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Genetic Control of *Salmonella typhimurium*-Induced Depression of Delayed-Type Hypersensitivity to Sheep Erythrocytes in Mice

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Infection of mice with a temperature-sensitive mutant of *Salmonella typhimurium* C5TS allowed the survival of genetically susceptible mice. The ability to mount a delayed-type hypersensitivity (DTH) response to sheep erythrocytes during infection with C5TS was studied in various inbred mouse strains, recombinant inbred strains derived from C57BL/6 (susceptible) and A/J (resistant) mice, and C3H congenic mice. Suppression of the DTH response to sheep erythrocytes was found in mice that carried the *Ity*^{s} allele, the *H-2*^{b} haplotype, or both. These genes are known to increase susceptibility to *S. typhimurium* infection. In contrast, no DTH response suppression was observed in mouse strains that carried other genes that increased susceptibility to *S. typhimurium*, e.g., DBA/2 and C3H/HeJ. Apart from a transient suppression in A/J mice, the DTH responses of resistant mice (A/J and CBA) were normal or increased. The DTH response to sheep erythrocytes could be restored in immunodepressed mice by increasing the immunizing dose, suggesting the possible role of activated macrophages in depression of the DTH response.

Natural resistance of mice to *Salmonella typhimurium* infection is regulated by several genes. The *Ity* gene controls the early phase of the infection (5, 23). The susceptibility allele *Ity*^{s} is present in C57BL/6 and BALB/c mice. The *Ity* gene controls the net growth rate of virulent strains of *S. typhimurium* (5). Yet the growth of some strains with low virulence (2) and the survival of temperature-sensitive mutants of *S. typhimurium* (7, 24) are not affected by the gene. Results of in vitro studies have shown that the *Ity* gene is expressed by the macrophages (4, 14, 26). The mechanism of action of the *Ity* gene is unknown. Its action is not dependent on the activation of macrophages by T cells (1, 18) or by lipopolysaccharide (1). Whether *Ity* modulates the rate of bacterial growth (1, 7) or bacterial killing (14, 26) remains controversial. Susceptibility to *S. typhimurium* is also increased in C3H/HeJ and CBA/N mice that carry the *Lps*^{d} or the *Xid* genes, respectively (19, 20). Moreover, a gene linked to the major histocompatibility complex (6) and another gene present in DBA/2 and C57L mice (21) increase the susceptibility of mice to *S. typhimurium* in the late phase of the infection.

S. typhimurium infection can induce immunodepression in some mouse strains. Depression of the proliferative response to B and T mitogens has been described in C3H (13) and C57BL/6 (3) mice. In this investigation we show that *S. typhimurium* infection can induce a depression of the delayed-type hypersensitivity (DTH) response to sheep erythrocytes and that this immunodepression appears to be associated with some of the genes that are known to increase susceptibility to *S. typhimurium* (*Ity*^{s} and *H-2*^{b}).

MATERIALS AND METHODS

Mice. C57BL/6-J-Pas, BALB/c-By-Pas, C3H/He-J-Pas, DBA/2-J-Pas, and A/J-Pas mice and F_1 hybrids from C57BL/6-J-Pas and DBA/2-J-Pas mice were obtained from the Pasteur Institute (Paris) animal breeding facility. CBA/J-Ico mice were obtained from Iffa Credo (L'Arbresle, France). Mice of the AXB and BXA set of recombinant inbred strains derived from A/J (A) and C57BL/6 (B) mice were bred at the Pasteur Institute from progenitors kindly provided by M. Nesbitt (University of California, La Jolla). C3H.B10 mice were obtained from M. Pla (Institut National de la Santé et de la Recherche Médicale, U93, St. Louis Hospital, Paris). Female mice between 6 and 12 weeks of age were used in all the experiments.

Bacteria. *S. typhimurium* C5TS, a temperature-sensitive mutant derived from the virulent C5 strain (7) and kindly provided by C. E. Hormaeche, was stored at −80°C. For each experiment a sample was thawed and grown for 24 h at 30°C in tryptic soy broth. Mice were inoculated intravenously (i.v.) with the indicated doses of *S. typhimurium* C5TS suspended in 0.2 ml of saline. Bacterial concentrations were always verified by quantitative plate counts on tryptic soy agar.

