INFECTION AND IMMUNITY, May 2006, p. 2787–2802 Vol. 74, No. 5
0019-9567/06/$08.00+0 doi:10.1128/IAI.74.5.2787–2802.2006

Influence of Slc11a1 on the Outcome of *Salmonella enterica* Serovar Enteritidis Infection in Mice Is Associated with Th Polarization

Judith Caron,[1,2] Line Larivière,[2] Mayss Nacache,[1,2] Mifong Tam,[2] Mary M. Stevenson,[2,3,5] Colin McKerly,[6] Philippe Gros,[2,4] and Danielle Malo[1,2,3]*

Department of Human Genetics, McGill University, Montreal, Québec,[1] Centre for the Study of Host Resistance, Montreal General Hospital, Montreal, Québec[2]; Department of Medicine, McGill University, Montreal, Québec,[3] Department of Biochemistry, McGill University, Montreal, Québec,[4] Centre for Host-Parasite Interactions, McGill University, Macdonald Campus, Ste. Anne de Bellevue, Québec,[5] and Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario,[6] Canada

Received 21 November 2005/Returned for modification 3 January 2006/Accepted 22 February 2006

Genetic analyses identified *Ses1* as a significant quantitative trait locus influencing the carrier state of 129S6 mice following a sublethal challenge with *Salmonella enterica* serovar Enteritidis. Previous studies have determined that *Slc11a1* was an excellent candidate gene for *Ses1*. Kinetics of infection in 129S6 mice and *Slc11a1*-deficient (129S6-*Slc11a1*^{tm1Mcg}) mice demonstrated that the wild-type allele of *Slc11a1* contributed to the *S. enterica* serovar Enteritidis carrier state as early as 7 days postinfection. Gene expression profiling demonstrated that 129S6 mice had a significant up-regulation of proinflammatory genes associated with macrophage activation at day 10 postinfection, followed by a gradual increase in immunoglobulin transcripts, whereas 129S6-*Slc11a1*^{tm1Mcg} mice had higher levels of immunoglobulins earlier in the infection. Quantitative reverse transcription-PCR revealed an increase in Th1 cytokine (*Ifng* and *Il12*) and Th1-specific transcription factor *Tbx21* expression during infection in both the 129S6 and 129S6-*Slc11a1*^{tm1Mcg} strains. However, the expression of *Gata3*, a transcription factor involved in Th2 polarization, *Cd28*, and *Il4* was markedly increased in *Slc11a1*-deficient mice during infection, suggesting a predominant Th2 phenotype in 129S6-*Slc11a1*^{tm1Mcg} animals following *S. enterica* serovar Enteritidis infection. A strong immunoglobulin G2a response, reflecting Th1 activity, was observed only in 129S6 mice. All together, these results are consistent with an impact of *Slc11a1* on Th cell differentiation during chronic *S. enterica* serovar Enteritidis infection. The presence of a Th2 bias in *Slc11a1*-deficient mice is associated with improved bacterial clearance.

Human infectious diseases are among the major causes of morbidity and mortality worldwide. Gram-negative bacteria of the genus *Salmonella* are ubiquitous in nature and inhabit the normal intestinal flora of multiple hosts. They can cause a variety of pathologies, including gastroenteritis, abortions, pneumonias, and lethal septicemias, in both humans and animals. *Salmonella* infections usually occur through ingestion of contaminated food or water and are responsible for two major disease patterns in humans, typhoid fever, a systemic disease, and salmonellosis, a self-containing gastrointestinal illness. *Salmonella* infection affects 1.4 million people annually in the United States alone, where *Salmonella enterica* serovars Enteritidis and Typhimurium account for more than half of the reported cases (16). Enteric fever is caused by the human pathogen *S. enterica* serovar Typhi and to a lesser extent by *S. enterica* serovar Paratyphi A and B. An estimated 21 million cases of typhoid fever are reported each year, with 200,000 associated fatalities (16). Approximately 5% of infected patients develop a chronic carrier state with active shedding of *Salmonella* for more than a year, whereas others become lifelong carriers (16). Chronic carriers are at an increased risk of undergoing relapses and developing other pathologies. They also become the new reservoir of the pathogen in the population, increasing the risks of infecting other people and thus playing a major role in the reemergence of epidemic-prone diseases.

In mice, *S. enterica* serovar Typhimurium causes a systemic disease resembling typhoid fever, and the outcome of infection relies on the activation of both the innate and adaptive immune responses of the host. Studies with mouse models of infection have identified several innate immune genes, including *Slc11a1* (solute carrier family 11 member 1, also known as *Nramp1*), *Tlr4* (Toll-like receptor 4), *Nos2* (inducible nitric oxide synthase), and that for NADPH oxidase, that influence the early phase of *S. enterica* serovar Typhimurium infection (reviewed in reference 58). Clearance of the bacteria from the reticuloendothelial system during the late stage of infection involves T and B lymphocytes, costimulatory molecules (CD28), T-cell receptor (TCR), and the major histocompatibility complex class II genes (70).

We have developed a chronic model of *Salmonella* infection based on the inoculation of a sublethal dose of *S. enterica* serovar Enteritidis into C57BL/6J and 129S6/SvEvTac (129S6) mice (13). *S. enterica* serovar Enteritidis infection of C57BL/6J and 129S6 animals does not cause a clinical disease; however, persistence of the bacteria within the spleen and mesenteric lymph nodes is observed for a prolonged period of time in 129S6 compared to C57BL/6J mice (13, 14). Previous single-

* Corresponding author. Mailing address: Departments of Medicine and Human Genetics, McGill University, McGill University Health Center, Montreal General Hospital, 1650 Cedar Avenue, local L11-144, Montreal, QC, Canada H3G 1A4. Phone: (514) 934-1934, ext. 44503. Fax: (514) 933-7146. E-mail: Danielle.malo@mcgill.ca.

locus linkage analysis and a genome-wide search for interacting loci in a (C57BL/6J × 129S6)F_2 segregating population have revealed that the genetic architecture of *Salmonella* persistence is different in females and males. In females, the genetic model included the individual effect of *Ses3* on chromosome 15 and two significant interactions between *Ses1* on chromosome 1 and *D7Mit267* and between *Ses1* and *DXMit48*, accounting for 47% of the total phenotypic variance. The model for males included the individual effect of *Ses1.1* (proximal chromosome 1) and three interactions (*Ses1-D9Mit218*, *D2Mit197-D4Mit2*, and *D3Mit256-D13Mit36*) and explained 47% of the phenotypic variance (13, 14). These analyses have clearly demonstrated that *Ses1*, which was validated with congenic mice, is the locus having the greatest impact on the phenotypic variance in both females and males (14).

We have previously reported that *Slc11a1* is located at the maximum peak logarithm of the odds score of the *Ses1* genetic interval, making this gene an excellent candidate based on its chromosomal position and its function (13). *Slc11a1* has been shown in multiple studies to be of primordial importance in the outcome of infections with intracellular pathogens including *S. enterica* serovar Typhimurium in mice (67, 68). A role for *SLC11A1* in the host defense against tuberculosis and leprosy was demonstrated in humans (6, 23, 24) and against salmonellosis and chronic *Salmonella* carriage in chickens (5, 31, 38). *Slc11a1* is involved in the control of bacterial growth in the reticuloendothelial system during the early phase of *Salmonella* infection. In mice, *Slc11a1* presents two allelic forms: a wild-type allele and a susceptible *Slc11a1* allele (*Slc11a1*^{s}) that has a nonconservative glycine-to-aspartic acid change at residue 169 which renders the mRNA too unstable to translate into a functional protein (42, 68). *Slc11a1* is located in the late endosome-lysosome compartment of resting phagocytes (29, 59) and is recruited to the membrane of phagosomes containing live bacteria (29). During *Salmonella* infection, the bacteria are phagocytosed by macrophages and polymorphonuclear cells (PMNs), in which fusions of early endosomes with lysosomes decrease the internal phagosomal pH and confer bactericidal properties on these cells (18, 72). To circumvent bacterial killing, salmonellae generate a specialized compartment named the *Salmonella*-containing vacuole (SCV), in which the environment is favorable to the survival of the bacteria. *Slc11a1* has been shown to have an impact on SCV maturation: SCVs formed in *Slc11a1*-deficient macrophages do not acquire M6PR (mannose 6-phosphate receptor), a protein known to regulate the delivery of a subset of lysosomal enzymes from the trans-Golgi network to the prelysosomal compartment (18, 29, 35, 72). In addition, Slc11a1 functions as a pH-dependent manganese (Mn^{2+}) and iron (Fe^{2+}) efflux pump (25, 33, 34) at the phagosomal membrane. Divalent cations like manganese and iron are critical for the survival of pathogens, and removal of these from the phagosome results in enhanced bacteriostatic and/or bactericidal activity. SCV maturation is restored in iron-depleted primary macrophages from *Slc11a1*-deficient mice, suggesting that Slc11a1 counteracts the ability of salmonellae to arrest phagosome maturation through depletion of iron and/or other cations (34).

The involvement of *Slc11a1* in controlling *S. enterica* serovar Enteritidis clearance was initially demonstrated with mice carrying a null allele at *Slc11a1* (129S6-*Slc11a1*^{tm1Mcg}) (13). In the experimental model of *S. enterica* serovar Enteritidis infection, the wild-type allele at *Slc11a1* contributes to *Salmonella* persistence in the spleens of 129S6 mice during the late phase of infection. The objective of the present study was to investigate this unexpected role of *Slc11a1* in the outcome of a chronic *Salmonella* infection. These analyses provide evidence that functional polymorphisms at *Slc11a1* are associated with T-helper cell polarization during a chronic *S. enterica* serovar Enteritidis infection, leading to either bacterial clearance (predominant Th2 response in 129S6-*Slc11a1*^{tm1Mcg} mice) or bacterial persistence (predominant Th1 response in 129S6 mice).

MATERIALS AND METHODS

Animals. The inbred mouse strains 129S6/SvEvTac (129S6) and C57BL/6J were obtained from Taconic (Germantown, NY) and The Jackson Laboratories (Bar Harbor, ME), respectively. The 129S6-*Slc11a1*^{tm1Mcg} mice were provided by Philippe Gros (McGill University, Montreal, Québec, Canada). The 129S6-*Slc11a1*^{tm1Mcg} mice were generated by crossing chimeric offspring created with embryonic stem cells of 129S6/SvEvTac origin with 129S6/SvEvTac mice. Prior to infection, all mice were maintained in our animal facilities under conditions specified by the Canadian Council on Animal Care.

***Salmonella* infection.** The *Salmonella* stock and infectious dose were prepared as previously described (60). Briefly, mice were infected intravenously with 1,000 CFU of *S. enterica* serovar Enteritidis strain 3b (William Kay, University of Victoria, Victoria, British Columbia, Canada) and sacrificed 1, 3, 5, 7, 10, or 42 days postinoculation by carbon dioxide asphyxiation. The spleens were removed aseptically and used for CFU counts, RNA extraction, or splenocyte purification. The *Salmonella* loads in the spleens were obtained, counted, and analyzed as described by us previously (13). The spleen index was determined as the root square of the spleen weight (×100) divided by the body weight (26).

Hematology. Blood samples were obtained by cardiac puncture from control animals and infected mice at 10 and 42 days postinoculation. The collected blood was diluted 1:200 in 1× phosphate-buffered saline (PBS) or 1:10 in 3% acetic acid to enumerate the erythrocytes (RBC) and leukocytes (WBC), respectively, and the numbers of cells were determined microscopically with a hemocytometer. The percentages of macrophages, lymphocytes, and polymorphonuclear cells were determined by differential counts of 400 cells on blood smears stained with Diff-Quick (Dade Behring, Zurich, Switzerland).

Tissue sample collection and histological evaluation. Mice were anesthetized with a mixture of ketamine and xylazine in saline. Anesthetized mice were perfused through cardiac puncture with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). The tissues were collected and kept in 20% sucrose-PBS for further histopathologic examination. Fixed tissue samples were processed, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin.

Flow cytometry. Spleens were aseptically removed from control and infected 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice. The red pulp was removed through gentle homogenization of the spleen between the frosted ends of two sterile glass slides, red blood cells were lysed, and splenocytes were extracted with Lympholyte M (Cedarlane Laboratories Limited, Hornby, Ontario, Canada). Splenocytes were counted and adjusted to the desired concentration in RPMI medium (Invitrogen Life Technologies, Burlington, Ontario, Canada). The cells were stained with 2 μg of monoclonal antibody (MAb) suspended in PBS supplemented with 1% bovine serum albumin and 0.1% sodium azide for 30 min at 4°C. After washes, the cells were suspended in PBS with 1% fetal calf serum (HyClone, Logan, Utah) and 0.1% sodium azide. These cells were immediately analyzed by FACScan (BD Biosciences, Mississauga, Ontario, Canada). Data analysis was performed with the CellQuest software (BD Biosciences, Mississauga, Ontario, Canada). The different splenic cell populations were determined by single-color staining of surface markers. Fluorescein isothiocyanate-conjugated MAbs were all purchased from PharMingen (BD Biosciences, Mississauga, Ontario, Canada). The MAbs used were against mouse CD3 (clone 145-2C11, hamster immunoglobulin G [IgG]), CD4 (clone RM4-5, rat IgG2a), CD8 (clone 53-6.7, rat IgG2a), B220 (clone RA3-6B2, rat IgG2a), MAC-1 (clone M1/70, rat IgG2b), F4/80 [clone 6F12, rat IgG2a(κ)], Gr-1 (clone RB6-8C5, rat IgG2b), αβ TCR [clone H57-597, Armenian hamster IgG2(λ1)], γδ TCR [clone GL3, Armenian hamster IgG2(κ)], and PanNK/CD49b [clone DX5, rat IgM(κ)].

