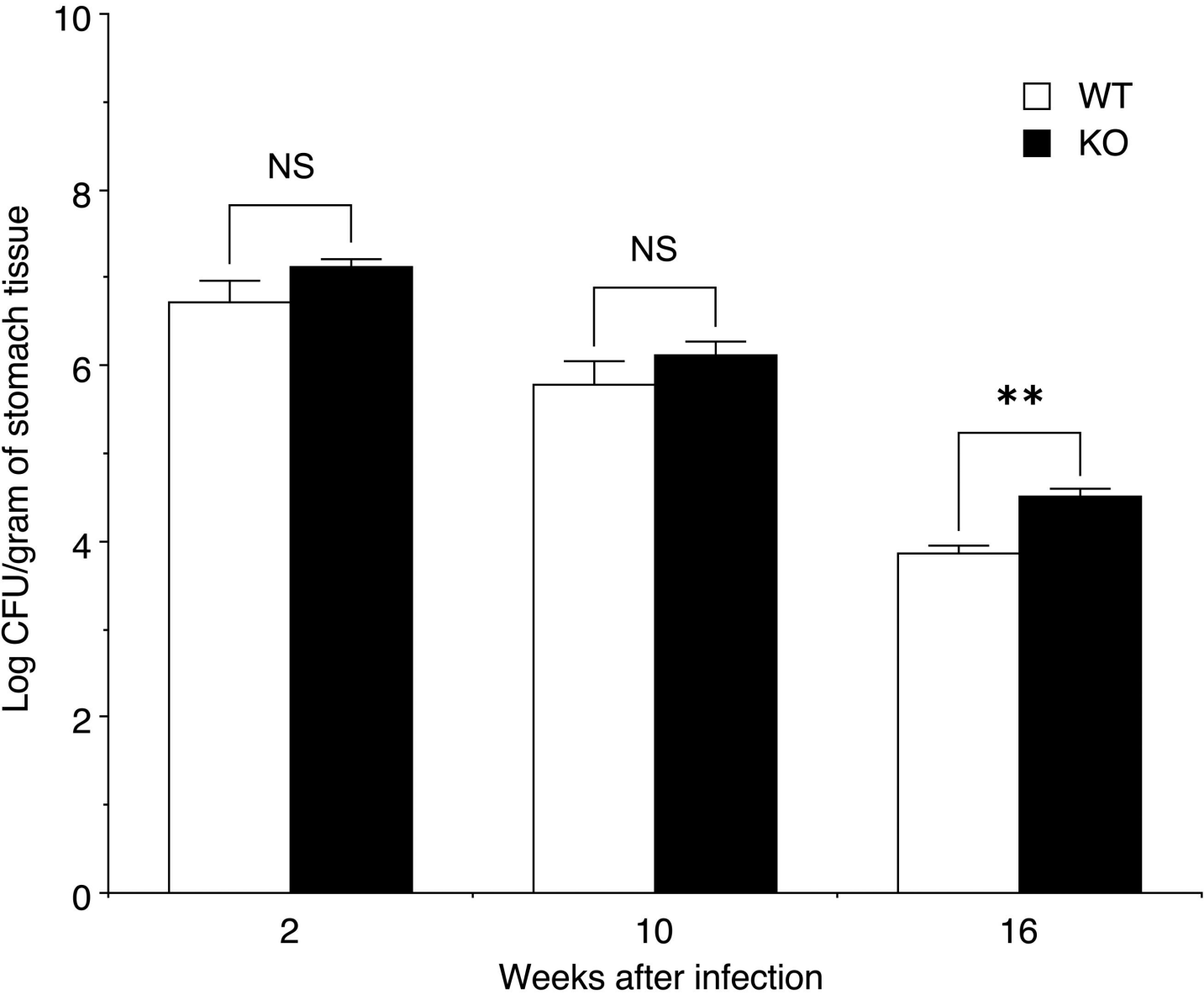
Figure 1 Higher colonization of *H. pylori* in the stomach of ASC-deficient mice . (**A**) Bacterial loads, assessed as $\log_{10}$CFU/gram of stomach, of both wild-type (WT) (open bars) and ASC-deficient mice (KO) (closed bars) show gradual decrease with time, and there is a tendency that ASC-deficient mice show higher CFU values. Colonization in ASC-deficient mice is significantly higher than that in wild-type mice at 16 weeks post-infection. **, p=0.01; NS, not significant. (**B**) Immunostaining for *H. pylori*. Tissue sections were obtained from the stomach of wild-type (left) and ASC-deficient mice (right) 16 weeks post-inoculation. *H. pylori* colonization was detected on the surface mucosa and in the gastric proper glands in both groups of mice. Bar = 100 μm.

