Comparative Protection of Mice against Virulent and Attenuated Strains of *Brucella abortus* by Passive Transfer of Immune T Cells or Serum

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Passively transferred immune serum provided significantly greater protection to BALB/c mice against attenuated *Brucella abortus* 19 than against virulent strain 2308, whether serum donors had been infected with strain 19 or 2308. In contrast, immune T cells conferred better protection upon recipients challenged with the homologous strain of *B. abortus*. It is hypothesized that strain 2308, but not strain 19, can survive in macrophages after opsonization and that epitopes which induce protective cell-mediated immunity may differ between strains 19 and 2308.

Evidence from the mouse model system indicates that both humoral and cell-mediated immune responses participate in protective immunity against the facultative intracellular bacterium *Brucella abortus* (1–3, 5–13, 15; L. N. Araya, P. H. Elzer, G. E. Rowe, F. M. Enright, and A. J. Winter, J. Immunol., in press). Virulent strains of *B. abortus* such as strain 2308 nevertheless possess the capacity to produce chronic infections in cattle (14) or BALB/c mice (8) in the face of the immune response of the host. The basis of this property remains unexplained and distinguishes virulent strains from attenuated strain 19, which has long been used as a living vaccine in cattle (14) and is eliminated much more rapidly from the tissues of BALB/c mice (8). In the studies reported here, we wished to determine the relative susceptibilities of virulent and attenuated strains of *B. abortus* to the protective effects of antibodies and T effector lymphocytes generated in response to infection. For this purpose, passive transfer experiments were performed to test the efficacy of immune serum and T cells in limiting infection in mice by strains 19 and 2308.

BALB/c ByJ female mice (Jackson Laboratory, Bar Harbor, Maine) was used as donors and recipients. Donors at 10 weeks of age were infected intravenously with approximately 5×10^4 CFU of either strain 19 (National Veterinary Services Laboratories, Ames, Iowa) or mouse-passaged strain 2308 in 100 μl of phosphate-buffered saline. Inocula were prepared from freshly thawed stock cultures, and the exact dose was determined retrospectively by viable counts (8, 16). Donors infected with strain 19 were killed at 6 weeks postinfection (p.i.) since it had been established that substantial concentrations of protective antibodies and immune T cells were present by this time (Araya et al., in press). Immune cells were isolated from spleens of strain 2308-infected mice at the end of the plateau phase at 8 weeks p.i. and during the phase of decline at 12 weeks p.i. (8), when the size and cellularity of the spleens resembled those of spleens at 4 and 5 weeks p.i., respectively, from mice infected with strain 19 (L. N. Araya, unpublished data).

To produce T-lymphocyte-enriched cell populations, we prepared single-cell suspensions of spleens in Hanks balanced salt solution containing 3 mg of DNase (Sigma Chemical Co., St. Louis, Mo.) by forcing the minced tissue through a 40-mesh stainless steel screen with a glass plunger. Erythrocytes were removed by exposure of the cells for 30 s to a lysing buffer containing 0.83 g of NH_4Cl, 100 mg of $KHCO_3$, and 1.72 mg of EDTA per 100 ml of distilled water. The leukocytes were washed, suspended in RPMI 1640 medium containing 10% fetal calf serum (Sigma), and depleted of B cells by panning (17) on plates coated with an affinity-purified goat anti-mouse immunoglobulin G [F(ab')$_2$ fragment specific] (Cappel Division, Organon Teknika, West Chester, Pa.). Nonadherent cells were washed, pooled, kept on ice for 30 min, and filtered again through the 40-mesh screen. The filtered cell suspension was washed twice with Dulbecco modified Eagle medium plus 1% fetal calf serum and resuspended in the same medium for injection into mice. The viability of panned cells, estimated by trypan blue exclusion, exceeded 95%. The percentage of B cells was determined by a direct immunofluorescence test with a fluorescein isothiocyanate-conjugated F(ab')$_2$ goat anti-mouse immunoglobulin M (Cappel). T-cell subsets were determined by indirect assays employing rat monoclonal antibodies of the immunoglobulin G2b isotype specific for CD4 (L3T4-positive cells; GK1.5, ATCC TIB 207) and CD8 (Lyt2-positive cells; 2.43, ATCC TIB 210) antigens, followed by a fluorescein isothiocyanate-conjugated goat anti-rat immunoglobulin G (heavy and light chains) (Cappel). The total percentage of CD4 and CD8 T cells in panned preparations from strain 19-infected donors infected 6 weeks earlier averaged 72%. Cells which did not express CD4 or CD8 surface markers had no role in passive protection, since protection was abolished by removal of both CD4 and CD8 T cells (Araya et al., in press). Panned cells from donors infected 8 or 12 weeks previously with strain 2308 contained 42 and 70% total T cells, respectively. B cells composed ≤3% in all preparations and had no role in protection as demonstrated by analysis of antibody responses in recipient and control groups (Araya et al., in press). Donors were bled from the retro-orbital sinus or from the heart at the time of sacrifice. Sera were diluted in an equal volume of phosphate-buffered saline, filter sterilized, and heat inactivated at 56°C

