INFECTION AND IMMUNITY, July 1992, p. 2855–2862
0019-9567/92/072855-08$02.00/0

Vol. 60, No. 7

Humoral and Cell-Mediated Immunity in Mice to a 17-Kilodalton Lipoprotein of *Francisella tularensis* Expressed by *Salmonella typhimurium*

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Received 16 December 1991/Accepted 29 April 1992

A 17-kDa lipoprotein, TUL4, of the facultative intracellular bacterium *Francisella tularensis* is one of several membrane proteins that induce an in vitro response in T cells from *F. tularensis*-primed humans. A DNA fragment of the live vaccine strain *F. tularensis* LVS encoding TUL4 was cloned into *Salmonella typhimurium* χ4072, an attenuated Δ*cya* Δ*crp* mutant. Expression of the protein by the recombinant *S. typhimurium* χ4072 (pTUL4-15) was maintained after passage in BALB/cJ mice. When mice were immunized with *S. typhimurium* χ4072(pTUL4-15), some animals showed an antibody response and a T-cell response to TUL4. When the immunized mice were challenged with the live vaccine strain *F. tularensis* LVS, bacterial counts in the liver and spleen were lower than in animals immunized with *S. typhimurium* χ4072. Immunization with *F. tularensis* LVS caused a much stronger protection against the challenge than did immunization with *S. typhimurium* χ4072(pTUL4-15). The present study demonstrated that the 17-kDa lipoprotein TUL4 of *F. tularensis* is involved in a protective immunity to tularemia. Possibly, several T-cell-reactive proteins of the organism have to contribute for optimal protection to be achieved.

A cell-mediated immune response is a key event in the mammalian host defense against facultative intracellular bacteria such as *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Listeria monocytogenes*, and *Francisella tularensis* (15). Much work is at present devoted to identifying the microbial antigens involved. Since a protective T-cell response is usually obtained only by vaccination with replicating live bacteria and not by nonviable antigens (4, 9, 15), the importance of a certain antigen is not easily evaluated.

In the live vaccine strain *F. tularensis* LVS, a multitude of membrane proteins has been identified which stimulate T cells from primed individuals (34, 38, 43). The gene encoding one such protein, a 17-kDa lipoprotein (41) denoted TUL4, has been cloned in *Escherichia coli* (39) and sequenced (40). The recombinant protein was recognized by T cells of LVS-vaccinated individuals (40). T-cell epitopes of the protein have been identified (40). The responding T cells produce interleukin 2 and gamma interferon, which indicates that the protein may be involved in the induction of a protective host response (40).

Recombinant techniques provide means of testing the immunogenic relevance of a specific protein of a pathogenic microorganism. When such techniques are used to search for protective antigens of intracellular bacteria, attenuated *Salmonella* strains are useful carriers owing to their ability to colonize and proliferate in the gut-associated lymphoid tissue as well as in the liver and spleen of experimental animals (11, 14, 25). Mutants dependent on aromatic amino acids (Δ*aroA*) and mutants lacking adenylate cyclase and cyclic AMP receptors (Δ*cya* Δ*crp*) are examples of such strains. An expression-cloning vector has been constructed for the purpose of high-level and stable expression of foreign antigen in the Δ*asd* mutant *S. typhimurium* χ4072 of a Δ*cya* Δ*crp* *S. typhimurium* strain (27). A complementing *asd* gene, encoding an enzyme essential for the synthesis of the cell wall peptidoglycan of gram-negative bacteria, is plasmid located in *S. typhimurium* χ4072. The loss of plasmid will consequently be lethal to the organism, and only those cells that harbor the plasmid are able to replicate (10).

In the present study, the gene encoding TUL4 was inserted in the Asd$^+$ plasmid of *S. typhimurium* χ4072. Expression of TUL4 by the recombinant was found to be maintained after passage in mice. In mice immunized with the recombinant, an antibody and a lymphocyte response to TUL4 and a protective immune response to *F. tularensis* LVS were demonstrated.

MATERIALS AND METHODS

Bacteria. The *S. typhimurium* SR-11 (7) Δ*cya* Δ*crp* strain χ3730 (11) and Δ*asd* Δ*cya* Δ*crp* strain χ4072 (27) were provided by R. Curtiss III, Washington University, St. Louis, Mo. The vaccine strain *F. tularensis* LVS was supplied by the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md. It has recently been shown that the vaccine strain is capable of proliferating intracellularly in murine macrophages (2). Bacteria were stored at −70°C.