Figure 2 Representative histopathology of the gastric mucosa of wild-type (left panels) and ASC-deficient mice (right panels) at 2 (**A** and **B**), 10 (**C** and **D**), and 16 weeks (**E** and **F**) after *H. pylori* infection. At 2 weeks after infection, both wild-type (**A**) and ASC-deficient mice (**B**) developed gastritis, with mild to moderate inflammatory cell infiltrate present within the lamina propria. At 10 weeks post-infection, the gastric mucosa from both groups of mice shows an inflammatory cell infiltrate similar in composition but slightly larger than that seen at 2 weeks post-infection (**C** and **D**). No significant histopathological difference is found between the two groups. At 16 weeks after infection, wild-type mice developed moderate gastritis (**E**), on the other hand, ASC-deficient mice had only mild gastritis (**F**). Inset in (**E**) shows inflammatory cells composed of lymphocytes and neutrophils. Bar = 100 μm.

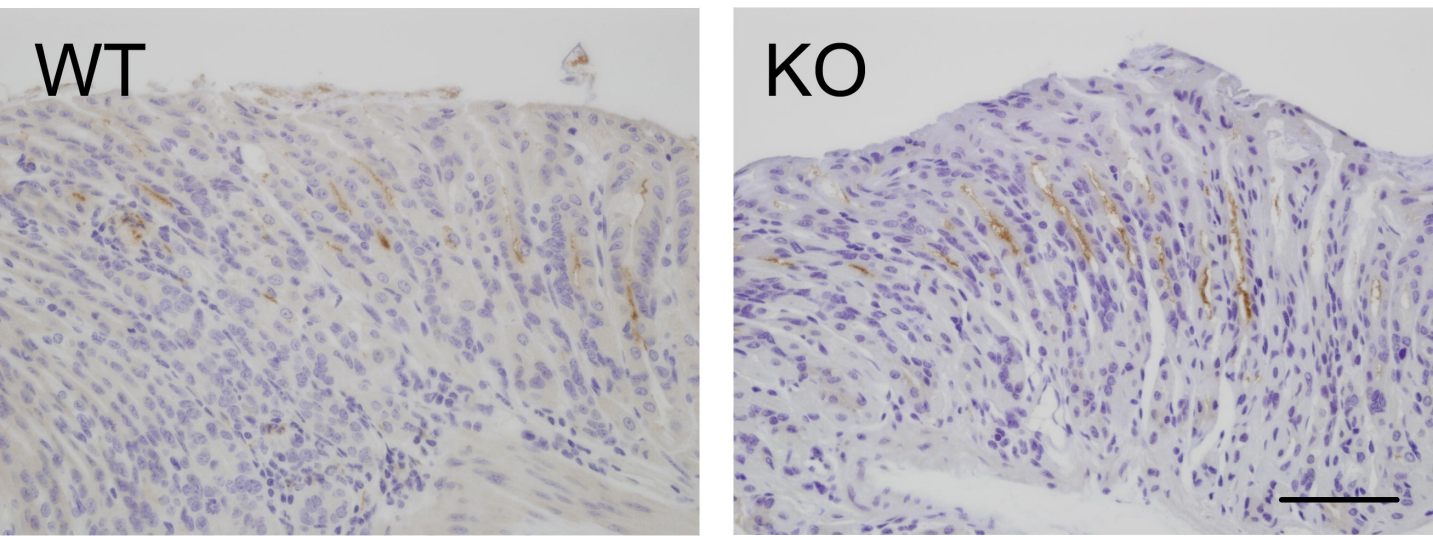
Figure 3 Assessment of chronic inflammation (**A**) and activity (**B**) induced by *H. pylori* infection in the stomach of wild-type (WT) (open bars) and ASC-deficient mice (KO) (closed bars). Gastric mucosal changes were assessed histologically based on the updated Sydney system. Scores for respective mononuclear and polymorph nuclear cell infiltrates at 10 weeks post-infection do not differ between the two groups. At 16 weeks post-infection, however, the score of activity in ASC-deficient mice is significantly lower than that in wild-type mice (**B**). The score for mononuclear cell infiltration is also lower in ASC-deficient mice compared to wild-type mice, although the difference is not statistically significant (**A**). Data are expressed as means ± SEM. *, $p<0.05$; NS, not significant.