Sensitization to sheep erythrocytes. Sheep erythrocytes were washed 3 times with saline. Mice were injected i.v. with 0.2 ml of a 0.01% sheep erythrocyte suspension in saline, unless stated otherwise and challenged 4 days later in a hind footpad with 40 μl of a 20% sheep erythrocyte suspension. Increases in footpad thickness were measured 24 and 48 h later with a dial gauge caliper. Mean values were determined from groups of at least five mice. The response of control mice peaked either at 24 or 48 h after footpad challenge. The highest value was selected and compared with the response of infected mice recorded at the same time.

The nonspecific responses of unimmunized animals were below 0.10 mm.

Enumeration of bacteria in the spleen. The spleen of each mouse was removed 21 days after infection and homogenized in 2 ml of distilled water with a motor-driven Teflon (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) pestel. Portions of 10-fold serial dilutions in saline were plated in tryptic soy agar. Colonies were counted after a 24 h of incubation at 30°C.

Statistical analysis. The statistical significance ($P < 0.05$) of differences among the various groups of mice were evaluated by Student's unpaired t test.

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RESULTS

DTH response to sheep erythrocytes in various mouse strains infected with *S. typhimurium* C5TS. Various mouse strains that differed in their susceptibility to *S. typhimurium* infection were inoculated i.v. with 10^6 C5TS. This bacterial strain replicates slowly in mice and allows the survival of susceptible mice and induces a protracted infection (16). The ability of infected mice to mount a DTH response to sheep erythrocytes was determined at various times postinfection and compared with the responses of control mice. The increase in footpad thickness of control mice ranged from 0.33 ± 0.08 mm in CBA mice to 0.84 ± 0.15 mm in DBA/2 mice. DTH responses of infected mice were expressed as a percentage of the response of the corresponding controls. The data reported in Fig. 1 indicate that infected C57BL/6 and BALB/c mice (both Ity^s) exhibited a strong depression of their DTH responses to sheep erythrocytes. In C57BL/6 mice the response was still depressed at day 28 (data not shown), whereas the response of BALB/c mice was restored at day 21. Mouse strains that possessed other genes that are known to increase susceptibility to *S. typhimurium*, such as C3H/HeJ (19) or DBA/2 (21), displayed normal responses. In resistant (Ity^r) mice, such as A/J and CBA, the responses were normal or increased. The only exception was a transient depression of the response of A/J mice at day 7.

The depressed response of (C57BL/6 × DBA/2)F_1 mice indicates that the infection-induced depression of DTH was dominant.

DTH response to sheep erythrocytes in infected recombinant inbred and congenic strains. AXB/BXA recombinant inbred strains were infected with 10^6 C5TS, and their ability to develop a DTH response to sheep erythrocytes was determined at day 14. The data reported above indicate that at this time the response to sheep erythrocytes was strongly depressed in C57BL/6 mice, whereas it was increased in A/J mice.

The results reported in Fig. 2 indicate that in the strains that carried the Ity^s allele (AXB-1, AXB-6, and BXA-6), DTH responses were strongly depressed, as in the parental C57BL/6 strain. Two recombinant inbred strains (AXB-2 and AXB-7) had normal responses, and the responses of the three other strains were moderately but significantly ($P < 0.05$) depressed. Two of these strains (AXB-4 and BXA-8) were $H\text{-}2^b$.

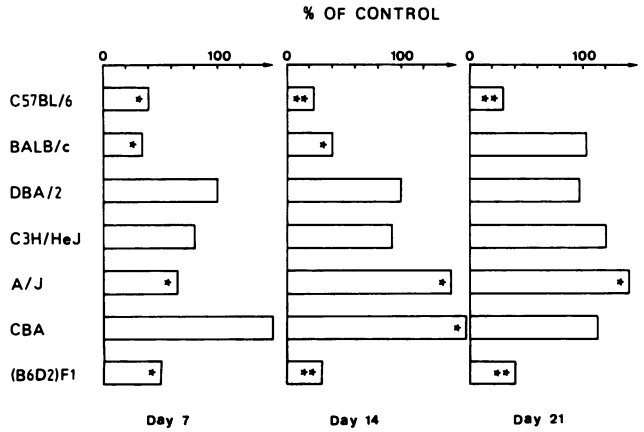

FIG. 1. DTH response to sheep erythrocytes in various mouse strains infected with 10^6 *S. typhimurium* C5TS. Groups of five mice were sensitized with sheep erythrocytes on the indicated days after infection, and footpad testing was carried out 4 days later. Results are expressed as a percentage of the response of controls. Results marked with an asterisk are significantly different ($P < 0.05$) from those of controls. (B6D2)F_1, (C57BL/6 × DBA/2)F_1.