Construction of plasmids. Manipulations of DNA were performed by using standard techniques (24). A 1.0-kb *Eco*RV-*Sph*I DNA fragment was prepared by the technique of Birnboim and Doly (6) from the plasmid pTUL4-9 (39), a derivative of pUC18. The fragment contains the gene encoding the T-cell-reactive 17-kDa protein TUL4 but contains no other expressed open reading frames (40). The isolated 1.0-kb fragment was ligated to the Asd$^+$ plasmid pYA248 (27) after the plasmid had been cleaved with *Eco*RI and *Sph*I and after the *Eco*RI site had been made blunt by using the Klenow fragment. The translation initiation codon of pYA248 was followed by a short sequence of 36 putatively encoding nucleotides of the *Francisella* DNA. The recombi-

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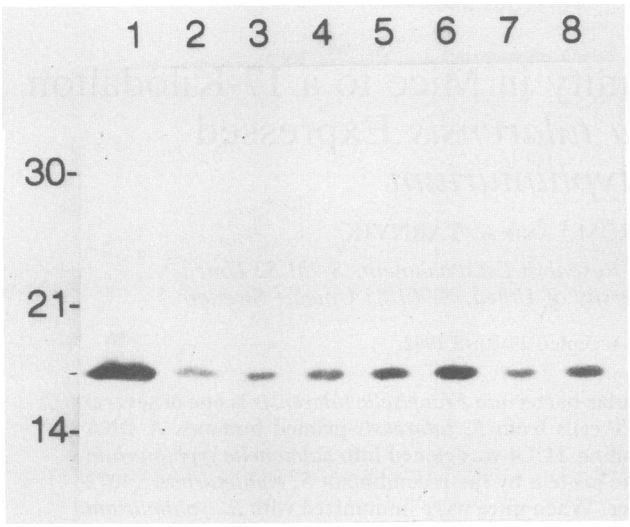

FIG. 1. Expression of TUL4 by *S. typhimurium* χ4072(pTUL4-15) isolated from spleens of infected BALB/cJ mice. Proteins of bacterial lysates were separated by SDS-PAGE and transferred to nitrocellulose for Western blot analysis. A monoclonal antibody raised against TUL4 of *F. tularensis* LVS was used as probe. Lanes: 1, *F. tularensis* LVS; 2 to 8, isolates of *S. typhimurium* χ4072 (pTUL4-15) from spleens of seven different mice. Molecular masses (in kilodaltons) indicated at left.

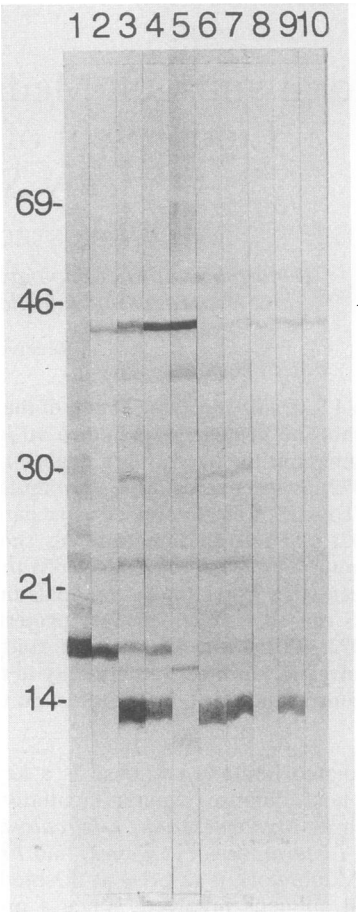

FIG. 2. Antibody response of individual BALB/cJ mice to TUL4. Membranes of *F. tularensis* LVS were separated by SDS-PAGE and transferred to nitrocellulose for Western blot analysis. Mice were bled 1 month after the completion of immunization with *S. typhimurium*. Sera were diluted 1/50 and analyzed. Lanes: 1, monoclonal antibody specific to TUL4; 2 to 4, sera from three mice immunized with *S. typhimurium* χ4072(pTUL4-15); 5 to 7, sera from three mice immunized with *S. typhimurium* χ4072; 8 to 10, sera from three nonimmunized mice. Molecular masses (in kilodaltons) indicated at left.

nant plasmid, denoted pTUL4-15, was transformed (16, 20) into *S. typhimurium* χ3730. By using a lysate of the χ3730 recombinant, transduction of phage P22 HT *int* into χ4072 was performed according to standard methods (12, 37). The presence of TUL4 in lysates from transduced strains was confirmed by Western blot analysis, and one of the strains, denoted *S. typhimurium* χ4072(pTUL4-15), was selected for the experiments.