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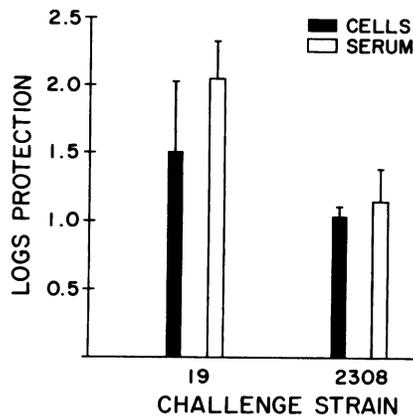† Present address: School of Veterinary Medicine, Universidad Nacional, Heredia, Costa Rica.

FIG. 1. Protection against challenge infection with *B. abortus* 19 or 2308 provided to BALB/c mice by passive transfer of T cells or serum from donors infected 6 weeks earlier with strain 19. Transfers were made with the same pool of donor cells and serum, 2 h after infection. Logs protection represent the difference between mean log numbers of *B. abortus* in spleens of principal and corresponding control groups at 1 week p.i. There were five mice per group. Bars indicate standard deviation.
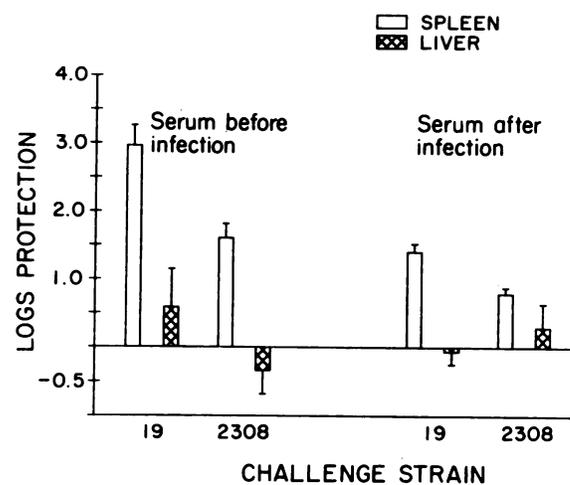

FIG. 2. Protection against challenge infection with *B. abortus* 19 or 2308 provided to BALB/c mice by transfer of serum from donors infected 6 weeks earlier with strain 19. Transfers were made 2 h before or 2 h after infection, in separate experiments. The same lot of antiserum was used in both experiments. Logs protection represent the difference between mean log numbers of *B. abortus* in spleens and livers of principal and corresponding control groups at 1 week p.i. There were five mice per group. Bars indicate standard deviation.

for 30 min. Sera were frozen at $-20°C$ unless used on the same day.

Ten-week-old recipient mice were injected intravenously with 3×10^7 T-lymphocyte-enriched spleen cells in 400 μl of medium or with 200 μl of serum diluted 1:2 in phosphate-buffered saline. Injections were made 2 h before or 2 h after challenge infections. Control mice were injected with an equivalent quantity of medium or phosphate-buffered saline, since it had been established that transfer of normal serum exerted no effect on the growth of the challenge strain (8; J. A. Montaraz and A. J. Winter, unpublished data), whereas transfer of normal T-cell-enriched splenocytes generally enhanced bacterial growth (8; Araya et al., in press). The reason for this enhancement is not known; it has been observed also in rats infected with *Listeria monocytogenes* (D. D. McGregor, personal communication). Recipients were killed at 1 week p.i., and numbers of *B. abortus* were determined in the spleen and in some experiments in the liver (8). At 1 week p.i., mean log numbers of brucellae of strain 19 or 2308 in the spleens of control groups ranged from 6.0 to 6.5, while numbers in the liver were more than 10-fold lower. Log transformations of the data and statistical analyses were performed by methods previously employed (8, 16).