RNA extraction. RNA was extracted from the spleens of control and infected animals with TRIZOL reagent according to the manufacturer's instructions (Invitrogen Life Technologies, Burlington, Ontario, Canada). Briefly, 30 to 50 μg

of the spleen was homogenized and placed in TRIZOL solution to extract the RNA. The RNA was purified with chloroform and precipitated in isopropanol. The RNeasy total RNA cleanup kit was used as an extra RNA-purifying step (QIAGEN, Mississauga, Ontario, Canada). The RNA was suspended in RNase-free diethyl pyrocarbonate water (Invitrogen Life Technologies, Burlington, Ontario, Canada) and quantitated by spectrophotometry, and its quality was observed by gel electrophoresis with morpholinepropanesulfonic acid (MOPS) buffer, as well as with the RNA 6000 Nano LabChip with the 2100 bioanalyzer (Agilent, Palo Alto, CA).

Microarray analysis. Total spleen RNAs of 129S6 and 129S6-*Slc11a1*^{tm1Mcg} female mice were extracted from control and *Salmonella*-infected animals sacrificed at days 10 and 42 postchallenge and were pooled to account for all possible intrastrain variation. These RNA pools were used to prepare biotinylated targets as previously described (65). Preparation of fluorescent cRNA, hybridization, and scanning of the arrays were done according to the manufacturer's instructions (Affymetrix, Santa Clara, CA). Briefly, 10 μg of RNA was reversed transcribed to double-stranded cDNA with an oligo(dT) primer containing a T7 RNA polymerase binding site. The cDNA was transcribed in vitro to cRNA with biotinylated dUTP and dCTP. Microarray hybridizations were performed with 10 μg of target cRNA on the Affymetrix U74Av2 GeneChip (Affymetrix, Santa Clara, CA). These microarrays contain probe sets for 12,000 mouse genes (6,000 known genes and 6,000 expressed sequence tags). Hybridized microarrays were scanned with a GeneArray scanner (Hewlett-Packard, Palo Alto, CA). Transcript expression levels were calculated from a single average difference ratio across 16 probe pairs, as well as a reliability score (ambiguous or absent [A], marginal [M], or present [P], based on the hybridization variability within each probe set) following scanning of the hybridized microarrays with the Affymetrix GeneChip Mas 4 software (Affymetrix, Santa Clara, CA).

The raw expression values were analyzed with the GeneSpring software package v7.0 (Silicon Genetics, Redwood City, CA). The data were normalized per chip to the 50th percentile and per gene by using the median. All values below 0.1 were reassigned a threshold value of 0.1. The probe sets showing an A reliability score at all of the time points were discarded. Once the normalization steps were done, we considered the genes with an expression change of twofold or more between the 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mouse strains at any time point for further analysis. GeneSpring allowed the regular retrieval of newly annotated expressed sequence tags from the National Center for Biotechnology Information (www.ncbi.nih.gov/). The selected genes were then organized in functional clusters. The genes that were scrutinized in more detail were the ones with known functions or those involved in pathways implicated in inflammation.

Quantification of the expression of genes influencing Th cell development. A set of genes known to be involved in the differentiation process of Th cells were selected for quantification and include the cytokine genes *Ifng*, *Il12*, and *Il4*; the transcription factor genes *Tbx21* and *Gata3*; and the costimulatory molecule genes *Cd28*, *Cd80*, and *Cd86*. Reverse transcription was performed with 5 μg of total RNA and Moloney murine leukemia virus reverse transcriptase (Invitrogen Life Technologies, Burlington, Ontario, Canada). Real-time PCR was performed in duplicate on each transcript with Brilliant SYBR green QPCR master mix according to the manufacturer's (Stratagene, La Jolla, CA) instructions. The primers used were designed from the coding sequences of the genes as obtained from the Affymetrix identification number and the Ensembl mouse genome server (www.ensembl.org/Mus_musculus/). The relative expression of the genes was normalized to the amount of TATA box-binding protein (Tbp) (endogenous reference) and compared to a calibrator (gene mRNA expression in naive animals) by using the $2^{-\Delta\Delta CT}$ method (40).

Measurements of cytokines in serum during infection. Interleukin-12 (IL-12), gamma interferon (IFN-γ), and IL-4 levels in serum were measured by enzyme-linked immunosorbent assay (ELISA) at days 1, 3, 5, 7, 10, and 42 postinfection with paired MAbs and recombinant mouse cytokines as standards according to the manufacturer's (R&D Systems, Minneapolis, MN) instructions.

Antibody response to *Salmonella* infection. Serum was obtained from the blood of control and *Salmonella*-infected mice. Total serum IgG, IgG1, IgG2a, IgG2b, IgG3, and IgM levels were measured with commercial ELISA kits (Bethyl Laboratories, Montgomery, TX). The anti-*Salmonella* IgG, IgG1, and IgG2a responses to *S. enterica* serovar Enteritidis were determined by ELISA with sonicated-killed *S. enterica* serovar Enteritidis as previously described (4).

Statistical analysis. Data were analyzed by analysis of variance and the Wilcoxon rank-sum test, where appropriate. Two-tailed P values of <0.05 were considered statistically significant. Where appropriate, P value adjustments for multiple testing were done by a Bonferroni method (69). All analyses were conducted with SAS 9.1 (69).

RESULTS

***Slc11a1*-deficient mice clear *S. enterica* serovar Enteritidis more efficiently.** We investigated the candidacy of *Slc11a1* as the gene underlying *Ses1* with mice carrying a null allele at *Slc11a1* (129S6-*Slc11a1*^{tm1Mcg}). Initial data have shown that 129S6-*Slc11a1*^{tm1Mcg} mice have a significantly lower spleen *S. enterica* serovar Enteritidis load compared to that of wild-type 129S6 mice at day 42 postinfection (13). To characterize the course of *S. enterica* serovar Enteritidis infection in mice carrying a null allele at *Slc11a1*, we infected 129S6-*Slc11a1*^{tm1Mcg} knockout mice with $\sim 10^3$ CFU of salmonellae and compared the bacterial loads in the spleens of these animals to those of their wild-type 129S6 counterparts on days 1, 3, 5, 7, 10, and 42 postinfection (Fig. 1A). Bacterial loads in the spleen were consistently lower in 129S6-*Slc11a1*^{tm1Mcg} mice in both the early (days 5, 7, and 10) and the late (day 42) phases of infection (Fig. 1A). The early difference in bacterial load observed at day 3 between the C57BL/6J and 129S6 mice (13) was not reproduced in this study, suggesting that the higher *S. enterica* serovar Enteritidis load observed in C57BL/6J mice is not explained by *Slc11a1* but rather by a C57BL/6J background effect. We observed an impact of gender on the spleen bacterial loads in both wild-type and 129S6-*Slc11a1*^{tm1Mcg} mice. Female mice had lower bacterial loads than the males at day 42 postinfection, consistent with our genetic models (Fig. 1B). *Slc11a1*-deficient mice present a phenotype very similar to that observed in 129S6.B6-*Ses1* congenic mice with respect to bacterial load and sex effect, providing further evidence that *Slc11a1* is the likely gene underlying *Ses1* (14).

Slc11a1-deficient mice develop a marked leukocytosis during infection which correlates with bacterial clearance. Hematological parameters of 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice were measured during infection with *S. enterica* serovar Enteritidis. As expected, all control mice had levels of RBC and WBC within the accepted norms (Fig. 2A and B). The proportions of monocytes, lymphocytes, and PMNs within the WBC compartment were also normal (Fig. 2C, D, and E). Following infection, only the 129S6 mice developed moderate anemia, as demonstrated by the lower erythrocyte levels at day 42 postinoculation compared to those of the noninfected mice (Fig. 2A). Decreases of 38% (day 10) and 17% (day 42) in the numbers of RBC were noted in the peripheral blood from 129S6 mice compared to that from 129S6-*Slc11a1*^{tm1Mcg} animals after infection. In 129S6 mice, total WBC were found to be at constant levels throughout the infection, compared to 129S6-*Slc11a1*^{tm1Mcg} mice, which showed an increase in total WBC at day 10 postinfection (Fig. 2B). The leukocytosis of 129S6-*Slc11a1*^{tm1Mcg} mice is explained by a sharp increase in monocyte and PMN levels at day 10 postinfection (Fig. 2C and E). In addition, 129S6-*Slc11a1*^{tm1Mcg} mice showed an increase in circulating lymphocyte counts throughout infection, whereas 129S6 mice developed a transient lymphopenia following *Salmonella* challenge characteristic of a more acute disease condition (Fig. 2D). Taken together, these results suggest that the hematological parameters reflect the progression of the infection in 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice and suggest that 129S6-*Slc11a1*^{tm1Mcg} animals are in a recovery phase of the *Salmonella* infection compared to 129S6 mice.

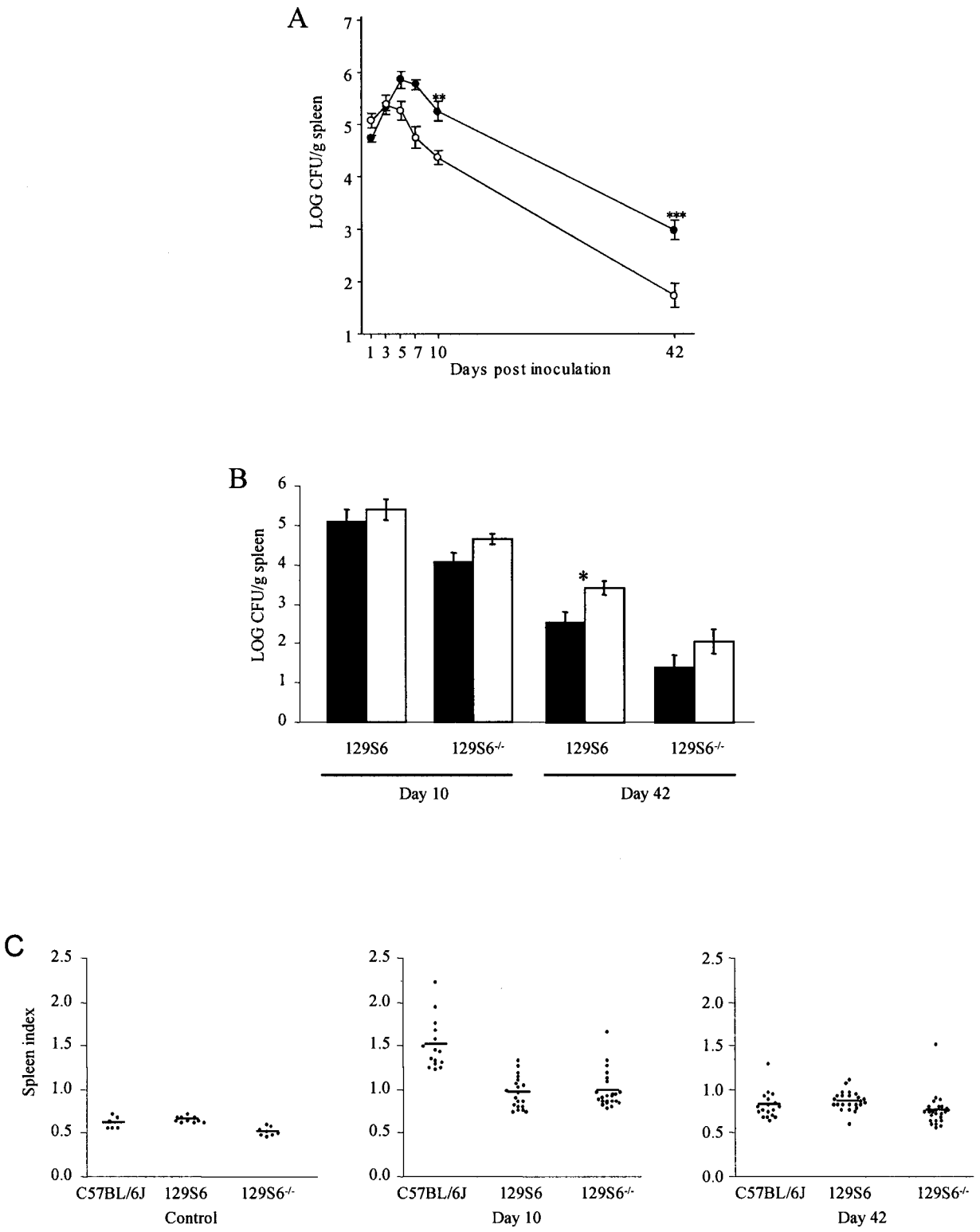

FIG. 1. (A) Kinetics of infection in inbred mice following administration of a sublethal inoculum of *S. enterica* serovar Enteritidis. Black and white circles represent groups of four to six 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice, respectively, that were sacrificed at different time points following intravenous infection with 1,000 CFU of *S. enterica* serovar Enteritidis strain 3b. The experiments were done in triplicate. The data are the average log CFU per gram of spleen ± the standard error of the mean. Asterisks represent the significance levels of the difference in the numbers of *Salmonella* CFU between the two strains of mice (**, $P < 0.01$; ***, $P < 0.001$). (B) Graphic representation of *Salmonella* carriage in female (black columns) and male (white columns) 129S6 and 129S6-*Slc11a1*^{tm1Mcg} (129S6$^{-/-}$) mice at days 10 and 42 following a challenge with *S. enterica* serovar Enteritidis. The asterisk represents the significance levels of the differences in *Salmonella* carriage between the female and male mice ($P < 0.05$). (C) Scatterplots demonstrating the spleen indexes of C57BL/6J, 129S6, and 129S6-*Slc11a1*^{tm1Mcg} (129S6$^{-/-}$) control mice and mice infected for 10 and 42 days with *S. enterica* serovar Enteritidis. The experiments were done in triplicate, and each dot represents an animal.