Mice. Mice (Bomholtgård, Ry, Denmark) were housed under conventional conditions and given food and water ad libitum. For animal passage of bacterial strains and for virulence determination, 8- to 10-week-old BALB/cJ mice were used. In immunization experiments, BALB/cJ mice were 4 to 5 weeks old and SJL, C57BL/10, and A/J mice were 8 to 10 weeks old when first inoculated.

Animal passage of bacterial strains. To retain bacterial virulence, BALB/cJ mice were fed *S. typhimurium* χ4072 (pTUL4-15) orally or were intraperitoneally injected with *F. tularensis* LVS. Five days later, spleens were removed and homogenized in sterile saline for the isolation of bacteria. One isolate of each species was stored frozen for use in the experiments.

Virulence determination. *S. typhimurium* χ4072 or *S. typhimurium* χ4072(pTUL4-15) was intraperitoneally injected into BALB/cJ mice at a dose of 5×10^3 bacteria. At regular intervals thereafter, animals were killed by decapitation and the skin was folded back over the abdomen to expose the peritoneum. Spleens, livers, and Peyer's patches were aseptically removed and homogenized in sterile saline for the determination of viable counts.

Immunization. *S. typhimurium* χ4072, *S. typhimurium* χ4072(pTUL4-15), and *F. tularensis* LVS were used for immunization. *S. typhimurium* was grown overnight at 37°C in Luria-Bertani broth (5). After being diluted 1/50 in the broth, bacteria were grown at 37°C for approximately 4 h to an optical density at 540 nm of 1.0. Cultures were centrifuged, and bacteria were resuspended in phosphate-buffered saline (PBS), pH 7.2. Mice were immunized twice, 4 weeks apart, by the intraperitoneal injection of 100 μl of PBS containing 5×10^3 (5×10^2 for the more susceptible mouse strain C57BL/10) organisms of *S. typhimurium* χ4072 or *S. typhimurium* χ4072(pTUL4-15).

F. tularensis LVS was grown on modified Thayer-Martin agar (35), suspended in PBS, and administered at a dose of 140 to 230 bacteria. The immunization schedule was the same as was used for *S. typhimurium*, although *F. tularensis* was intravenously administered.

Determination of antibody titers. Sera were drawn 1 month after the completion of immunization. Mice were locally anesthetized prior to the puncture of the retroorbital vein. Serum was tested by an enzyme-linked immunosorbent assay (ELISA) for the presence of antibodies to each of 13 overlapping synthetic peptides (see Fig. 3), corresponding to the sequence of 149 amino acids of TUL4. Each well of a microplate was coated with 2 μg of peptide, and wells were thereafter blocked with PBS containing 0.05% (vol/vol)

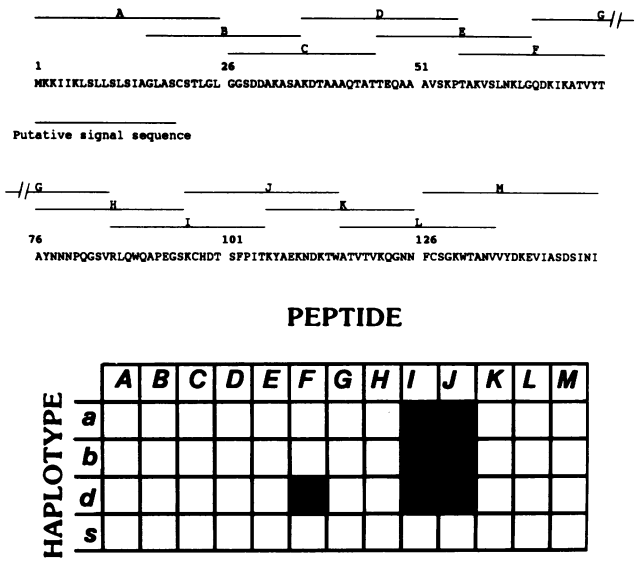

FIG. 3. Antibody response of immunized mice to overlapping synthetic peptides spanning the sequence of TUL4. Mice were immunized with *S. typhimurium* χ4072(pTUL4-15) and bled 1 month later. Sera from five mice of each haplotype were pooled, diluted 1/100, and analyzed by an ELISA. Filled box indicates that the mean value of a triplicate sample exceeded the mean + 3 SDs of wells lacking peptide. The amino acid sequence of TUL4 is indicated in single-letter code, and the positions of the synthetic peptides are also indicated.