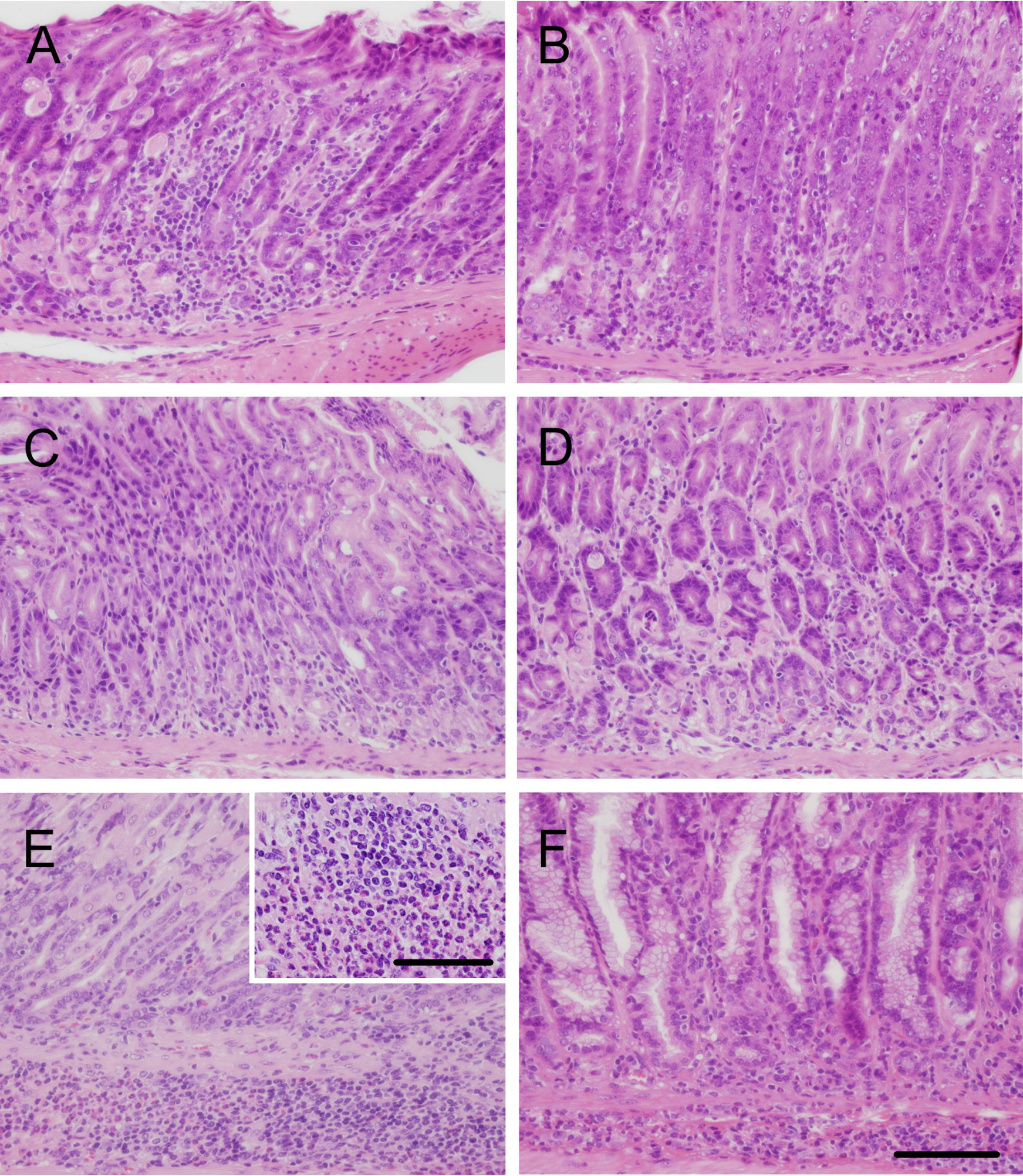
Figure 4 (**A**) Gene expression of IFN-γ, TNF-α, and IL-10 in the stomach of wild-type (WT) (open bars) and ASC-deficient mice (KO) (closed bars) at 16 weeks after infection with *H. pylori*, assessed by quantitative RT-PCR. ASC-deficient mice have lower levels of IFN-γ mRNA than wild-type mice. On the other hand, the expression levels of TNF-α and IL-10 do not differ significantly between the two groups. Data are expressed as means ± SEM. *, $p<0.05$; NS, not significant. (**B**) Serum protein level of IL-18 as assessed by ELISA. Sera were taken from ASC-deficient (close bar) and wild-type mice (open bar) at 16 weeks after infection. ASC deficiency completely abolishes IL-18 secretion. Data are represented as means ± SEM. ND, not determined