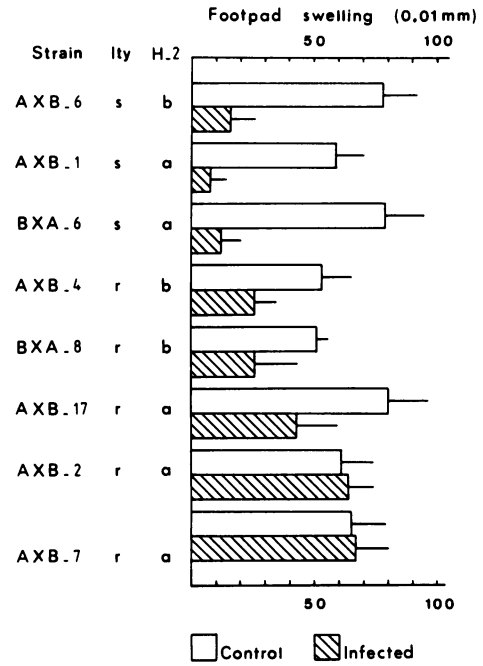

FIG. 2. DTH response to sheep erythrocytes in recombinant inbred strains infected with 10^6 *S. typhimurium* C5TS 14 days before sensitization with sheep erythrocytes. Footpad challenge was carried out 4 days after sensitization in infected and control animals (five mice per group).

The results reported in Table 1 indicate that the DTH response to sheep erythrocytes was significantly ($P < 0.01$) depressed in C3H.B10 ($H\text{-}2^b$) mice, whereas the response was unaffected in C3H/HeJ ($H\text{-}2^k$) mice.

Relationship between DTH response suppression and bacterial spleen counts. During the first week of infection with C5TS, spleen counts are similar in various mouse strains (7, 16), but differences in the rate of clearance occur later (16). The results of spleen counts and spleen weights determined in various mouse strains 21 days after the inoculation of 10^6 C5TS are shown in Table 2. The highest spleen counts were present in C57BL/6 and AXB-6 mice, and the highest spleen weights were found in C57BL/6 and AXB-1 mice. These mouse strains exhibited a strong depression of the DTH response to sheep erythrocytes. However, other mouse strains such as BALB/c, (C57BL/6 × DBA/2)F_1, and BXA-6 were also suppressed, although their spleen counts and spleen weights were similar to those of mouse strains that exhibited normal DTH responses to sheep erythrocytes.

TABLE 1. DTH response to sheep erythrocytes in infected C3H congenic mice

Strain	*H-2* allele	DTH response (mm) of the following mice:	
		Control	Infected (% of control)[a]
C3H/HeJ	*k*	0.38 ± 0.10	0.36 ± 0.05 (95)
C3H.B10	*b*	0.48 ± 0.19	0.15 ± 0.10 (31)

[a] Mice were infected i.v. with 10^6 C5TS 14 days before sensitization with sheep erythrocytes. Footpad testing was performed 4 days after sensitization.

TABLE 2. Spleen counts and spleen weights in various mouse strains 21 days after infection with 10^6 C5TS

Strain	Log_{10} spleen counts[a]	Spleen weight (mg)[a]	DTH response suppression[b]
C57BL/6	4.35 ± 0.63	653 ± 167	++
BALB/c	2.11 ± 0.35	208 ± 61	++
DBA/2	2.38 ± 0.20	198 ± 62	−
C3H/HeJ	1.51 ± 0.32	206 ± 18	−
A/J	2.30 ± 0.45	158 ± 37	−
CBA	2.74 ± 0.61	133 ± 50	−
(B6D2)F_1[c]	2.53 ± 0.15	230 ± 27	++
AXB-1	3.03 ± 0.74	403 ± 132	++
AXB-2	2.65 ± 0.30	307 ± 57	−
AXB-4	2.98 ± 0.24	250 ± 55	+
AXB-6	4.14 ± 0.34	370 ± 38	++
AXB-7	2.52 ± 0.26	170 ± 26	−
AXB-17	3.10 ± 0.31	239 ± 47	+
BXA-6	2.82 ± 0.61	219 ± 95	++
BXA-8	2.92 ± 0.21	171 ± 30	+