Tween 20. Serum diluted 1/100 in PBS containing Tween 20 was added, and plates were incubated for 4 h at 37°C. After washing, alkaline phosphatase-conjugated secondary antibody (Dakopatts A/S, Copenhagen, Denmark) was added, and plates were incubated for 2 h. Finally, the substrate was added, and absorbance at 405 nm was recorded by using an automated ELISA reader (Titertek Multiskan; Flow Laboratories). Values were considered positive when the mean value of a triplicate sample exceeded the mean plus 3 standard deviations (SDs) of wells lacking peptide.

Assay of proliferative response of spleen cells. One month after the last injection, spleens were removed from immunized mice. Cells were prepared by gently squeezing the organs, washing the cells twice, and resuspending them in RPMI 1640-HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 15% inactivated fetal calf serum, 1% (wt/vol) gentamicin, 5×10^{-5} M β-mercaptoethanol, and 2 mM L-glutamine. Each culture (200 μl) contained 6×10^5 spleen cells. As a stimulating agent, TUL4 (1 μg/ml), heat-killed *F. tularensis* LVS (10^5 bacteria per ml), or heat-killed *S. typhimurium* (10^5 bacteria per ml) was used. In preliminary experiments, these doses of antigen were found to be optimal. To estimate the proliferative response, cultures were incubated at 37°C for 4 to 5 days, pulsed for 6 h with 1.0 μCi of [^{3}H]thymidine (18 Ci/mmol), and harvested with an automated cell harvester (Inotech, Basel, Switzerland).

Challenge of immunized mice with *F. tularensis* LVS. Three months after the completion of immunization, mice were intravenously challenged with *F. tularensis* LVS at a dose of 160 to 230 bacteria. At various intervals thereafter, mice were sacrificed for the enumeration of bacteria in the liver and spleen. The CFU per organ were recorded.

The lethal dose for 50% of the mice (LD_{50}) was calculated for the intravenous route by administration in one series of 80, 800, 8,000, and 80,000 bacteria and in another series of 180, 1,800, and 18,000 bacteria. Each dose of bacteria was given to 5 to 10 animals.

Administration of cyclosporin A. Cyclosporin A (Sandimmun; Sandoz AG, Basel, Switzerland) was solubilized in olive oil at a concentration of 20 mg/ml, and a daily dose of 50 mg/kg of body weight was subcutaneously injected into *S. typhimurium*-immunized and nonimmunized BALB/cJ mice, starting 2 days before challenge and continuing to the end of the experiment. Control animals received olive oil.

SDS-PAGE and Western blot analysis. *F. tularensis* or *S. typhimurium* cells were harvested at mid-exponential phase, pelleted by centrifugation, and washed twice. Bacterial membranes were prepared by sonication, ultracentrifugation, and Sarkosyl treatment according to a procedure previously used for *F. tularensis* (34). The membranes were resuspended in sample buffer (62.5 mM Tris [pH 6.8], 1% sodium dodecyl sulfate [SDS], 20% β-mercaptoethanol, 10% glycerol) and heated at 100°C for 5 min, whereafter proteins (10 to 15 μg per sample) were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (19) on gels containing a linear gradient of acrylamide (10 to 17.5%). In some experiments, washed bacteria were lysed in sample buffer and separated by SDS-PAGE. Western blot analysis was performed as described by Swanson et al. (44). A murine monoclonal antibody (39) raised against TUL4 or sera from *Salmonella*-immunized mice (drawn 1 month after the completion of immunization) were used as primary antibodies at a dilution of 1/50. Secondary antibodies were alkaline phosphatase conjugated.

Statistical analysis. For statistical analysis, Student's *t* test, Fisher's exact test, or analysis of variance was used.

RESULTS

Expression of TUL4 by the recombinant strain of *S. typhimurium*. A Western blot analysis of a whole cell lysate and of a membrane preparation showed that the recombinant strain χ4072(pTUL4-15) expressed TUL4 (data not shown). The expression was, however, not strong enough to allow detection in Coomassie-stained gels. Seven to 30 days after intraperitoneal injection of 5×10^3 cells of *S. typhimurium* χ4072(pTUL4-15) in BALB/cJ mice, serial dilutions of homogenates from liver, spleen, and feces were plated. TUL4 was present in all of 40 isolates (Fig. 1). Thus, as expected from the nature of the Δ*asd* host system (10), all bacterial clones had retained the plasmid. The plasmid still expressed the *F. tularensis* antigen.