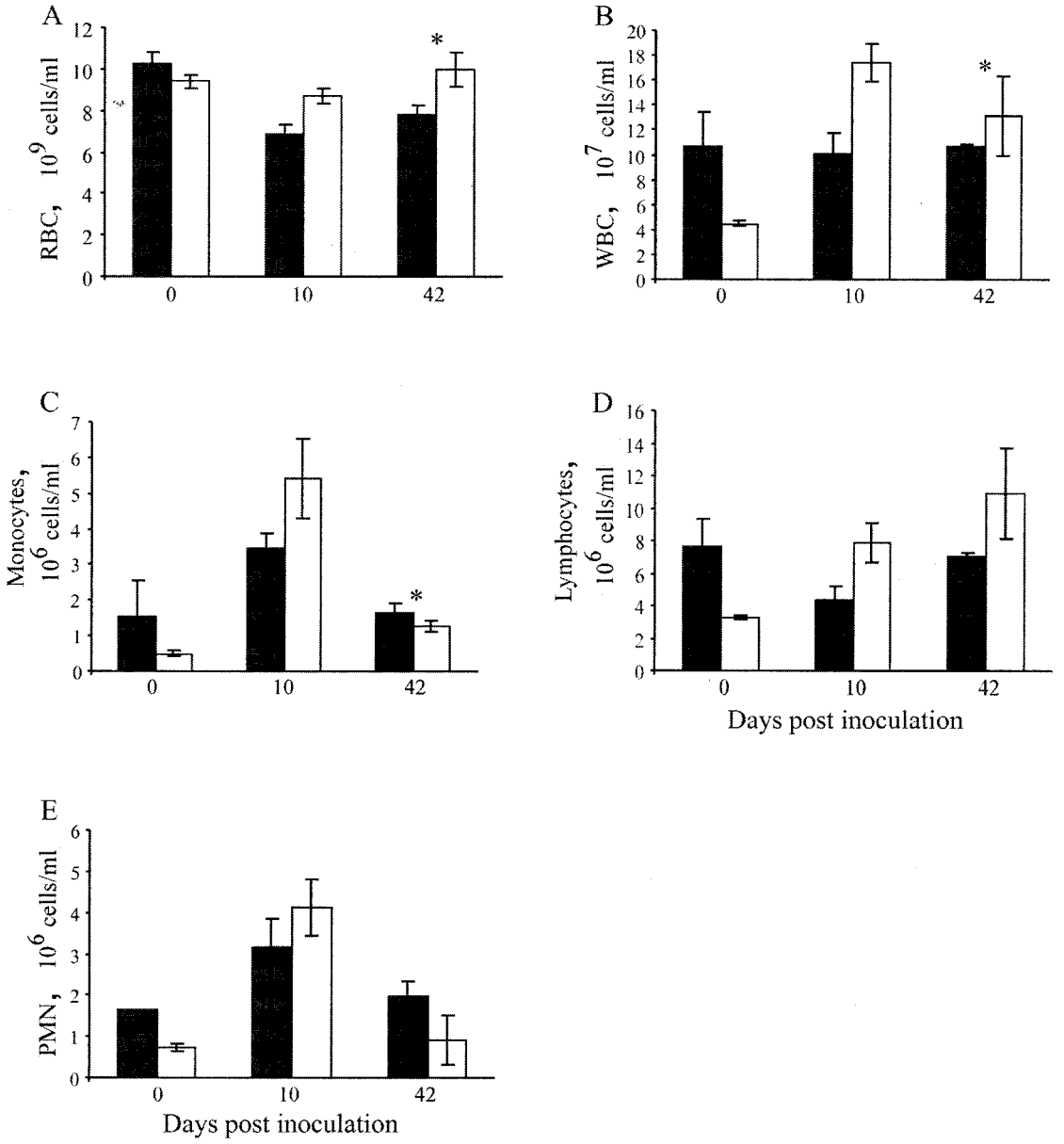

FIG. 2. Hematological analysis of 129S6 (black columns) and 129S6-*Slc11a1*^{tm1Mcg} (white columns) mice following an intravenous challenge with 1,000 CFU of *S. enterica* serovar Enteritidis. The total numbers of RBC (A) and WBC (B), as well as the levels of circulating monocytes (C), lymphocytes (D), and PMNs (E) were evaluated in naive (day 0) and infected (days 10 and 42) animals. The data are expressed as the mean of the total number of cells per milliliter ± the standard error of the mean for groups of two to six control and infected animals, respectively. An asterisk indicates a significant difference in a hematological parameter between the two strains of mice ($P < 0.05$).

***Slc11a1* has no impact on the macroscopic and histopathologic phenotypes and cellularity of the spleen during *S. enterica* serovar Enteritidis infection.** The finding that the wild-type allele at *Slc11a1* influences the outcome of a persistent *S. enterica* serovar Enteritidis infection was unexpected since it has been shown in several studies to be protective against highly virulent *S. enterica* serovar Typhimurium infections. We hypothesized that the absence of a functional Slc11a1 protein may cause increased recruitment of inflammatory cells at the site of infection, which in turn, by secreting specific cytokines or other inflammatory mediators, are able to more efficiently eliminate the bacteria later during infection. To investigate this hypothesis, we measured the spleen index, performed a histopathologic analysis of the spleen, and analyzed spleen cell types by fluorescence-activated cell sorter (FACS) in control and infected 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice.

The spleen index was measured in 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice and compared to that of the C57BL/6J (*Slc11a1*^{Asp169}) strain. All mice presented a transient splenomegaly following infection with *S. enterica* serovar Enteritidis (Fig. 1C). Control 129S6-*Slc11a1*^{tm1Mcg} animals had a significantly lower spleen index average (0.52 ± 0.02; $P < 0.0001$) than

TABLE 1. Distribution of cell types in the spleens of 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice

Strain	No. of days p.i.[c]	Total no. of cells[a]	Total no. of stained cells/spleen (10^6)[a]									
			CD3	CD4	CD8	B220	MAC	F4/80	αβ	γδ	Gr1	Pan-NK
129S6	0	20.0 ± 0.0	7.1 ± 0.5	5.2 ± 0.2	1.8 ± 0.2	12.3 ± 0.7	1.2 ± 0.2	1.5 ± 0.8	6.6 ± 0.3	0.4 ± 0.1	1.5 ± 0.1	0.4 ± 0.1
129S6	10	170.0 ± 19.3	70.8 ± 10.0	46.1 ± 6.7	19.6 ± 3.0	59.1 ± 6.0	27.2 ± 3.9	20.9 ± 2.9	76.3 ± 10.8	37.1 ± 4.1	20.1 ± 3.0	10.4 ± 1.6
129S6	42	16.3 ± 1.8	7.5 ± 0.9	5.9 ± 1.4	1.7 ± 0.2	9.3 ± 1.0	1.2 ± 0.2	1.9 ± 0.3	7.8 ± 1.2	1.7 ± 0.4	2.1 ± 0.4	0.4 ± 0.1
129S6$^{-/-b}$	0	13.9 ± 6.1	5.1 ± 2.6	3.9 ± 2.2	1.2 ± 0.5	8.9 ± 3.3	0.6 ± 0.3	0.5 ± 0.2	4.4 ± 3.4	0.2 ± 0.1	0.8 ± 0.4	0.2 ± 0.1
129S6$^{-/-b}$	10	149.0 ± 22.5	53.7 ± 4.5	38.1 ± 3.3	14.4 ± 1.4	52.4 ± 8.3	22.5 ± 4.1	16.8 ± 3.4	60.2 ± 6.3	33.2 ± 6.7	18.5 ± 3.1	9.4 ± 1.8
129S6$^{-/-b}$	42	17.3 ± 2.7	6.7 ± 1.2	4.9 ± 4.7	1.8 ± 0.3	8.9 ± 1.6	1.8 ± 0.7	1.3 ± 0.2	7.4 ± 1.2	1.1 ± 0.2	2.7 ± 0.7	0.3 ± 0.0

[a] Data are represented as the mean ± the standard error of the mean.
[b] *Slc11a1*^{tm1Mcg} knockout mice.
[c] Data were obtained from averages of two, four, and six mice at days 0, 10, and 42 postinfection (p.i.), respectively.

the spleen index average of the 129S6 (0.63 ± 0.01) mice (Fig. 1C), although they were both within normal limits. The C57BL/6J mice presented the highest spleen index (1.50 ± 0.30) at day 10 postinfection. The 129S6 and 129S6-*Slc11a1*^{tm1Mcg} strains had a significant and comparable increase in their spleen indexes 10 days postinfection (~0.95 to 1.13). By day 42 postinfection, the spleen index decreased in all mouse strains, with the 129S6-*Slc11a1*^{tm1Mcg} mice having significantly lower values than their wild-type counterparts ($P = 0.0342$) (Fig. 1C). These data showed that *Slc11a1*-deficient mice behave similarly to 129S6.B6-*Ses1* mice (not shown) with respect to splenomegaly and that *Slc11a1* has little or no effect on the development of this phenotype during infection with *S. enterica* serovar Enteritidis.

We next examined the consequence of infection with *S. enterica* serovar Enteritidis for the spleens of the C57BL/6J, 129S6, congenic 129.B6-*Ses1*, and 129S6-*Slc11a1*^{tm1Mcg} mouse strains at day 10 and day 42 postinfection. Microscopic examination of histological sections of the spleens showed similar lesions in all mice, consisting of mild lymphoid atrophy of the periarteriolar lymphoid sheaths at day 10. Splenic thrombosis was also noted in 129S6, 129S6.B6-*Ses1*, and 129S6-*Slc11a1*^{tm1Mcg} mice. At day 42, there were no significant histological changes in the spleen sections from any of the strains analyzed (data not shown).

We further investigated the recruitment of immune cells to the spleen by FACS analysis in 129S6 and 129S6-*Slc11a1*^{tm1Mcg} naive and infected mice. Single-cell suspensions were prepared from the spleen and analyzed for surface markers expressed on mononuclear phagocytes (Mac1 and F4/80), granulocytes (Gr-1), T lymphocytes (CD3, CD4, CD8, and αβ and γδ TCRs), B lymphocytes (B220), and NK cells (PanNK). The total cellularity and the different cellular compartments were comparable in uninfected 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice (Table 1). Both strains showed significant increases in the total number of spleen cells at day 10 postinfection, although the increase was more pronounced in 129S6 mice (Table 1). This increase affected all cellular compartments but was not significantly different between the 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mouse spleens. The number of positively stained cells returned to uninfected levels in both strains at 42 days postinfection (Table 1).

Overall, the histopathologic changes in the spleen and the cell types involved in splenomegaly were very similar in 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice following *S. enterica* serovar Enteritidis infection. These results did not suggest a difference in the type and extent of cellular inflammation in the spleens of 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice that could explain the difference in bacterial clearance between the two strains.

Impact of *Slc11a1* on gene expression profiles in the spleen during infection with *S. enterica* serovar Enteritidis. To further investigate the specific activation of inflammatory cells involved in the ability of 129S6-*Slc11a1*^{tm1Mcg} mice to clear an *S. enterica* serovar Enteritidis infection, we performed gene expression profiling with commercial Affymetrix DNA chips. Because the spleen is the key tissue for *S. enterica* serovar Enteritidis persistence and since bacterial clearance is more efficient in females compared to males (13, 14), we used spleen RNAs prepared from female 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice to study the effect of *Slc11a1* on the expression profile of genes influencing the adaptive and cellular immune responses. In this analysis, we selected the genes that had a minimum 2.0-fold change in their expression levels between the control and infected mice, as well as between the 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice, at any one of the time points studied. As expected from the histopathology and FACS analyses, the majority of up- and down-regulated genes in the spleen during a *Salmonella* infection were found at day 10 postinoculation (Table 2). The numbers of genes transcriptionally altered at day 10 and day 42 postinfection were 149 and 74, respectively. A large proportion of these genes (about 30%) have been shown to be involved in immunity to infection. At day 10 postinfection, the 129S6 mice had higher expression of the type 1 cytokine gene *Ifng* associated with cell-mediated immunity genes and of additional genes involved in the early response to infection, including those for acute-phase proteins (*Saa3* and *Irg1*); proinflammatory molecules (*Il1a* and *Tnfa*); T-cell-, monocyte-, and granulocyte-tropic chemokines or receptors (*Ccl2*, *Cxcl5*, *Ccl7*, *Ccl8*, and *Fpr1*); and macrophage receptors (*Marco*, *Msr1*, and *Cd14*) (Table 2). At day 10 postinfection, the *Slc11a1*-deficient mice presented higher Ig levels and showed an increase in the expression in inflammatory genes, although not as strong as the response mounted by their wild-type counterparts (Table 2). Other host response genes, including the cell surface lymphocyte antigens (*Cd8b* and *Klra7*) and the cell adhesion molecule *Icam1*, were up-regulated in *Slc11a1*-deficient mice.