[a] Mean ± standard deviations of groups at least of five mice.
[b] Sensitization to sheep erythrocytes was carried out 14 days postinfection. Symbols: ++, DTH response inferior to 25% of the control; +, DTH response inferior to 50% of the control; −, no DTH response suppression.
[c] (B6D2)F_1, (C57BL/6 × DBA/2)F_1.

Moreover, by varying the dose of bacteria injected, we showed that the inoculation of 10^4 C5TS induced a significant depression of the DTH response in C57BL/6 mice, whereas the response of DBA/2 mice was not modified by 10^7 bacteria (Table 3).

Effect of the immunizing dose on DTH response to sheep erythrocytes in C57BL/6 mice. When the immunizing dose of sheep erythrocytes was given by the i.v. route, the optimal DTH response was induced by a low dose of antigen. An increase in the dose of antigen induces a decrease in the DTH response (11). In C57BL/6 mice infected with 10^6 C5TS, the dose response to sheep erythrocytes was different. An increase in the immunizing dose increased the DTH response (Table 4).

DISCUSSION

Results of previous studies have shown that infection with *S. typhimurium* C5TS induces an impairment of splenocyte proliferative responses to B and T mitogens and of interleukin 2 production in C57BL/6 mice (3). The results reported here indicate that C5TS infection also induces a depression of an in vivo immune response, i.e., the DTH response to sheep erythrocytes.

Depression of the DTH response to sheep erythrocytes appears to be genetically controlled. Mice that carried the *Ity*^{s} allele exhibited a strong depression of the DTH response. No depression of the DTH response to sheep erythrocytes has been found in infected DBA/2 and C3H/HeJ mice that are *Ity*^{r} but that carry other genes that increase their susceptibility to *S. typhimurium* (19, 21). Depression of the DTH response to sheep erythocytes was also present in infected mice that carried the *H-2*^{b} haplotype. This haplotype has been shown to be associated with a low rate of clearance of C5TS from the spleen (C. Nauciel, E. Ronco, M. Pla, and J.-L. Guenet, submitted for publication) and with an increased susceptibility of mice of the C57BL/10 background to a strain of *S. typhimurium* of low virulence (6). The longer duration of depression of the DTH response in infected C57BL/6 mice may be related to the fact that this mouse strain carries both the *Ity*^{s} gene and the *H-2*^{b} haplotype. The moderate depression of the DTH response in infected AXB-17 mice suggests that additional genes could be involved.

TABLE 3. Effect of the dose of bacteria on the DTH response to sheep erythrocytes in susceptible and resistant mice

Mouse strain	Dose of bacteria	DTH (% of control)[a]
C57BL/6	10^4	53
	10^5	32
	10^6	14
DBA/2	10^6	101
	10^7	100

[a] Groups of five mice were sensitized with a 0.01% suspension of sheep erythrocytes 14 days after infection with C5TS.

TABLE 4. DTH response to sheep erythrocytes in control and infected C57BL/6 mice sensitized with various doses of sheep erythrocytes

Dose (%) of sheep erythrocytes[a]	Increase in footpad thickness (10^{-2} mm ± SD) in the following mice:	
	Control	Infected[b]
0.01	68 ± 6	16 ± 8
0.1	44 ± 15	33 ± 10
1	19 ± 9	49 ± 8
10	11 ± 2	53 ± 13

[a] Mice were sensitized i.v. with 0.2 ml of the indicated concentration of sheep erythrocytes.
[b] Mice were infected i.v. with 10^6 C5TS 20 days before sensitization with sheep erythrocytes.