Retained virulence of the recombinant strain of *S. typhimurium*. *S. typhimurium* χ4072 and χ4072(pTUL4-15) showed very similar growth rates in vitro (data not shown). After BALB/cJ mice were orally inoculated, the recombinant strain χ4072(pTUL4-15) was isolated from the liver, spleen, and Peyer's patches. After intraperitoneal administration, the two strains showed similar multiplication rates in the liver and spleen (data not shown). Four and five weeks after the intraperitoneal administration of 5×10^3 cells of either of the strains, bacteria ($<10^2$) were still present in the organs of some of the animals. After 6 weeks, no bacteria were isolated.

Antibody and lymphocyte responses of immunized mice. Sera were obtained from BALB/cJ mice 1 month after the completion of intraperitoneal immunization. Sera from some

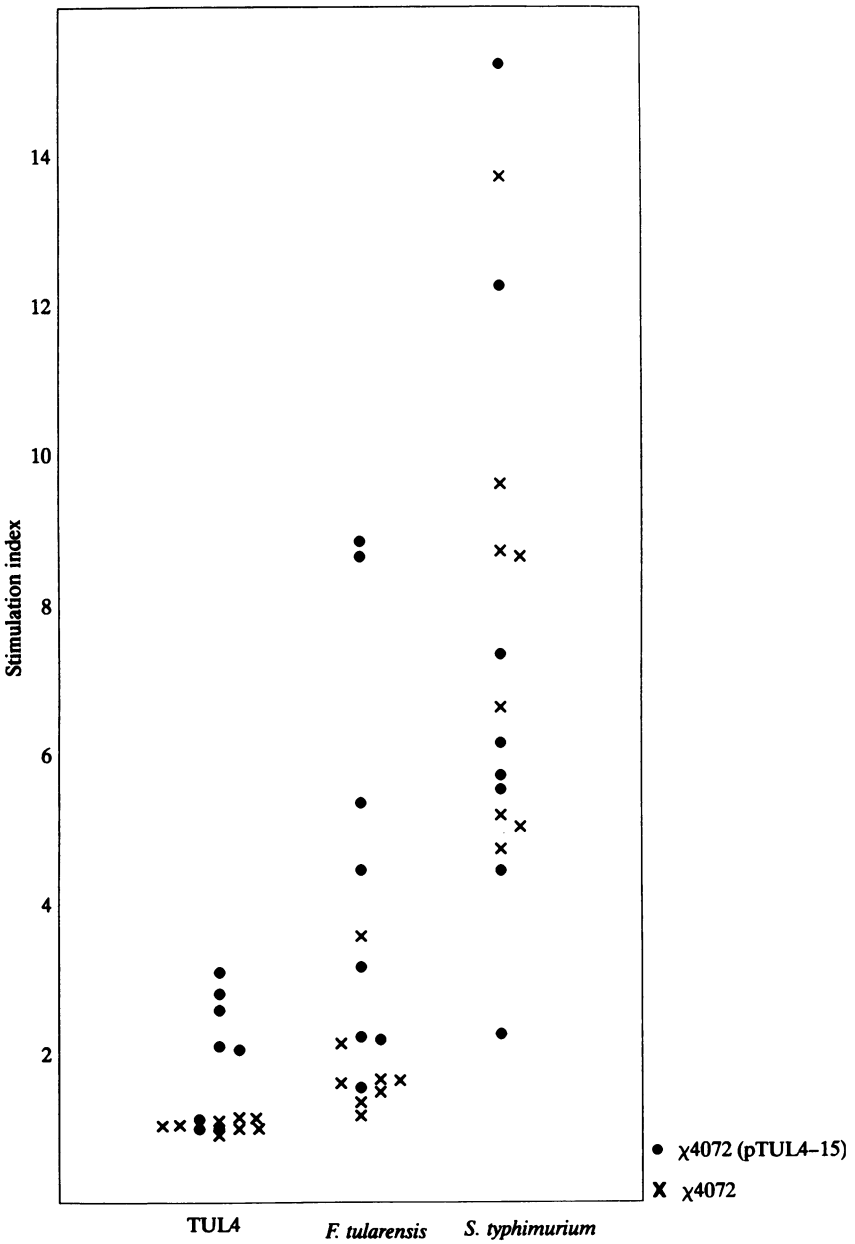

FIG. 4. Responses of spleen cells to TUL4. Spleen cells from eight mice previously immunized with *S. typhimurium* χ4072 and from eight mice immunized with *S. typhimurium* χ4072(pTUL4-15) were assayed for reactivity to TUL4 (1 μg/ml), heat-killed *F. tularensis* (10^5 bacteria per ml), or heat-killed *S. typhimurium* (10^5 bacteria per ml). Stimulatory index = mean cpm of five cultures containing antigen/mean cpm of 5 cultures lacking antigen. Mean counts per minute of cultures lacking antigen ranged from 4,400 to 11,100. SDs were less than 22% of the means.