At day 42 postinfection, we observed a decrease in the expression of inflammatory genes in both strains of mice (Table 3). Interestingly, there was an up-regulation of genes (*Slpi* and *Sli12*) known to have an inhibitory effect on the inflammatory response in 129S6 mice concomitantly with an increase in the expression of several Ig genes (Table 3). The expression of Ig

TABLE 2. Genes differentially expressed in 129S6 versus 129S6-*Slc11a1*^{tm1Mcg} mice at day 10 following an *S. enterica* serotype Enteritidis challenge[a]

Group and designation	Gene name or product[b]	Identifier[c]	Function	Fold change
Immune response				
Saa3	**Serum amyloid A3**	Mm.14277	Acute immune response	5.51
Irg1	Immunoresponsive gene 1	Mm.4662	Inflammation	4.83
Ccl8	Chemokine (C-C motif) ligand 8	Mm.42029	Inflammation	4.20
Marco	Macrophage receptor	Mm.1856	Scavenger receptor	3.88
Cxcl5	Chemokine (C-X-C motif) ligand 5	Mm.4660	Inflammation	3.45
Msr1	Macrophage scavenger receptor 1	Mm.239291	Endocytosis	3.19
Tnfa	Tumor necrosis factor alpha	Mm.1293	Inflammation	2.88
Il1a	IL-1α	Mm.15534	Cytokine	2.85
Ifng	IFN-γ	Mm.240327	Inflammation	2.84
Chia pending	Chitinase (pending)	NA	Eosinophil chemotactic cytokine	2.77
Fpr1	*N*-Formyl peptide chemotactic receptor	Mm.56951	Chemotaxis	2.48
Aif1	Allograft inflammatory factor 1	Mm.10742	Inflammation	2.37
Ccl2	Chemokine (C-C motif) ligand 2	Mm.290320	Inflammation	2.33
Lilrb4	Leukocyte immunoglobulin-like receptor B4	Mm.34408	Immune response	2.22
Ier3	Immediate-early response 3	Mm.25613	Acute immune response	2.20
Ccr5	Chemokine (C-C motif) receptor 5	Mm.14302	Chemokine receptor	2.17
Cd14	CD14 antigen	Mm.3460	Inflammation	2.16
Bnip3	BCL2/adenovirus E1B-interacting protein 1, NIP3	Mm.2159	Apoptosis	2.04
Ccl7	Cytokine gene	Mm.341574	Chemotaxis	2.03
Ccl21a	Chemokine (C-C motif) ligand 21a (leucine)	Mm.348219	Chemokine	−2.01
Bcl2a1a	B-cell leukemia/lymphoma 2-related protein A1a	Mm.196770	Apoptosis	−2.03
Ifrd1	IFN-related developmental regulator 1	Mm.168	Cell differentiation	−2.07
Icam1	Intercellular adhesion molecule	Mm.90364	Defense response	−2.23
Klra13	**Killer cell lectin-like receptor A, member 13**	Mm.333431	Defense response	−2.29
Cd8b	CD8 antigen, beta chain	Mm.333148	Immune response	−2.31
Klra8	**Killer cell lectin-like receptor A, member 8**	Mm.358615	Defense response	−2.66
Klra7	Killer cell lectin-like receptor A, member 7	Mm.193478	Defense response	−2.81
Klra3	**Killer cell lectin-like receptor A, member 3**	Mm.358615	Defense response	−3.87
Immunoglobulin				
Igh-6	Ig heavy chain 6 of IgM	Mm.342177	Antigen binding	2.56
H2-Eb1	Major histocompatibility complex class II antigen Eβ	Mm.22564	Antigen presentation	2.51
Sema4a	Sema and Ig domains (semaphorin) 4A	Mm.22061	Immunoglobulin	2.04
Gm900	Gene model 900	Mm.360834	Immunoglobulin	−2.05
LOC434035	IgV(κ) chain 1	Mm.305094	Immunoglobulin	−2.35
Igk-V8	Immunoglobulin κ chain variable 8	Mm.333117	Immunoglobulin	−2.50
Igh-VJ558	Immunoglobulin heavy chain (J558 family)	Mm.240437	Immunoglobulin	−2.63
Nucleic acid binding				
Cntn1	Contactin 1	Mm.4911	DNA binding	6.54
Upp	Uridine phosphorylase	Mm.4610	Nucleoside metabolism	4.82
Cbx2	Chromobox homolog 2	Mm.14547	Chromatin	3.48
Lass2	Longevity assurance homolog 2	Mm.181009	Transcription factor activity	2.53
Hoxd3	Hoxd-3	Mm.3578607	Regulation transcription	2.35
Dmc1h	Disrupted meiotic cDNA 1 homolog	Mm.2524	DNA repair	2.05
Dlx6	Distal-less homeobox 6	Mm.5152	DNA binding	−2.02
Hspa1a	Heat shock protein 1A	Mm.6388	DNA repair	−2.06
Sox6	SRY box-containing gene 6	Mm.323365	Transcription factor activity	−2.09
Setdb1	SET domain, bifurcated 1	Mm.181661	DNA binding	−2.21
Ddx6	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 6	Mm.267061	RNA binding	−2.82
Mef2c	Myocyte enhancer factor 2C	Mm.24001	Transcription factor activity	−2.96
Nab1	Ngfi-A binding protein 1	Mm.25903	Transcription repression	
Zfp40	Zinc finger protein 40	Mm.21025	Nucleic acid binding	−3.42
Protein binding				
Nedd8	Neural, developmentally down-regulated gene 8	Mm.296566	Protein binding	2.91
Snx10	Sorting nexin 10	Mm.294166	Protein transport	2.08
Vps45	Vacuolar protein sorting 45 (yeast)	Mm.263185	Protein transport	2.07
Ltbp4	Latent transforming growth factor beta binding protein 4	Mm.272251	Protein binding	−2.04
Dsc2	Desmocollin 2	Mm.280547	Protein binding	−2.06
Epb4.1	Erythrocyte protein band 4.1	Mm.30038	Actin binding	−2.06
Sos1	Son of sevenless homolog 1 (*Drosophila*)	Mm.60975	Protein binding	−2.42
Arl4	ADP-ribosylation factor-like 4	Mm.12723	Protein transport	−2.76

Continued on following page

TABLE 2—*Continued*

Group and designation	Gene name or product[b]	Identifier[c]	Function	Fold change
Metabolism				
Chi3l1	Chitinase 3-like 1	Mm.38274	Metabolism	3.29
Acrp30	Adipocyte complement-related protein	Mm.3969	Glucose metabolism	2.61
Clecsf8	C-type lectin, superfamily member 8	Mm.299633	Sugar binding	2.35
Bpgm	2,3-Bisphosphoglycerate mutase	Mm.282863	Metabolism	−2.00
Lipe	Hormone-sensitive lipase	Mm.333679	Cholesterol metabolism	−2.04
Enzyme activity				
Ptgs2	Prostaglandin endoperoxide synthase 2	Mm.292547	Oxidoreductase activity	3.34
Dapk2	**Death-associated kinase 2**	Mm.335252	Protein kinase	2.88
Ctla2b	Cytotoxic T-lymphocyte-associated protein 2β	Mm.358584	Cysteine protease inhibitor	2.51
Aoah	Acyloxyacyl hydrolase	Mm.314046	Hydrolase activity	2.48
Timp1	Tissue inhibitor of metalloproteinase 1	Mm.8245	Metalloendopeptidase inhibitor	2.46
Ptpro	Protein tyrosine phosphatase receptor type O	Mm.186361	Hydrolase activity	2.43
Gzmk	*Mus musculus* granzyme K gene	Mm.56993	Proteolysis and peptidolysis	2.19
Blmh	Bleomycin hydrolase	Mm.22876	Proteolysis and peptidolysis	2.17
Bst1	Bone marrow stromal cell antigen 1	Mm.246332	Hydrolase activity	2.07
Cd160	CD160 antigen	Mm.34693	Oxidoreductase activity	2.02
Atp2a3	ATPase, Ca^{2+} transporting	Mm.6306	ATPase	−2.01
Cat	Catalase	Mm.4215	Oxidoreductase activity	−2.05
Adam22	A disintegrin and metalloprotease domain 22	Mm.275895	Proteolysis and peptidolysis	−2.12
Pdpk1	3-Phosphoinositide-dependent protein kinase 1	Mm.10504	Protein kinase	−2.37
Oaz1	Ornithine decarboxylase antizyme	Mm.683	Enzyme inhibitor	−2.41
Eps8	Epidermal growth factor receptor pathway substrate 8	Mm.235346	Proteolysis and peptidolysis	−2.43
Cpe	Carboxypeptidase E	Mm.31395	Proteolysis and peptidolysis	−2.84
Reln	Reelin	Mm.3057	Endopeptidase activity	−3.02
Acat2	Acetyl-coenzyme A acetyltransferase 2	Mm.360538	Acetyl-coenzyme A acetyltransferase	−3.06
Cell maintenance				
Socs3	Suppressor of cytokine signaling 3	Mm.3468	Signaling	3.09
Slfn4	Schlafen 4	Mm.38192	Cell proliferation	2.45
Emp1	Emp-1	Mm.182785	Cell growth	2.07
Cdkn1a	Cyclin-dependent kinase inhibitor 1A	Mm.195663	Cell cycle	2.01
Other				
Arfgef1	ADP-ribosylation factor guanine nucleotide-exchange factor 1	Mm.229141	NA[d]	2.47
Procr	Protein C receptor, endothelial	Mm.3243	Blood coagulation	2.24
Edr	Erythroid differentiation regulator	Mm.358827	NA	2.09
Gp49a	Glycoprotein 49A	Mm.358601	Cell surface antigen	2.03
Serf2	Small EDRK-rich factor 2	Mm.262252	NA	−2.01
Snca	Synuclein alpha	Mm.17484	Synaptic vesicle transport	−2.05
Ddit4	DNA damage-inducible transcript 4	Mm.21697	NA	−2.13
Mdm1	Transformed mouse 3T3 cell double minute 1	Mm.101191	NA	−2.14
Cdr2	Cerebellar degeneration-related 2	Mm.1640	NA	−2.18
Clcn3	Chloride channel 3	Mm.259751	Ion transport	−2.21
Sparcl1	Extracellular matrix-associated protein (Sc1)	Mm.29027	Calcium binding	−2.25
Als2cr3	Amyotrophic lateral sclerosis 2 candidate 3	Mm.222887	γ-Aminobutyric acid receptor binding	−2.58
Shoc2	Soc-2 (suppressor of clear) homolog (*Caenorhabditis elegans*)	Mm.33376	NA	−2.59
FHOS2	Diaphanous protein homolog 3	Mm.329322	NA	−2.67
Mab21l2	MAb 21-like 2 (*C. elegans*)	Mm.214385	Development	−3.54
Adm	Adrenomedullin	Mm.1408	Neuropeptide signaling pathway	−3.99

[a] The list includes the annotated genes only.
[b] Genes in bold represent the genes that had a minimum difference change of 2.0-fold between the two strains of mice at days 10 and 42 postinfection.
[c] The identifier represents the unigene accession number found at the National Center for Biotechnology Information browser (www.ncbi.nlm.nih.gov).
[d] NA, not available.

genes was down regulated in the 129S6-*Slc11a1*^{tm1Mcg} mice at day 42 postinfection. Overall, the transcriptional activity was returned to uninfected status by day 42 in *Slc11a1*-deficient mice. It is clear from these experiments that the reprogramming of the transcriptome during an *S. enterica* serovar Enteritidis infection is temporally different in 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice. In addition, preferential early induction of genes in specific cells is different in 129S6 and 129S6-*Slc11a1*^{tm1Mcg} spleens such that genes involved with macrophages and/or dendritic cell activation predominate in 129S6 mice whereas induction of genes involved in lymphocyte activation are present in 129S6-*Slc11a1*^{tm1Mcg} mice.