The relationship between immunodepression and genes that are known to increase susceptibility to *S. typhimurium* infection remains to be determined. Immunodepression in susceptible mice is not merely related to the heavier colonization of the organs. Although some *Ity*^{s} strains (C57BL/6 and AXB-6) exhibited high spleen counts, other *Ity*^{s} strains (BALB/c, AXB-1, and BXA-6) did not harbor more bacteria than *Ity*^{r} mice. The induction of depression of the DTH response to sheep erythrocytes in mice with similar spleen counts thus depends on the mouse genotype. In other reports it has been shown that in *S. typhimurium* infection, genetically susceptible mice develop a depression of the proliferative responses to mitogens (3) and of the DTH response to *Salmonella* antigens (9, 10), whereas resistant mice are not suppressed. A correlation between susceptibility and immunodepression has also been reported in *Trypanosoma cruzi* (25) and *Leishmania tropica* (12, 17) infections. Thus, it seems that susceptible animals are more prone to develop immunodepression during the course of infection.

It is generally assumed that immunodepression increases susceptibility to infectious agents. However, in previous studies it has been shown that C57BL/6 mice infected with C5TS develop within a few days an increased resistance to reinfection with *S. typhimurium* or *Listeria monocytogenes* (16). Immunodepression and increased resistance to infection may thus occur at the same time in infected animals. Similar findings have been made in mice of the C3H lineage infected with *S. typhimurium* SL3235 (9, 13). This paradoxical situation may be explained by macrophage activation (8, 13). It has been shown during the course of rickettsial infection that activated macrophages can display simultaneously an increase in their bactericidal and tumoricidal activities and a suppressive effect on the proliferation of lymphocytes (8). A suppressive effect of macrophages on the

proliferative response to mitogens has been observed during *S. typhimurium* infection (3, 13). The dose response to sheep erythrocytes in C57BL/6 mice also suggests that activated macrophages play a role in the suppression of the DTH response induced by *S. typhimurium* infection. A similar restoration of the DTH response to sheep erythrocytes by increasing the immunizing dose has been reported in mice infected with *Plasmodium* spp. (15) and *Mycobacteria* spp. (22). This phenomenon has been ascribed to excess antigen catabolism or defective antigen presentation by macrophages from infected animals (15, 22). It is interesting that there is evidence that the *Ity* gene controls the interaction between macrophages and *S. typhimurium* (4, 14, 26). This raises the possibility that the functional changes induced by *S. typhimurium* infection differ in macrophages from Ity^s and Ity^r mice.