mice (21 of 59 serum samples from separate mice) immunized with *S. typhimurium* χ4072(pTUL4-15) showed reactivity to an antigen with the same electrophoretic mobility as TUL4 (Fig. 2). Sera of mice immunized with *S. typhimurium* χ4072 and sera of nonimmunized mice showed no reactivity to this antigen. Irrespective of immunization, however, sera from BALB/cJ mice showed reactivity to one or two other antigens of *F. tularensis* LVS (Fig. 2). By ELISA, antibody responses of pooled sera from five to six animals of each haplotype were tested towards overlapping synthetic peptides spanning the entire sequence of TUL4. BALB/cJ ($H\text{-}2^d$) mice recognized three peptides, two of which were the only peptides recognized by A/J ($H\text{-}2^a$) and C57BL/10 ($H\text{-}2^b$) mice (Fig. 3). In sera from SJL mice ($H\text{-}2^s$), no specific antibodies were found, irrespective of which peptide was used as the antigen. These results indicated that the TUL4-expressing recombinant induced an antibody response to TUL4 in mice of several haplotypes.

Lymphocyte responses to TUL4 of BALB/cJ mice were determined 1 month after the completion of immunization. Significant proliferative responses ($P < 0.05$) were demonstrated for five of eight mice immunized with *S. typhimurium*