Transcriptional regulation of Th1 and Th2 cells by Slc11a1. To evaluate the possibility that *Slc11a1* has an impact on bac-

TABLE 3. Genes differentially expressed in 129S6 versus 129S6-*Slc11a1*^{tm1Mcg} mice at day 42 following an *S. enterica* serotype Enteritidis challenge[a]

Group and designation	Gene name, product, or description[b]	Identifier[c]	Function	Fold change
Immunoglobulin				
LOC213156	Gamma variable region	Mm.313444	Antigen binding	5.00
LOC382694	Similar to Ig heavy chain	Mm.304472	Antigen binding	3.02
LOC382692	Similar to Ig heavy chain	Mm.313423	Antigen binding	2.87
Igh-6	Ig heavy chain of IgM	Mm.342177	Antigen binding	2.57
LOC435905	Similar to Ig light chain	Mm.333142	Antigen binding	2.51
Igh-VJ558	Similar to IgH chain VJ558	Mm.300219	Antigen binding	2.42
Igh-1a	Ig heavy chain 1a	Mm.342177	Antigen binding (IgG2a)	2.42
LOC381784	Similar to IgV(κ) gene	Mm.305097	Antigen binding	2.33
Igh-VS107	Ig heavy-chain S107 family	Mm.234287	Antigen binding	2.24
Igh-V	Ig heavy-chain variable region	Mm.313476	Antigen binding	2.16
Igh-VJ558	Ig heavy chain	Mm.313411	Antigen binding	2.01
Gm1067	Gene model 1067	Mm.333118	Antigen binding	2.01
Immune response				
Pik3cd	Phosphatidylinositol 3-kinase catalytic delta	Mm.229108	Signal transduction B cell	2.35
Ncf1	Neutrophil cytosolic factor 1	Mm.4149	Inflammation	2.09
Saa3	**Serum amyloid A3**	Mm.14277	Acute immune response	2.03
Klrd1	Killer cell lectin-like receptor D, member 1	Mm.8186	Defense response	−2.00
Ifit1	IFN-induced protein with tetratricopeptide repeats 1	Mm.6718	Inflammation	−2.28
Klra3	**Killer cell lectin-like receptor A, member 3**	Mm.333433	Defense response	−2.34
Klra13	**Killer cell lectin-like receptor A, member 13**	Mm.333434	Defense response	−2.34
Ifi202a	IFN-induced protein	Mm.218770	Inflammation	−2.38
Klra8	**Killer cell lectin-like receptor A, member 8**	Mm.321961	Defense response	−2.61
DNA binding				
Nr3c1	Nuclear receptor 3, group C, member 1	Mm.129481	Regulation of transcription	2.51
Cbx1	Chromobox homolog 1	Mm.29055	Chromatin binding	2.30
Swap70	SWAP complex protein	Mm.334144	ATP/DNA binding	2.26
Lipid metabolism				
Psap	Prosaposin gene	Mm.277498	Lipid metabolism	3.34
Pla2g12	Phospholipase A2, group XII	Mm.151951	Lipid metabolism	2.48
Pltp	Matrix metalloproteinase 13	Mm.6105	Lipid transport	−2.02
Fabp7	Fatty acid binding protein 7	Mm.3644	Lipid transport	−2.37
Enzyme activity				
Spi12	Serine protease inhibitor 12	Mm.36526	Inhibitor of leukoproteinase	3.34
Arg2	Arginase type II	Mm.3506	Arginase	2.58
Slpi	Secretory leukoprotease inhibitor	Mm.371583	Inhibitor of leukoproteinase	2.54
Ptpn12	**Protein tyrosine phosphatase, nonreceptor type 12**	Mm.319117	Phosphatase	2.27
St6gal1	β-Galactoside α2,6-sialyltransferase 1	Mm.149029	Transferase	2.26
Mapkapk2	Mitogen-activated protein kinase-activated protein kinase 2	Mm.221235	Protein kinase	2.09
Dapk2	**Death-associated kinase 2**	Mm.335252	Protein kinase	2.08
Usp22	Ubiquitin-specific protease 22	Mm.30602	Peptidase	2.02
Mmp13	RIKEN cDNA 4833432B22	Mm.5022	Proteolysis and peptidolysis	−2.01
Mmp3	Matrix metalloproteinase 3	Mm.4993	Proteolysis and peptidolysis	−2.06
Plau	Phospholipid transfer protein	Mm.4183	Proteolysis and peptidolysis	−2.17
Other				
F13b	Coagulation factor XIIIβ	Mm.30105	Coagulation	3.46
Pdlim3	PDZ and LIM domain 3	Mm.282900	Cytoskeleton	2.31
Xin	Cardiac morphogenesis	Mm.10117	Cell adhesion	2.22
Fbxw1b	F box and WD40 domain	Mm.28017	Ubiquitin cycle	2.17
Sept6	Septin6	Mm.260036	Cell cycle	−2.16

[a] The list includes the annotated genes only.
[b] Genes in bold represent the genes that had a minimum difference change of 2.0-fold between the two strains of mice at days 10 and 42 postinfection.
[c] The identifier represents the unigene accession number found at the National Center for Biotechnology Information browser (www.ncbi.nlm.nih.gov).

terial persistence in the spleen through local Th cell polarization, we selected cytokines known to be specific to the Th1 (IL-12 and IFN-γ) or Th2 (IL-4) response for quantitative PCR validation. In addition, we selected costimulatory molecules (CD28, CD80, and CD86) that were shown to have a key role in regulating T-cell activation and T-dependent B-cell responses, as well as critical transcription factors that have a role in gene expression of Th1 (T-box transcription factor T-bet or Tbx21) and Th2 (the zinc finger transcription factor Gata3) cells.

The Th1 cytokines (*Ifng* and *Il12*) and transcription factor

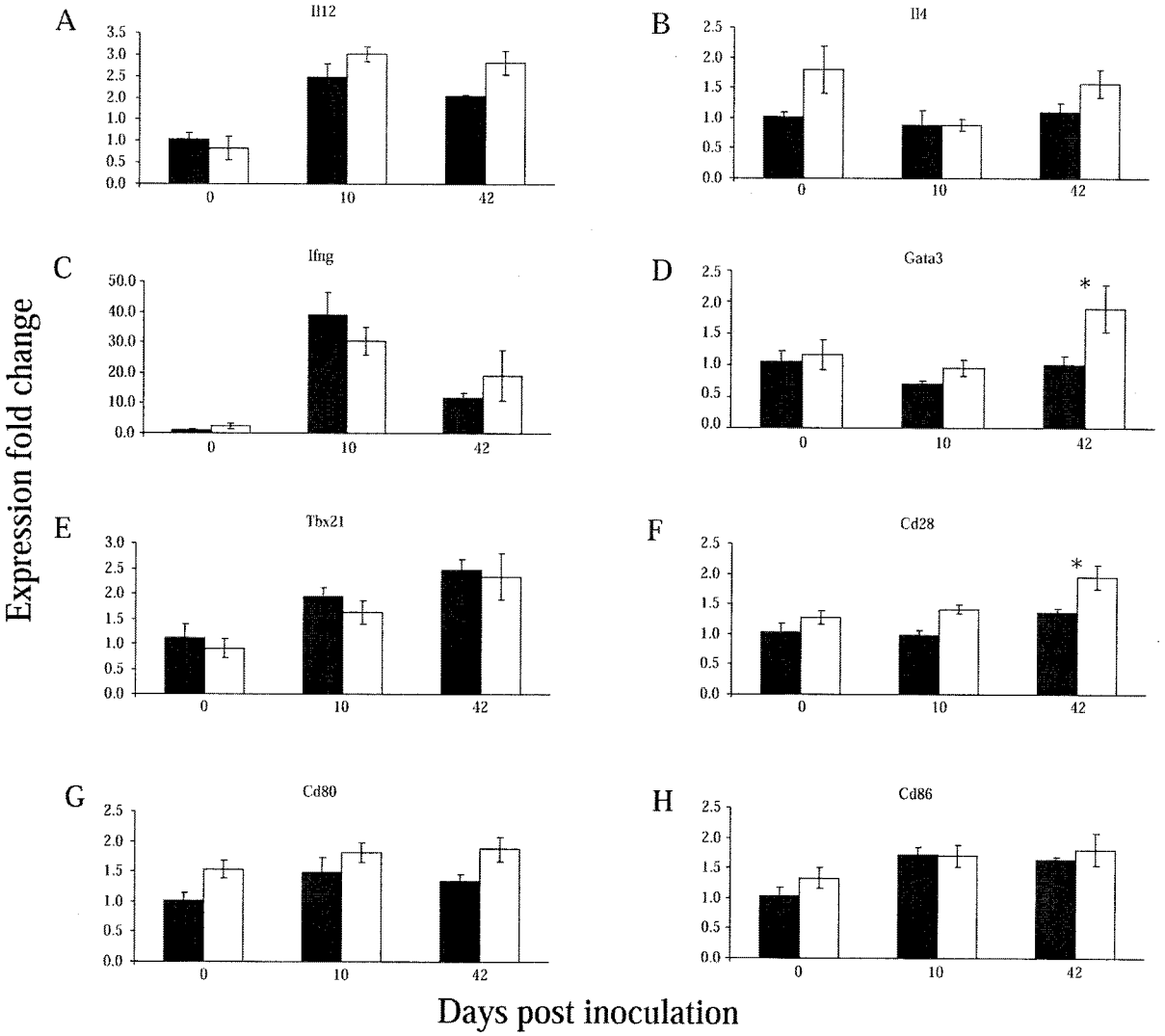

FIG. 3. Gene expression profiling by quantitative reverse transcription-PCR. (A to H) Real-time PCR analyses of selected genes induced during *S. enterica* serovar Enteritidis infection. The *y* axis shows the fold changes in *Salmonella*-responsive gene expression in infected 129S6 (black columns) and 129S6-*Slc11a1tm1Mcg* (white columns) mice compared to control mice and each other over time. The results were normalized to the endogenous levels of TATA box-binding protein (Tbp). The error bars were calculated on the relative fold changes in expression. The experiments were done in duplicate with RNAs from four to eight mice. An asterisk indicates a significant difference in the fold change in expression between the two strains of mice ($P < 0.05$).

(*Tbx21*) were up-regulated during infection in both 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice (Fig. 3A, C, and E). At day 10, higher expression of *Ifng* was observed in 129S6 mice although it did not reach the significance threshold (Fig. 3C). The expression of the Th2 cytokine gene *Il4* was constant throughout infection in both 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice (Fig. 3B). The expression of *Gata3*, a transcription factor known to stimulate the expression of *Il4*, was markedly increased in *Slc11a1*-deficient mice at day 42 postinfection (Fig. 3D). Different studies have proposed that costimulatory molecules may have a role in determining the dominance of Th cell responses. We detected a progressive up-regulation of *Cd28* in 129S6-*Slc11a1*^{tm1Mcg} mice; the expression levels of *Cd28* were significantly higher in *Slc11a1*-deficient mice at day 42 postinfection (Fig. 3F). Expression levels of *Cd80* and *Cd86* were not significantly regulated during infection, although *Cd80* transcripts were more abundant in 129S6-*Slc11a1*^{tm1Mcg} mice (Fig. 3G and H). These results complement the observations made by transcription analyses and suggest that bacterial clearance in 129S6-*Slc11a1*^{tm1Mcg} mice is associated with a local mixed Th1 and Th2 response, with a predominant Th2 bias. Determining Th profiles based on the expression of cytokines at specific time points after infection may not be optimal because of their temporal expression. To support and complement these studies, we measured cytokines in the serum at early time points and evaluated Ig class switch recombination following *S. enterica* serovar Enteritidis infection in 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice.

Serum cytokine levels in *S. enterica* serovar Enteritidis-infected wild-type and *Slc11a1* knockout mice. *S. enterica* serovar

Enteritidis infection induced modest IL-12 p70 production in vivo in both wild-type and *Slc11a1* knockout mice at all time points. No significant differences were noted between the two groups, reflecting the expression data (Fig. 4A). We also observed significant increases in serum IFN-γ levels in wild-type and *Slc11a1* knockout mice compared to those in the uninfected controls (Fig. 4B). IFN-γ levels were, however, significantly higher in *Slc11a1*-deficient mice of both sexes on day 1 ($P = 0.0146$) postinfection compared to the levels observed in 129S6 mice. At day 10, levels of IFN-γ in the serum were slightly higher in wild-type compared to 129S6-*Slc11a1*^{tm1Mcg} mice, which was consistent with the expression studies. At day 42, IFN-γ levels returned to control values in both groups. IL-4 was detected only in the serum of *Slc11a1* knockout mice at day 10 postinfection ($P = 0.0210$), which may reflect the constitutively higher expression levels of *Il4* in these mice (Fig. 4C).

Slc11a1 influences IgG class switching during infection with *S. enterica* serovar Enteritidis. Th1 cells are known to enhance IgG2a synthesis by B cells through IFN-γ, whereas Th2 cells induce IgE and IgG1 production and secretion by IL-4. Total serum levels of IgG, IgG1, IgG2a, IgG2b, IgG3, and IgM were quantitated in naive and infected 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice. As shown in Fig. 5A, the concentration of total IgG increased in both strains of mice following infection. 129S6-*Slc11a1*^{tm1Mcg} mice presented higher serum IgG levels at day 0 compared to 129S6 mice and lower levels at day 42 postinoculation ($P = 0.0313$), consistent with the expression analyses. The high IgG levels present in 129S6 mice at day 42 were explained by high IgG2a levels. More importantly, the 129S6 animals had significantly higher levels of IgG2a ($P < 10^{-4}$) compared to 129S6-*Slc11a1*^{tm1Mcg} mice. IgG1 levels decreased throughout the infection in both strains; however, the IgG1 concentration remained significantly higher at day 42 postinfection in 129S6-*Slc11a1*^{tm1Mcg} mice in comparison to that in wild-type animals ($P = 0.0137$) (Fig. 5B). An increase in IgG2b and IgG3 production was detected only in 129S6 mice during the course of infection (Fig. 5D and E). IgM levels did not increase significantly following *Salmonella* infection and were not different between the two groups of mice (Fig. 5F). *S. enterica* serovar Enteritidis-specific IgG1 and IgG2a responses were measured at day 42 postinfection (Fig. 6). Levels of *Salmonella*-specific IgG1 were similar in wild-type and *Slc11a1* knockout mice; IgG1 titers were 3.9 ± 0.1 and 3.5 ± 0.2 in 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice, respectively. However, evident differences in the IgG2a isotype between wild-type and *Slc11a1*-deficient mice were observed at day 42 (Fig. 6). Infected wild-type 129S6 mice had significantly higher levels of IgG2a than their *Slc11a1* knockout mouse counterparts. Differences in IgG2a titers were more pronounced in female mice (Fig. 6A) than in male mice (Fig. 6B). *Slc11a1* knockout mice have a profound deficiency in the development of *Salmonella*-specific IgG2a and do not appear to develop a compensatory IgG1 response. All together, these results suggest that persistence of bacteria in the spleens of 129S6 mice correlates with a predominant Th1 differentiation in response to infection and that clearance of the bacteria in *Slc11a1*-deficient mice is associated with a Th2 response.