Immune cells or serum from strain 19-infected donors conferred significant protection upon recipients challenged with strain 19 or 2308 ($P < 0.01$ to $P < 0.001$) (Fig. 1). However, levels of protection provided by cells (1.5 versus 1.0 logs; $P = 0.06$) or serum (2.1 versus 1.2 logs; $P < 0.001$) were greater in groups challenged with strain 19 (Fig. 1). In a repetition of this experiment (data not shown), recipients challenged with strain 19 again demonstrated higher levels of protection than those challenged with strain 2308 after transfer of immune cells ($P < 0.01$) or serum ($P = 0.06$). Further experiments were performed with serum from strain 19 donors to determine whether these relationships were influenced by the time of passive transfer in relation to infection. Spleen counts in all groups given serum were significantly below those of corresponding control groups ($P < 0.01$ to $P < 0.001$) (Fig. 2). However, the level of

protection in spleens provided against strain 19 was again greater than that provided against 2308, whether serum was transferred before infection ($P < 0.001$) or after infection ($P < 0.01$) (Fig. 2). In accord with prior observations (Araya et al., in press), protection afforded against challenge infection with each individual strain was significantly greater ($P < 0.001$) when serum was given before infection (Fig. 2). These relationships were unaffected by the small differences in numbers of brucellae recovered from the liver (Fig. 2), because liver counts represented 10% or less of those in the spleen. In reciprocal experiments, protection provided to recipients challenged with strain 19 was found to be significantly greater than that provided to recipients challenged with strain 2308, whether the recipients had received antiserum specific for strain 19 ($P < 0.01$) or for strain 2308 ($P < 0.01$) (Fig. 3). These data therefore demonstrate a consistently decreased effectiveness of antibodies in protecting against virulent strain 2308. The basis for this effect, which cannot be ascribed to differences in the specificity of protective antibodies induced by the two strains (Fig. 3), has yet to be established. It may be related to the possession by virulent strains, but not by strain 19, of properties which allow survival in macrophages (4) even after opsonization with antibody.

In both experiments performed with immune T cells from donors infected with strain 2308, significant protection ($P < 0.01$) approaching 1 log was provided to recipients challenged with strain 2308 (Fig. 4). Mean levels of protection against strain 19 were in both instances lower (Fig. 4) and achieved statistical significance with 12-week ($P < 0.05$) but not with 8-week donor cells. Differences in protection between groups challenged with strains 19 and 2308 were not significant in either experiment owing to the unusually large within-group variations in mice challenged with strain 19 (Fig. 4). Therefore, in contrast to the effects of immune serum, T cells derived from strain 2308-infected donors did not provide better protection against challenge with strain 19. Rather, the data suggest that protection afforded by
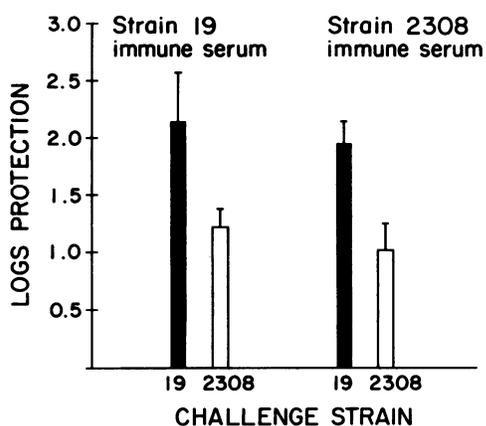
FIG. 3. Protection against challenge infection with *B. abortus* 19 or 2308 provided to BALB/c mice by transfer of serum from donors infected 6 weeks earlier with strain 19 or 2308. Transfers were made 2 h after infection. Separate experiments were performed with serum from strain 19- and strain 2308-infected donors. Enzyme-linked immunosorbent assay titers of the sera were 1,600 and 3,200, respectively. Logs protection represent the difference between mean log numbers of *B. abortus* in spleens of principal and corresponding control groups at 1 week p.i. There were five mice per group. Bars indicate standard deviation.

passively transferred T cells was better against the challenge strain used to induce the donor immune response (Fig. 1 and 4). This could have reflected antigenic differences between the two strains in T-cell epitopes involved in the induction of protective cell-mediated immune responses. In experiments under way, lymphocyte transformation assays are being used to test cross-reactivities of strain 19 and a variety of virulent field isolates of *B. abortus* against immune T cells of homologous and heterologous origin.
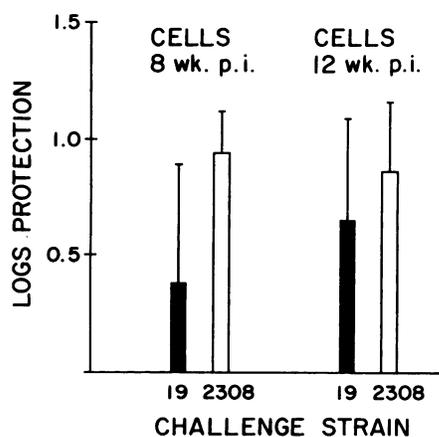

FIG. 4. Protection against challenge infection with *B. abortus* 19 or 2308 provided to BALB/c mice by transfer of T cells from donors infected 8 or 12 weeks earlier with strain 2308. Transfers were made 2 h after infection, in separate experiments with the two sets of donors. Logs protection represent the difference between mean log numbers of *B. abortus* in spleens of principal and corresponding control groups at 1 week p.i. There were five mice per group. Bars indicate standard deviation.

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