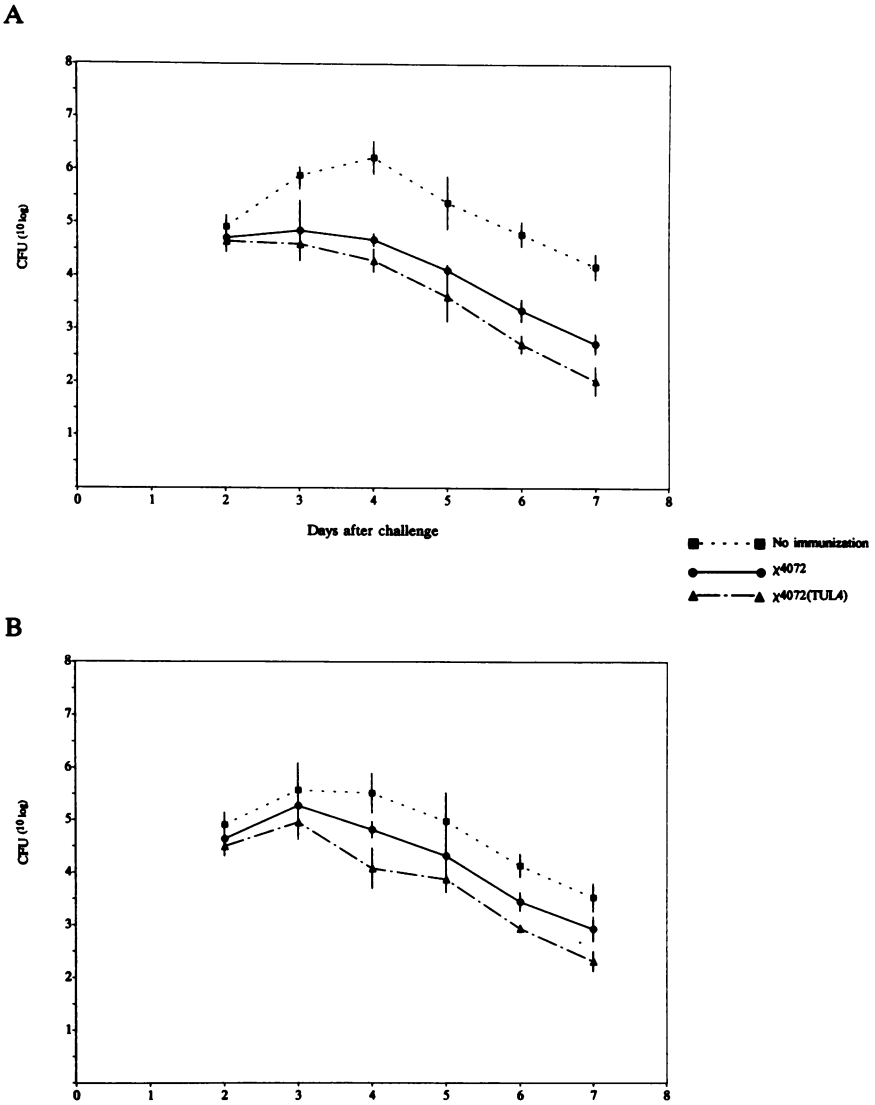

FIG. 5. Kinetics of bacterial growth of *F. tularensis* LVS in BALB/cJ mice immunized with *S. typhimurium* χ4072 or *S. typhimurium* χ4072(pTUL4-15) and in nonimmunized mice. Three months after the completion of immunization, animals were challenged with 180 bacteria of *F. tularensis* LVS. Beginning on day 2, mice were sacrificed and the numbers of CFU from livers (A) and spleens (B) were determined. The $\log_{10}$ number of CFU (mean ± SD) of five mice is indicated.

χ4072(pTUL4-15) (Fig. 4). Five *S. typhimurium* χ4072 (pTUL4-15)-immunized mice (the same five as above) also responded ($P < 0.01$) to heat-killed *F. tularensis* LVS (Fig. 4). Mice immunized with *S. typhimurium* χ4072 showed no significant proliferative responses (Fig. 4) to TUL4, whereas two of eight mice showed significant responses ($P < 0.05$) to *F. tularensis* LVS. When heat-killed *S. typhimurium* was used, similar degrees of responses were seen in χ4072(pTUL4-15)- and χ4072-immunized animals (Fig. 4).

Protection of immunized BALB/cJ mice to challenge with *F. tularensis* LVS. The $\log_{10}$ LD_{50} values after intravenous challenge of *F. tularensis* LVS in mice immunized with *S. typhimurium* χ4072(pTUL4-15) or *S. typhimurium* χ4072 were similar: 3.50 versus 3.38 in one experiment and 3.76 versus 3.57 ($P = 0.22$) in another experiment. Unexpectedly, the $\log_{10}$ LD_{50} values not only of the χ4072(pTUL4-15)-immunized mice but also of the χ4072-immunized mice were significantly higher ($P < 0.005$) than those of nonimmunized mice (2.90 and 2.84 in the two experiments). A high degree of protection was induced in mice immunized with *F. tularensis* LVS ($\log_{10}$ LD_{50} values of 5.47 and 5.19).

According to classical work on intracellular bacteria (22, 23), the enumeration of bacterial numbers in infected organs is a more suitable method than LD_{50} determination for the assessment of protection. By this approach, a significant protective effect against *F. tularensis* LVS could be ascribed to the expression of recombinant TUL4. Hence, viable counts in the liver, as well as in the spleen, were lower in animals immunized with *S. typhimurium* χ4072(pTUL4-15) than in those immunized with *S. typhimurium* χ4072 ($P < 0.01$) (Fig. 5). The protection was, however, more pronounced when the mice were immunized with *F. tularensis* LVS ($P < 0.001$) (Fig. 6). Also, when this method was used,