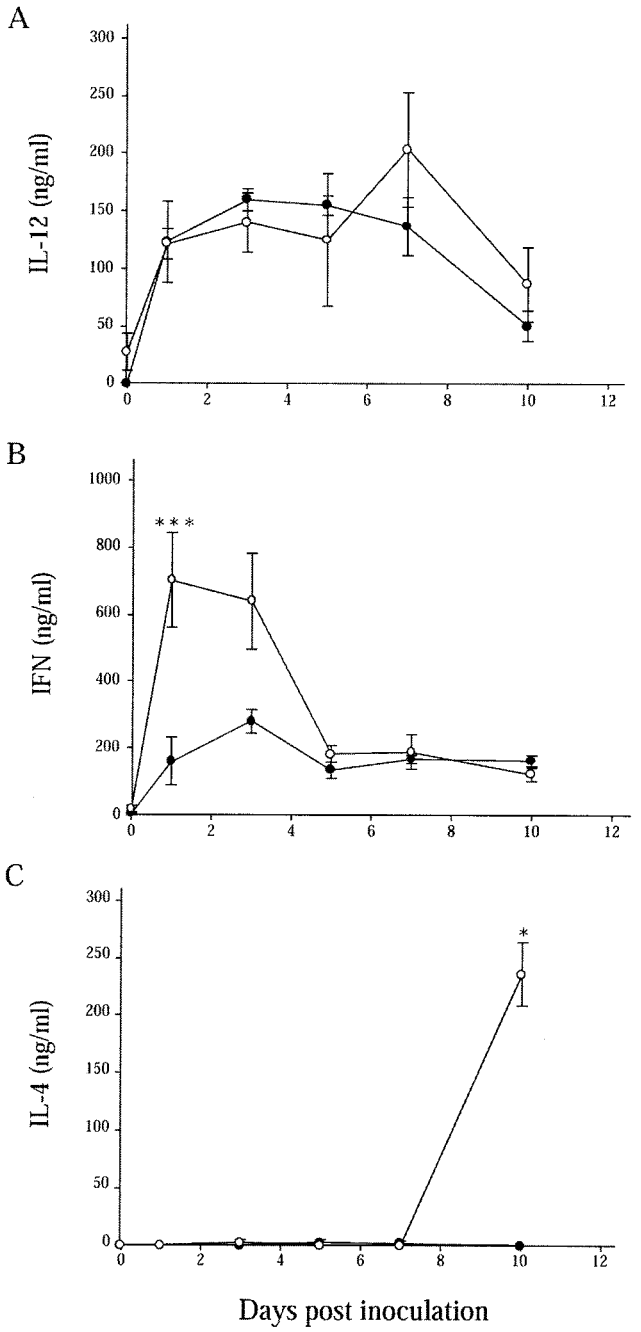

FIG. 4. Cytokine responses in 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice infected with *S. enterica* serovar Enteritidis. IL-12 (A), IFN-γ (B), and IL-4 (C) levels in serum have been quantitated by ELISA following an intravenous challenge with 1,000 CFU of *S. enterica* serovar Enteritidis in 129S6 (black circles) and 129S6-*Slc11a1*^{tm1Mcg} (white circles) mice. The data are the mean concentrations of cytokine (nanograms per milliliter) ± the standard error of the mean from groups of six mice at 1, 3, 5, 7, and 10 days postinfection. The serum of each mouse was tested in triplicate. Asterisks indicate significant differences between the cytokine levels of the two strains of mice (*, $P < 0.05$; ***, $P < 0.001$).

DISCUSSION

In the present study, we investigated the role of *Slc11a1* in the chronic persistence of *S. enterica* serovar Enteritidis in mice. The candidacy of *Slc11a1* was based on the previous

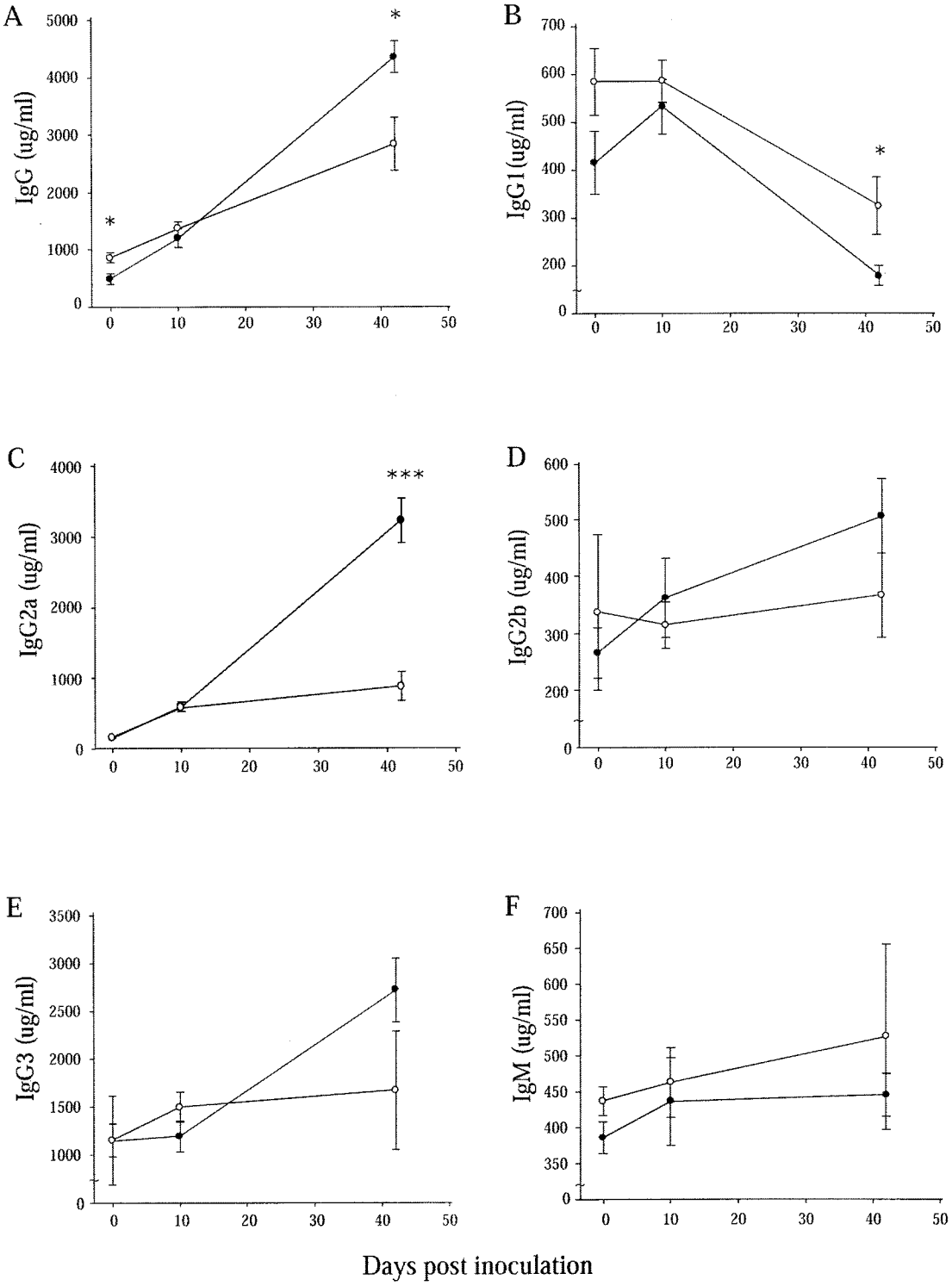

FIG. 5. Kinetics of serum antibodies following *S. enterica* serovar Enteritidis infection. Ig isotypes were quantitated by ELISA following an intravenous challenge with 1,000 CFU of *S. enterica* serovar Enteritidis in 129S6 (black circles) and 129S6-*Slc11a1*^{tm1Mcg} (white circles) mice. Total IgG (A), IgG1 (B), IgG2a (C), IgG2b (D), IgG3 (E), and IgM (F) levels were measured. The data obtained from groups of four control and six infected (days 10 and 42) mice are represented as the mean concentration of antibodies (micrograms per milliliter) ± the standard error of the mean. Each serum was tested twice in triplicate. Asterisks represent significant differences between the antibody titers of the two strains of mice (*, $P < 0.05$; ***, $P < 0.001$).

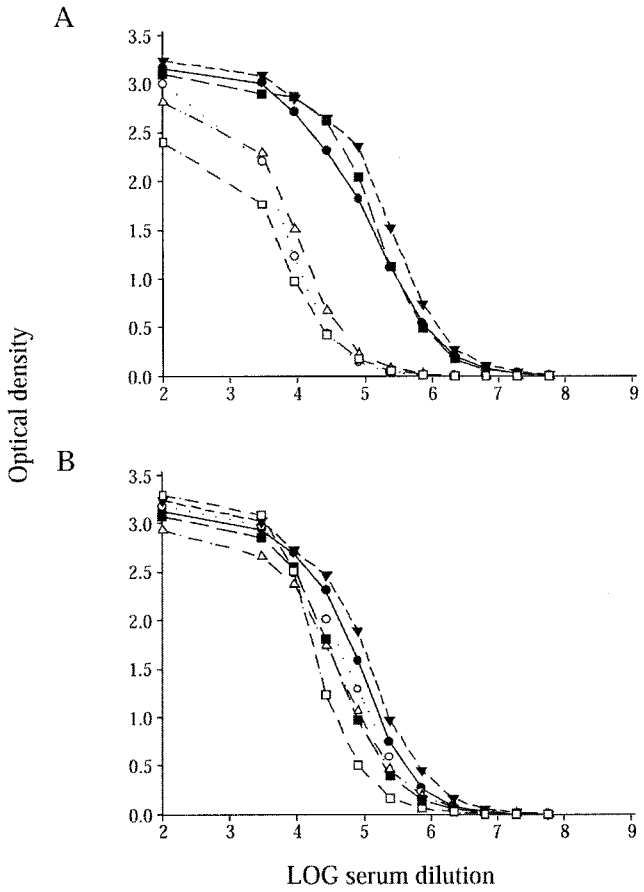

FIG. 6. *S. enterica* serovar Enteritidis-specific IgG2a antibody levels of individual female (A) and male (B) mice 42 days after infection. Black and white symbols represent the log of the antibody concentrations in the sera of infected 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice, respectively. The experiments were done in duplicate, and each line represents the average log antibody concentration value obtained for one mouse.

identification of a locus named *Ses1* that was shown to control spleen bacterial clearance in C57BL/6J mice by linkage and genome-wide two-locus interaction analyses (13, 14). *Slc11a1* is located at the maximum peak logarithm of the odds score of *Ses1* and is known to be a critical component of the innate immune response of the host to infection with highly virulent *S. enterica* serovar Typhimurium. In the chronically *S. enterica* serovar Enteritidis-infected mouse model, *Slc11a1* does not appear to be as critical in the initial phase of infection in that mice carrying a null allele at *Slc11a1* survive as well as mice carrying the wild-type allele; however, in this model, the wild-type allele at *Slc11a1* has been associated with persistent infection (13, 14). The kinetics of a sublethal challenge with *S. enterica* serovar Enteritidis in 129S6 and 129S6-*Slc11a1* deficient mice demonstrated that *Slc11a1* had an impact on bacterial clearance starting at day 7 postinfection. By day 42, 129S6-*Slc11a1*^{tm1Mcg} mice presented an 18-fold decrease in the spleen bacterial load compared to 129S6 mice. Congenic mice carrying the *Ses1* interval from C57BL/6J (Asp^{169}) mice on a 129S6 (Gly^{169}) background presented kinetics of infection that were similar to those observed in 129S6-*Slc11a1*^{tm1Mcg} animals, lending further support for the candidacy of *Slc11a1* as the molecular determinant of *Ses1*.

There was a clear influence of gender on the phenotype in both mouse strains, consistent with previous observations that females had a significantly better rate of *S. enterica* serovar Enteritidis clearance (13, 14). Sexual dimorphism in the extent of the host response to infection has been reported with other type of infections (2, 32, 64). Estrogen is known to regulate the differentiation, survival, and function of diverse immune cells (30, 37, 50). In addition, female mice produce significantly more specific antibody in response to various infections (27). The difference in gender may also be explained by the immunoregulatory effect of testosterone, which is know to act directly on CD4$^+$ T lymphocytes to increased IL-10 production (39). The difference between genders in our study was observed in wild-type and *Slc11a1* knockout mice and is clearly not dependent on Slc11a1.