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LITERATURE CITED

1. **Briles, D. E., W. Benjamin, Jr., B. Posey, S. M. Michalek, and J. R. McGhee.** 1986. Independence of macrophage activation and expression of the alleles of the *Ity* (immunity to *typhimurium*) locus. Microb. Pathogen. **1**:33–41.
2. **Benjamin, W. H., Jr., C. L. Turnbough, Jr., B. S. Posey, and D. E. Briles.** 1986. *Salmonella typhimurium* virulence genes necessary to exploit the $Ity^{s/s}$ genotype of the mouse. Infect. Immun. **51**:872–878.
3. **Deschenes, M., M. Guenounou, E. Ronco, F. Vacheron, and C. Nauciel.** 1986. Impairment of lymphocyte proliferative responses and interleukin-2 production in susceptible (C57BL/6) mice infected with *Salmonella typhimurium*. Immunology. **58**: 225–230.
4. **Harrington, K. A., and C. E. Hormaeche.** 1986. Expression of the innate resistance gene *Ity* in mouse Kupffer cells infected with *Salmonella typhimurium in vitro*. Microb. Pathogen. **1**: 269–274.
5. **Hormaeche, C. E.** 1979. Natural resistance to *Salmonella typhimurium* in different inbred mouse strains. Immunology **37**:311–318.
6. **Hormaeche, C. E., K. A. Harrington, and H. S. Joysey.** 1985. Natural resistance to *Salmonellae* in mice: control by genes within the major histocompatibility complex. J. Infect. Dis. **152**:1050–1056.
7. **Hormaeche, C. E., R. A. Pettifor, and J. Brock.** 1981. The fate of temperature-sensitive Salmonella mutants *in vivo* in naturally resistant and susceptible mice. Immunology **42**:569–576.
8. **Jerrels, T. R.** 1985. Immunosuppression associated with the development of chronic infection with *Rickettsia tsutsugamushi*: adherent suppressor cells activity and macrophage activation. Infect. Immun. **50**:175–182.
9. **Killar, L. M., and T. K. Eisenstein.** 1984. Differences in delayed-type hypersensitivity responses in various mouse strains in the C3H lineage infected with *Salmonella typhimurium* strain SL3235. J. Immunol. **133**:1190–1196.
10. **Killar, L. M., and T. K. Eisenstein.** 1986. Delayed-type hypersensitivity and immunity to *Salmonella typhimurium*. Infect. Immun. **52**:504–508.
11. **Lagrange, P. H., G. B. Mackaness, and T. E. Miller.** 1974. Influence of dose and route of antigen injection on the immunological induction of T cells. J. Exp. Med. **139**:528–559.
12. **Leclerc, C., F. Modabber, E. Deriaud, J. Djoko-Tamnou, and L. Chedid.** 1982. Visceral *Leishmania tropica* infection of BALB/c mice: cellular analysis of in vitro unresponsiveness to sheep erythrocytes. Infect. Immun. **37**:895–902.
13. **Lee, J. C., C. W. Gibson, and T. K. Eisenstein.** 1985. Macrophage-mediated mitogenic suppression induced in mice of the C3H lineage by a vaccine strain of *Salmonella typhimurium*. Cell. Immunol. **91**:75–91.
14. **Lissner, C. R., R. N. Swanson, and A. D. O'Brien.** 1983. Genetic control of the innate resistance of mice to *Salmonella typhimurium*: expression of the *Ity* gene in peritoneal and splenic macrophages isolated in vitro. J. Immunol. **131**:3006–3013.
15. **Michel, J. C., P. H. Lagrange, and B. Hurtel.** 1979. Modulation by malaria infection of induction of T lymphocyte-dependent delayed-type hypersensitivity and antibody formation to sheep erythrocytes in mice. Parasite Immunol. **1**:267–275.
16. **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.
17. **Nikol, A. D., and P. F. Bonventre.** 1985. Visceral leishmaniasis in congenic mice of susceptible and resistant phenotypes: immunosuppression by adherent spleen cells. Infect. Immun. **50**: 160–168.
18. **O'Brien, A. D., and E. F. Metcalf.** 1982. Control of early *Salmonella typhimurium* growth in innately *Salmonella*-resistant mice does not require functional T lymphocytes. J. Immunol. **129**:1349–1351.
19. **O'Brien, A. D., D. L. Rosenstreich, I. Scher, G. H. Campbell, R. P. MacDermott, and S. B. Formal.** 1980. Genetic control of susceptibility to *Salmonella typhimurium* in mice: role of the *Lps* gene. J. Immunol. **124**:20–24.
20. **O'Brien, A. D., I. Scher, and E. F. Metcalf.** 1981. Genetically conferred defect in anti-*Salmonella* antibody formation renders CBA/N mice innately susceptible to *Salmonella typhimurium* infection. J. Immunol. **126**:1368–1372.
21. **O'Brien, A. D., B. A. Taylor, and D. L. Rosenstreich.** 1984. Genetic control of natural resistance to *Salmonella typhimurium* in mice during the late phase of infection. J. Immunol. **133**:3313–3318.
22. **Orme, I. M, and F. M. Collins.** 1984. Immune response to atypical mycobacteria: immunocompetence of heavily infected mice measured in vivo fails to substantiate immunosuppression data obtained in vitro. Infect. Immun. **43**:32–37.
23. **Plant, J., and A. A. Glynn.** 1976. Genetics of resistance to infection with *Salmonella typhimurium* in mice. J. Infect. Dis. **133**:72–78.
24. **Swanson, R. N., and A. D. O'Brien.** 1983. Genetic control of the innate resistance of mice to *Salmonella typhimurium*: Ity gene is expressed in vivo by 24 hours after infection. J. Immunol. **131**:3014–3020.
25. **Tanowitz, M. B., N. Minato, R. Lalonde, and M. Wittner.** 1981. *Trypanosoma cruzi*: correlation of resistance and susceptibility in infected mice with the in vivo primary antibody response to sheep red blood cells. Exp. Parasitol. **52**:233–242.
26. **Van Dissel, J. T., P. C. J. Leijh, and R. Van Furth.** 1985. Differences in initial rate of intracellular killing of *Salmonella typhimurium* by resident, peritoneal macrophages from various mouse strains. J. Immunol. **134**:3404–3410.