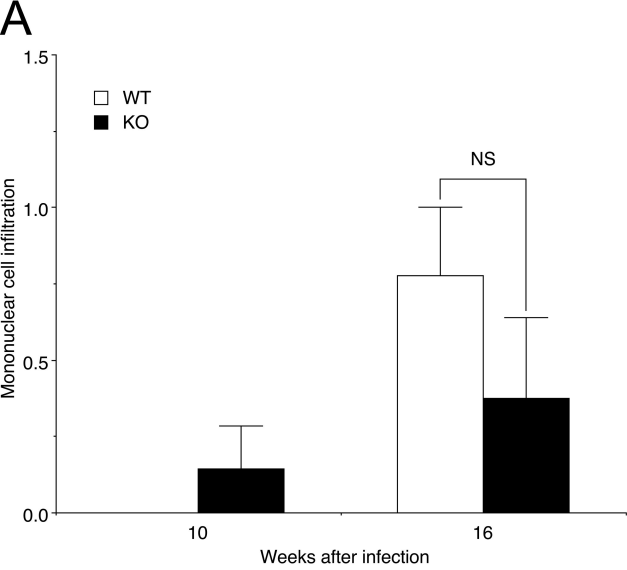
A

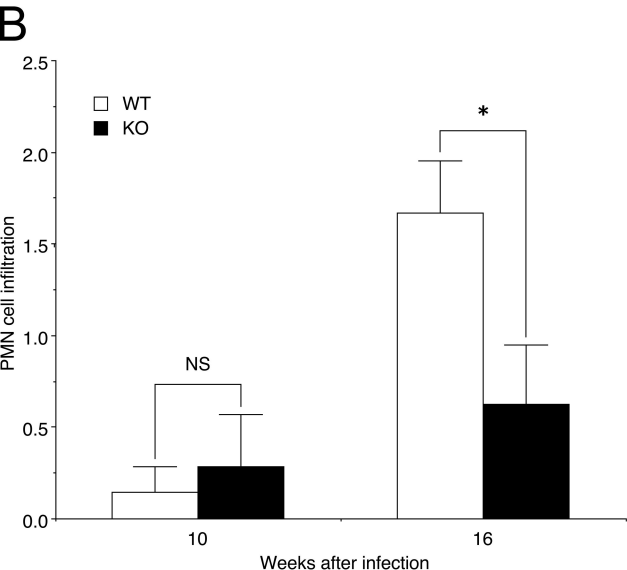

10
8
6
4
2
0
Log CFU/gram of stomach tissue
WT
KO
NS
NS
**
2
10
16
Weeks after infection

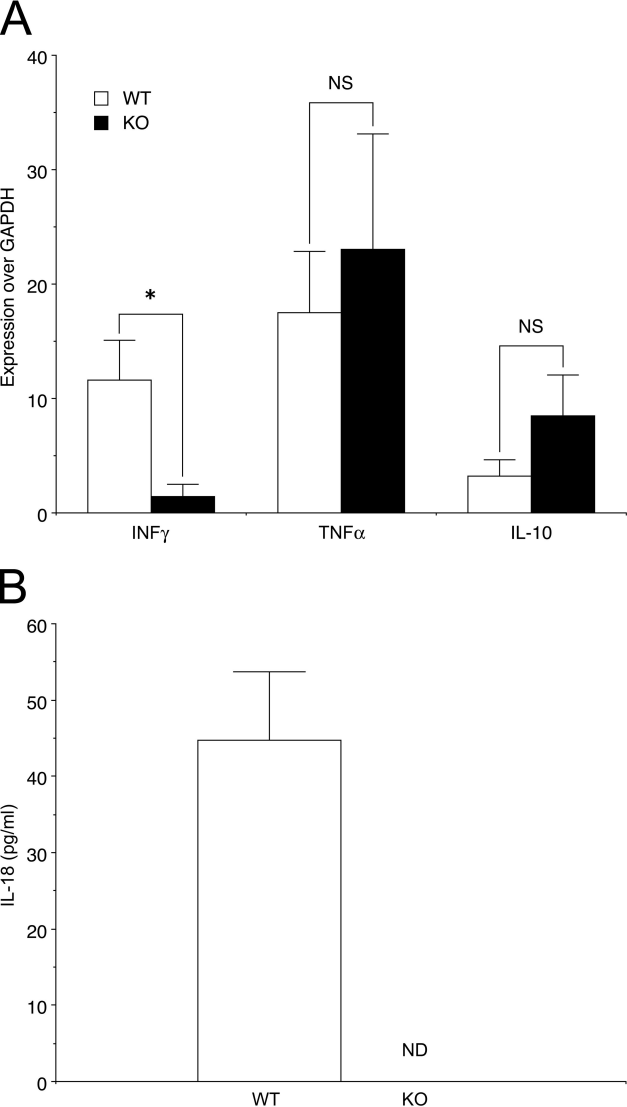
B


WT
KO


A
B
C
D
E
F


A
1.5
1.0
0.5
0.0
WT
KO
NS
Mononuclear cell infiltration
10
16
Weeks after infection


B
2.5
2.0
1.5
1.0
0.5
0.0
WT
KO
NS
*
PMN cell infiltration
10
16
Weeks after infection


A
40
30
20
10
0
Expression over GAPDH
WT
KO
*
NS
NS
INFγ
TNFα
IL-10
B
60
50
40
30
20
10
0
IL-18 (pg/ml)
ND
WT
KO