To get a better understanding of the impact of *Slc11a1* on the *Salmonella* carrier state, we examined different aspects of the immune response of 129S6-*Slc11a1*^{tm1Mcg} mice during infection with *S. enterica* serovar Enteritidis. A significant feature of *Salmonella* pathogenesis is the requirement of both innate and adaptive immune systems for the clearance of infections (43, 49, 58). Global gene expression profiling of the whole spleen with microarrays in conjunction with quantitative PCR analyses revealed different molecular signatures of specific cell types in 129S6-*Slc11a1*^{tm1Mcg} and 129S6 mice during a chronic *S. enterica* serovar Enteritidis infection. Not surprisingly, inflammatory genes constitute the major class of mRNAs expressed in response to *S. enterica* serovar Enteritidis infection. Several of these genes were in common with the core set of human genes, defining a shared host transcriptional response to various pathogens (8, 20, 36, 52). A high representation of microbially activated macrophage genes known to have a role during acute *Salmonella* infection, including those for macrophage receptors (*Cd14*, *Marco*, and *Msr*), proinflammatory cytokines (*Ifng*, *Il1a*, and *Tnfa*), and several chemokines (*Ccl2*, *Ccl7*, *Ccl8*, and *Cxcl5*), were up-regulated in 129S6 mice at day 10 postinfection. Increased T-cell-, monocyte-, and granulocyte-tropic chemokines may have had an impact on the higher number of cells recruited to the spleens of 129S6 mice during chronic infection with *S. enterica* serovar Enteritidis. In 129S6-*Slc11a1*^{tm1Mcg} mice, genes associated with activated T- and B-cell populations, including cell surface lymphocyte antigen (*Cd8b* and *Klra7*), costimulatory molecule (*Cd28*), and Ig genes, were more abundantly expressed during infection. The preferential induction of cell-specific clusters of genes in 129S6 and 129S6-*Slc11a1*^{tm1Mcg} mice was consistent with the activation of different cell populations during the immune response.

Transcriptional profiling also suggests that Th polarization may be involved in the host response to infection with *S. enterica* serovar Enteritidis in *Slc11a1*-deficient animals. The cytokine microenvironment is a critical determinant for Th cell lineage development. The proinflammatory cytokines IL-12 and IFN-γ play a fundamental role in Th1 phenotype differentiation and provide a link between innate and adaptive immunity, whereas IL-4 drives Th2 cells (51). In addition, the combined action of several cytokines, including IFN-γ, IL-12, and TNF, is essential to suppress the growth of the bacteria in target organs during infection and coincides with the formation

of macrophage-rich granulomas and nitric oxide and NADPH oxidase macrophage-mediated killing of the bacteria (48, 66). In humans, mutations identified in the genes *IFNGR1*, *IFNGR2*, *IL12B*, and *IL12RB1* clearly affect susceptibility to persistent infections with salmonellae (15). In our model, we observed an early (day 1) IFN-γ response in serum of *Slc11a1* knockout mice infected with *S. enterica* serovar Enteritidis which may be important in establishing an effective innate immune response that can control early bacterial replication and limit chronic infection. The early IFN-γ production observed in 129S6-*Slc11a1*^{tm1Mcg} mice may contribute to the more rapid decrease in *Salmonella* CFU observed in the spleens of these mice. Comparable up-regulation of *Ifng* and *Il12* was detected at day 10 postinfection in both strains of mice, correlating with the control of the bacterial load in the spleen. At the same time period, up-regulation of the transcription factor gene *Gata3* and production of IL-4 in the serum were observed only in *Slc11a1*-deficient mice. Gata3 is a zinc finger protein activated by Stat6 and is known to play a role in the transcriptional regulation of Th2 cytokines, including IL-4 (73).

Clearance of salmonellae from tissues requires efficient CD4$^+$ T-cell activation by TCR and costimulatory signals (44). *Slc11a1*-deficient mice showed a higher basal expression of *Cd80* and *Cd86* and a progressive up-regulation of *Cd28* during infection, coinciding with the more efficient bacterial clearance seen in these animals. CD28 costimulation has been reported to increase the expression of Gata3 (57) and to play an important role in resistance to *S. enterica* serovar Typhimurium infection, T-cell differentiation, and Ig class switching (47, 61). Mice deficient in both the *Cd80* and *Cd86* genes also have an impaired ability to undergo Ig class switching (9). The CD80/CD86/CD28 costimulatory pathway not only promotes initial T-cell activation but also contributes to the down-regulation of the immune response. Furthermore, it stimulates Th2 cell differentiation by transducing a positive signal to B cells that increases IgG1 and IgE production (11, 12, 41, 55, 56). Mice that are persistently infected with *S. enterica* serovar Typhimurium present high anti-*Salmonella* IgG titers (47). Our study has shown that *Slc11a1* has a profound impact in the induction of IgG2a class switching.

Th polarization of the immune response to salmonellae was shown to be influenced by the quantity the antigen and the context in which it is presented. Low-dose flagellin (a major antigen for the CD4$^+$ T-cell response to salmonellae) or defined peptide antigens promote a Th2 response, whereas high doses induce Th1 cells in vivo (17, 54). In addition, Th1 or Th2 phenotypes can be induced in mice depending on how flagellin is presented (19, 21, 45). Soluble flagellin and polymerized flagellin induce a strong Th2 response, while flagellin presented on live salmonellae induces a Th1 response, in C57BL/6J mice (19, 21, 45). A role for *Slc11a1* in the adaptive immune response to salmonellae was proposed previously but remains controversial. *Slc11a1* has been shown to increase the surface expression of major histocompatibility complex class II molecules and production of inflammatory cytokines by macrophages stimulated with lipopolysaccharide (7). Other studies have reported that cytokine production and antibody response following a *Salmonella* infection are not influenced by *Slc11a1* (22, 53, 62). A vaccine *S. enterica* serovar Typhimurium strain was shown previously to induce different Th subsets in wild-type and *Slc11a1* mutant mice on a C57BL/10J background; however, there was no impact of the *Slc11a1* genotype on the clearance of *S. enterica* serovar Typhimurium (63). The complex interactions between the host genetic background (C57BL/10J versus 129S6) and the pathogen (avirulent *S. enterica* serovar Typhimurium versus *S. enterica* serovar Enteritidis) may explain the different impacts of *Slc11a1* in different models of *Salmonella* infection.

Our experiments demonstrate that the *Slc11a1* influence on the outcome of an *S. enterica* serovar Enteritidis infection is associated with Th polarization. We have shown that the presence of *Slc11a1* promotes the development of a robust proinflammatory response in 129S6 mice, which has the consequence of delaying the activation of the adaptive immunity essential for clearance of the bacteria. The exact molecular mechanisms involved are not known; however, we can postulate that *Slc11a1* may have an impact on the quantity and/or the processing of the antigen presented to T cells because of its known function in controlling bacterial proliferation and phagosome maturation (18, 34). On the other hand, *Slc11a1* may act on bacterial mechanisms of persistence. A wild-type genotype at *Slc11a1* has been shown to promote the expression of specific *Salmonella* genes both in vivo and in vitro (71, 72). Two of these genes, *sitA* and *mntH*, encode high-affinity metal ion uptake systems in *S. enterica* serovar Typhimurium (10). These genes are essential for the survival and dissemination of salmonellae and therefore may play a role in promoting the persistence of *Salmonella* infection in mice with a wild-type genotype at *Slc11a1*.

In humans, *SLC11A1* polymorphisms have been associated with the progression of tuberculosis (1, 3, 28) and with the clinical manifestations of leprosy (46), suggesting that variation within *SLC11A1* may influence the type of cellular immune response. In our chronic model of infection, we can conclude that 129S6 mice are not inherently susceptible to *S. enterica* serovar Enteritidis infection but that the presence of *Slc11a1* contributes to the establishment of a *Salmonella* carrier state in these mice. Although the exact mechanisms are not completely understood, we have provided good evidence that *Slc11a1* plays a role during chronic *S. enterica* serovar Enteritidis infection by influencing the adaptive immune response of the host.

ACKNOWLEDGMENTS

We thank Rosalie Wilkinson and Line Laroche for technical expertise. We also thank Laurent Salez for help with the microarray analyses.

This work was supported by grants from the Canadian Institutes of Health Research (CIHR) and the Howard Hughes Medical Institute (HHMI; Infectious Diseases and Parasitology Program). J.C. is the recipient of a CIHR fellowship. D.M. is a scholar of CIHR and an International Research Scholar of the HHMI. P.G. is a Distinguished Scientist of the CIHR.

REFERENCES

1. **Abel, L., F. O. Sanchez, J. Oberti, N. V. Thuc, L. V. Hoa, V. D. Lap, E. Skamene, P. H. Lagrange, and E. Schurr.** 1998. Susceptibility to leprosy is linked to the human NRAMP1 gene. J. Infect. Dis. **177:**133–145.
2. **Aebischer, T., S. Laforsch, R. Hurwitz, F. Brombacher, and T. F. Meyer.** 2001. Immunity against *Helicobacter pylori*: significance of interleukin-4 receptor alpha chain status and gender of infected mice. Infect. Immun. **69:** 556–558.
3. **Alcais, A., F. O. Sanchez, N. V. Thuc, V. D. Lap, J. Oberti, P. H. Lagrange, E. Schurr, and L. Abel.** 2000. Granulomatous reaction to intradermal injec-

tion of lepromin (Mitsuda reaction) is linked to the human NRAMP1 gene in Vietnamese leprosy sibships. J. Infect. Dis. **181:**302–308.
4. **Beal, R. K., P. Wigley, C. Powers, S. D. Hulme, P. A. Barrow, and A. L. Smith.** 2004. Age at primary infection with Salmonella enterica serovar Typhimurium in the chicken influences persistence of infection and subsequent immunity to re-challenge. Vet. Immunol. Immunopathol. **100:**151–164.
5. **Beaumont, C., J. Protais, F. Pitel, G. Leveque, D. Malo, F. Lantier, F. Plisson-Petit, P. Colin, M. Protais, P. Le Roy, J. M. Elsen, D. Milan, I. Lantier, A. Neau, G. Salvat, and A. Vignal.** 2003. Effect of two candidate genes on the Salmonella carrier state in fowl. Poult. Sci. **82:**721–726.
6. **Bellamy, R.** 2003. Susceptibility to mycobacterial infections: the importance of host genetics. Genes Immun. **4:**4–11.
7. **Blackwell, J. M.** 1996. Structure and function of the natural-resistance-associated macrophage protein (Nramp1), a candidate protein for infectious and autoimmune disease susceptibility. Mol. Med. Today **2:**205–211.
8. **Boldrick, J. C., A. A. Alizadeh, M. Diehn, S. Dudoit, C. L. Liu, C. E. Belcher, D. Botstein, L. M. Staudt, P. O. Brown, and D. A. Relman.** 2002. Stereotyped and specific gene expression programs in human innate immune responses to bacteria. Proc. Natl. Acad. Sci. USA **99:**972–977.
9. **Borriello, F., M. P. Sethna, S. D. Boyd, A. N. Schweitzer, E. A. Tivol, D. Jacoby, T. B. Strom, E. M. Simpson, G. J. Freeman, and A. H. Sharpe.** 1997. B7-1 and B7-2 have overlapping, critical roles in immunoglobulin class switching and germinal center formation. Immunity **6:**303–313.
10. **Boyer, E., I. Bergevin, D. Malo, P. Gros, and M. F. Cellier.** 2002. Acquisition of Mn(II) in addition to Fe(II) is required for full virulence of *Salmonella enterica* serovar Typhimurium. Infect. Immun. **70:**6032–6042.
11. **Broeren, C. P., G. S. Gray, B. M. Carreno, and C. H. June.** 2000. Costimulation light: activation of $CD4^+$ T cells with CD80 or CD86 rather than anti-CD28 leads to a Th2 cytokine profile. J. Immunol. **165:**6908–6914.
12. **Burr, J. S., S. L. Kimzey, D. R. Randolph, and J. M. Green.** 2001. CD28 and CTLA4 coordinately regulate airway inflammatory cell recruitment and T-helper cell differentiation after inhaled allergen. Am. J. Respir. Cell Mol. Biol. **24:**563–568.
13. **Caron, J., J. C. Loredo-Osti, L. Laroche, E. Skamene, K. Morgan, and D. Malo.** 2002. Identification of genetic loci controlling bacterial clearance in experimental Salmonella enteritidis infection: an unexpected role of Nramp1 (Slc11a1) in the persistence of infection in mice. Genes Immun. **3:**196–204.
14. **Caron, J., J. C. Loredo-Osti, K. Morgan, and D. Malo.** 2005. Mapping of interactions and mouse congenic strains identified novel epistatic QTLs controlling the persistence of Salmonella Enteritidis in mice. Genes Immun. **6:**500–508.
15. **Casanova, J. L., and L. Abel.** 2004. The human model: a genetic dissection of immunity to infection in natural conditions. Nat. Rev. Immunol. **4:**55–66.
16. **Centers for Disease Control and Prevention.** December 2003. Centers for Disease Control and Prevention report. Division of Bacterial and Mycotic Diseases. Disease Information. Centers for Disease Control and Prevention, Atlanta, Ga.
17. **Constant, S., C. Pfeiffer, A. Woodard, T. Pasqualini, and K. Bottomly.** 1995. Extent of T cell receptor ligation can determine the functional differentiation of naive $CD4^+$ T cells. J. Exp. Med. **182:**1591–1596.
18. **Cuellar-Mata, P., N. Jabado, J. Liu, W. Furuya, B. B. Finlay, P. Gros, and S. Grinstein.** 2002. Nramp1 modifies the fusion of Salmonella typhimurium-containing vacuoles with cellular endomembranes in macrophages. J. Biol. Chem. **277:**2258–2265.
19. **Cunningham, A. F., M. Khan, J. Ball, K. M. Toellner, K. Serre, E. Mohr, and I. C. MacLennan.** 2004. Responses to the soluble flagellar protein FliC are Th2, while those to FliC on Salmonella are Th1. Eur. J. Immunol. **34:**2986–2995.
20. **Detweiler, C. S., D. B. Cunanan, and S. Falkow.** 2001. Host microarray analysis reveals a role for the Salmonella response regulator phoP in human macrophage cell death. Proc. Natl. Acad. Sci. USA **98:**5850–5855.
21. **Didierlaurent, A., I. Ferrero, L. A. Otten, B. Dubois, M. Reinhardt, H. Carlsen, R. Blomhoff, S. Akira, J. P. Kraehenbuhl, and J. C. Sirard.** 2004. Flagellin promotes myeloid differentiation factor 88-dependent development of Th2-type response. J. Immunol. **172:**6922–6930.
22. **Eckmann, L., J. Fierer, and M. F. Kagnoff.** 1996. Genetically resistant (Ityr) and susceptible (Itys) congenic mouse strains show similar cytokine responses following infection with Salmonella dublin. J. Immunol. **156:**2894–2900.
23. **Fitness, J., S. Floyd, D. K. Warndorff, L. Sichali, S. Malema, A. C. Crampin, P. E. Fine, and A. V. Hill.** 2004. Large-scale candidate gene study of tuberculosis susceptibility in the Karonga district of northern Malawi. Am. J. Trop. Med. Hyg. **71:**341–349.
24. **Fitness, J., K. Tosh, and A. V. Hill.** 2002. Genetics of susceptibility to leprosy. Genes Immun. **3:**441–453.
25. **Forbes, J. R., and P. Gros.** 2003. Iron, manganese, and cobalt transport by Nramp1 (Slc11a1) and Nramp2 (Slc11a2) expressed at the plasma membrane. Blood **102:**1884–1892.
26. **Forget, A., E. Skamene, P. Gros, A. C. Miailhe, and R. Turcotte.** 1981. Differences in response among inbred mouse strains to infection with small doses of *Mycobacterium bovis* BCG. Infect. Immun. **32:**42–47.
27. **Fuller, A. C., B. Kang, H. K. Kang, H. Yahikozowa, M. C. Dal Canto, and B. S. Kim.** 2005. Gender bias in Theiler's virus-induced demyelinating disease correlates with the level of antiviral immune responses. J. Immunol. **175:**3955–3963.
28. **Greenwood, C. M., T. M. Fujiwara, L. J. Boothroyd, M. A. Miller, D. Frappier, E. A. Fanning, E. Schurr, and K. Morgan.** 2000. Linkage of tuberculosis to chromosome 2q35 loci, including NRAMP1, in a large aboriginal Canadian family. Am. J. Hum. Genet. **67:**405–416.
29. **Gruenheid, S., E. Pinner, M. Desjardins, and P. Gros.** 1997. Natural resistance to infection with intracellular pathogens: the Nramp1 protein is recruited to the membrane of the phagosome. J. Exp. Med. **185:**717–730.
30. **Hall, J. M., J. F. Couse, and K. S. Korach.** 2001. The multifaceted mechanisms of estradiol and estrogen receptor signaling. J. Biol. Chem. **276:**36869–36872.
31. **Hu, J., N. Bumstead, P. Barrow, G. Sebastiani, L. Olien, K. Morgan, and D. Malo.** 1997. Resistance to salmonellosis in the chicken is linked to NRAMP1 and TNC. Genome Res. **7:**693–704.
32. **Huygen, K., and K. Palfliet.** 1984. Strain variation in interferon gamma production of BCG-sensitized mice challenged with PPD II. Importance of one major autosomal locus and additional sexual influences. Cell. Immunol. **85:**75–81.
33. **Jabado, N., F. Canonne-Hergaux, S. Gruenheid, V. Picard, and P. Gros.** 2002. Iron transporter Nramp2/DMT-1 is associated with the membrane of phagosomes in macrophages and Sertoli cells. Blood **100:**2617–2622.
34. **Jabado, N., P. Cuellar-Mata, S. Grinstein, and P. Gros.** 2003. Iron chelators modulate the fusogenic properties of Salmonella-containing phagosomes. Proc. Natl. Acad. Sci. USA **100:**6127–6132.
35. **Jabado, N., A. Jankowski, S. Dougaparsad, V. Picard, S. Grinstein, and P. Gros.** 2000. Natural resistance to intracellular infections: natural resistance-associated macrophage protein 1 (Nramp1) functions as a pH-dependent manganese transporter at the phagosomal membrane. J. Exp. Med. **192:**1237–1248.
36. **Jenner, R. G., and R. A. Young.** 2005. Insights into host responses against pathogens from transcriptional profiling. Nat. Rev. Microbiol. **3:**281–294.
37. **Kanda, N., and S. Watanabe.** 2004. 17β-Estradiol enhances the production of granulocyte-macrophage colony-stimulating factor in human keratinocytes. J. Investig. Dermatol. **123:**329–337.
38. **Leveque, G., V. Forgetta, S. Morroll, A. L. Smith, N. Bumstead, P. Barrow, J. C. Loredo-Osti, K. Morgan, and D. Malo.** 2003. Allelic variation in TLR4 is linked to susceptibility to *Salmonella enterica* serovar Typhimurium infection in chickens. Infect. Immun. **71:**1116–1124.
39. **Liva, S. M., and R. R. Voskuhl.** 2001. Testosterone acts directly on $CD4^+$ T lymphocytes to increase IL-10 production. J. Immunol. **167:**2060–2067.
40. **Livak, K. J., and T. D. Schmittgen.** 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C(T)}$ method. Methods **25:**402–408.
41. **Lumsden, J. M., J. A. Williams, and R. J. Hodes.** 2003. Differential requirements for expression of CD80/86 and CD40 on B cells for T-dependent antibody responses in vivo. J. Immunol. **170:**781–787.
42. **Malo, D., K. Vogan, S. Vidal, J. Hu, M. Cellier, E. Schurr, A. Fuks, N. Bumstead, K. Morgan, and P. Gros.** 1994. Haplotype mapping and sequence analysis of the mouse Nramp gene predict susceptibility to infection with intracellular parasites. Genomics **23:**51–61.
43. **Mastroeni, P.** 2002. Immunity to systemic Salmonella infections. Curr. Mol. Med. **2:**393–406.
44. **Mastroeni, P., and N. Menager.** 2003. Development of acquired immunity to Salmonella. J. Med. Microbiol. **52:**453–459.
45. **McSorley, S. J., B. T. Cookson, and M. K. Jenkins.** 2000. Characterization of $CD4^+$ T cell responses during natural infection with Salmonella typhimurium. J. Immunol. **164:**986–993.
46. **Meisner, S. J., S. Mucklow, G. Warner, S. O. Sow, C. Lienhardt, and A. V. Hill.** 2001. Association of NRAMP1 polymorphism with leprosy type but not susceptibility to leprosy per se in West Africans. Am. J. Trop. Med. Hyg. **65:**733–735.
47. **Mittrucker, H. W., A. Kohler, T. W. Mak, and S. H. Kaufmann.** 1999. Critical role of CD28 in protective immunity against Salmonella typhimurium. J. Immunol. **163:**6769–6776.
48. **Monack, D. M., D. M. Bouley, and S. Falkow.** 2004. Salmonella typhimurium persists within macrophages in the mesenteric lymph nodes of chronically infected $Nramp1^{+/+}$ mice and can be reactivated by IFN-γ neutralization. J. Exp. Med. **199:**231–241.
49. **Monack, D. M., A. Mueller, and S. Falkow.** 2004. Persistent bacterial infections: the interface of the pathogen and the host immune system. Nat. Rev. Microbiol. **2:**747–765.
50. **Mouihate, A., X. Chen, and Q. J. Pittman.** 1998. Interleukin-1β fever in rats: gender difference and estrous cycle influence. Am. J. Physiol. **275:**R1450–R1454.
51. **Murphy, K. M., and S. L. Reiner.** 2002. The lineage decisions of helper T cells. Nat. Rev. Immunol. **2:**933–944.
52. **Nau, G. J., J. F. Richmond, A. Schlesinger, E. G. Jennings, E. S. Lander, and R. A. Young.** 2002. Human macrophage activation programs induced by bacterial pathogens. Proc. Natl. Acad. Sci. USA **99:**1503–1508.

53. **Nauciel, C., F. Vilde, and E. Ronco.** 1985. Host response to infection with a temperature-sensitive mutant of *Salmonella typhimurium* in a susceptible and a resistant strain of mice. Infect. Immun. **49:**523–527.
54. **Parish, C. R., and F. Y. Liew.** 1972. Immune response to chemically modified flagellin. 3. Enhanced cell-mediated immunity during high and low zone antibody tolerance to flagellin. J. Exp. Med. **135:**298–311.
55. **Podojil, J. R., N. W. Kin, and V. M. Sanders.** 2004. CD86 and β2-adrenergic receptor signaling pathways, respectively, increase Oct-2 and OCA-B expression and binding to the 3′-IgH enhancer in B cells. J. Biol. Chem. **279:**23394–23404.
56. **Podojil, J. R., and V. M. Sanders.** 2003. Selective regulation of mature IgG1 transcription by CD86 and β2-adrenergic receptor stimulation. J. Immunol. **170:**5143–5151.
57. **Rodriguez-Palmero, M., T. Hara, A. Thumbs, and T. Hunig.** 1999. Triggering of T cell proliferation through CD28 induces GATA-3 and promotes T helper type 2 differentiation in vitro and in vivo. Eur. J. Immunol. **29:**3914–3924.
58. **Roy, M. F., and D. Malo.** 2002. Genetic regulation of host responses to Salmonella infection in mice. Genes Immun. **3:**381–393.
59. **Searle, S., N. A. Bright, T. I. Roach, P. G. Atkinson, C. H. Barton, R. H. Meloen, and J. M. Blackwell.** 1998. Localisation of Nramp1 in macrophages: modulation with activation and infection. J. Cell Sci. **111**(Pt. 19)**:**2855–2866.
60. **Sebastiani, G., L. Olien, S. Gauthier, E. Skamene, K. Morgan, P. Gros, and D. Malo.** 1998. Mapping of genetic modulators of natural resistance to infection with Salmonella typhimurium in wild-derived mice. Genomics **47:**180–186.
61. **Shahinian, A., K. Pfeffer, K. P. Lee, T. M. Kundig, K. Kishihara, A. Wakeham, K. Kawai, P. S. Ohashi, C. B. Thompson, and T. W. Mak.** 1993. Differential T cell costimulatory requirements in CD28-deficient mice. Science **261:**609–612.
62. **Sirova, M., O. Hovorka, I. Riha, B. Rihova, M. Baudys, S. W. Kim, and E. Skamene.** 1997. The in vivo antibody response against exogenous antigens is not influenced by the mouse Bcg (Nramp1) gene. Immunology **90:**626–631.
63. **Soo, S. S., B. Villarreal-Ramos, C. M. Anjam Khan, C. E. Hormaeche, and J. M. Blackwell.** 1998. Genetic control of immune response to recombinant antigens carried by an attenuated *Salmonella typhimurium* vaccine strain: Nramp1 influences T-helper subset responses and protection against leishmanial challenge. Infect. Immun. **66:**1910–1917.
64. **Su, Z., and M. M. Stevenson.** 2000. Central role of endogenous gamma interferon in protective immunity against blood-stage *Plasmodium chabaudi* AS infection. Infect. Immun. **68:**4399–4406.
65. **Tamayo, P., D. Slonim, J. Mesirov, Q. Zhu, S. Kitareewan, E. Dmitrovsky, E. S. Lander, and T. R. Golub.** 1999. Interpreting patterns of gene expression with self-organizing maps: methods and application to hematopoietic differentiation. Proc. Natl. Acad. Sci. USA **96:**2907–2912.
66. **Vazquez-Torres, A., J. Jones-Carson, P. Mastroeni, H. Ischiropoulos, and F. C. Fang.** 2000. Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. I. Effects on microbial killing by activated peritoneal macrophages in vitro. J. Exp. Med. **192:**227–236.
67. **Vidal, S., M. L. Tremblay, G. Govoni, S. Gauthier, G. Sebastiani, D. Malo, E. Skamene, M. Olivier, S. Jothy, and P. Gros.** 1995. The Ity/Lsh/Bcg locus: natural resistance to infection with intracellular parasites is abrogated by disruption of the Nramp1 gene. J. Exp. Med. **182:**655–666.
68. **Vidal, S. M., D. Malo, K. Vogan, E. Skamene, and P. Gros.** 1993. Natural resistance to infection with intracellular parasites: isolation of a candidate for Bcg. Cell **73:**469–485.
69. **Westfall, P. H., R. D. Tobias, D. Rom, R. D. Wolfinger, and Y. Hochberg.** 1999. Multiple comparisons and multiple tests using the SAS system, p. 231–235. SAS Institute, Inc., Cary, N.C.
70. **Young, D., T. Hussell, and G. Dougan.** 2002. Chronic bacterial infections: living with unwanted guests. Nat. Immunol. **3:**1026–1032.
71. **Zaharik, M. L., and B. B. Finlay.** 2004. Mn^{2+} and bacterial pathogenesis. Front. Biosci. **9:**1035–1042.
72. **Zaharik, M. L., B. A. Vallance, J. L. Puente, P. Gros, and B. B. Finlay.** 2002. Host-pathogen interactions: host resistance factor Nramp1 up-regulates the expression of Salmonella pathogenicity island-2 virulence genes. Proc. Natl. Acad. Sci. USA **99:**15705–15710.
73. **Zheng, W., and R. A. Flavell.** 1997. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell **89:**587–596.

Editor: A. D. O'Brien