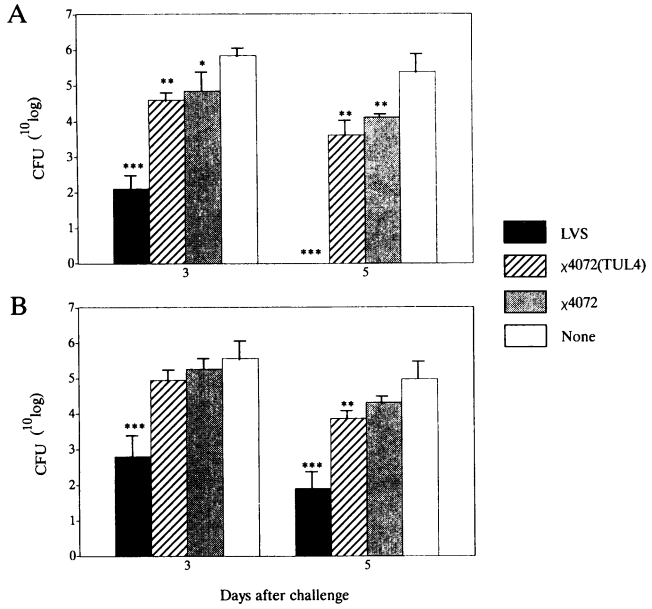

FIG. 6. Challenge with *F. tularensis* LVS of BALB/cJ mice immunized with *F. tularensis* LVS, *S. typhimurium* χ4072(pTUL4-15), or *S. typhimurium* χ4072 and of nonimmunized mice. Three months after the completion of immunization, animals were challenged with 180 bacteria of *F. tularensis* LVS. Mice were sacrificed at the times indicated, and the numbers of CFU from livers (A) and spleens (B) were determined. The $\log_{10}$ number of CFU (mean ± SD) of five mice is indicated. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$ (compared with nonimmunized mice). Student's *t* test was used.

a significant degree of protection against *F. tularensis* LVS was recorded for BALB/cJ mice that were immunized with *S. typhimurium* χ4072 ($P < 0.001$) (Fig. 5).

Effect of cyclosporin A on resistance to *F. tularensis* of immunized mice. Nonimmunized BALB/cJ mice or mice immunized 3 months previously with *S. typhimurium* χ4072(pTUL4-15) or *S. typhimurium* χ4072 were treated with cyclosporin A. The treatment rendered mice of all three groups highly and equally susceptible to challenge with *F. tularensis* LVS. Data on animals immunized with *S. typhimurium* χ4072(pTUL4-15) is shown in Fig. 7 ($P < 0.001$).

DISCUSSION

The relevance of the T-cell-reactive 17-kDa lipoprotein for protection against *F. tularensis* LVS has been assessed here. Immunization of mice with an attenuated strain of *S. typhimurium* that expresses the lipoprotein conferred host resistance to *F. tularensis* LVS, as determined by bacterial growth in the liver and spleen. A lymphocyte response to TUL4 in recombinant-immunized mice was also demonstrated. Although the mechanism behind the protective effect of TUL4 has not been fully clarified, the inhibitory effect of cyclosporin A on protection indicated that T cells were involved. Cyclosporin A is a potent inhibitor of T-cell activation (36) and has been found to increase the susceptibility of mice to *F. tularensis* LVS (3). It should be noted that cyclosporin A abolished the resistance to *F. tularensis*, even though the animals had acquired antibodies to TUL4 by being immunized with the recombinant. Thus, TUL4 antibodies alone did not seem to afford any protection against *F. tularensis* LVS. This is in accordance with previous data indicating that a humoral antibody response of mice to *F. tularensis* is of little value to host resistance (3). It has recently been demonstrated, however, that antibodies may protect against intraperitoneally administered *F. tularensis* LVS (13).

Attenuated *Salmonella* mutants have been used to induce protective immunity in animals against virulent strains of the species (14, 33). Moreover, the mutants have been used for immunization with heterologous antigens of various microorganisms, such as *Plasmodium berghei*, *M. leprae*, *Chlamydia trachomatis*, *E. coli*, *Vibrio cholerae*, *Streptococcus pyogenes*, *Brucella abortus*, and *Leishmania major* (1, 8, 17, 25, 30, 32, 42, 45). Generally, a humoral response to the cloned antigen has been demonstrated, whereas cell-mediated responses have been studied only rarely (1, 42, 45). A $CD4^+$ T-cell response and host protection to the intracellular parasite *L. major* were elicited by oral administration of an attenuated *aroA* mutant of *S. typhimurium* expressing a membrane glycoprotein of the parasite (45). That study and the present one show that *Salmonella* strains expressing heterologous antigens are useful models for studies of mechanisms behind a protective cell-mediated immune response of mammals against intracellular parasites.

An unexpected finding was the protection conferred by *S. typhimurium* χ4072 to challenge with *F. tularensis* LVS. It is well known that nonspecific, T-cell-independent mechanisms may contribute to host protection for some time after immunization (28, 29). Resistance did, however, last for as long as 3 months and was apparently T-cell dependent. A more probable explanation for the protection afforded by the nonrecombinant strain is the presence of cross-reactive T-cell epitopes in the two species. Individuals without a history of tularemia or tularemia vaccination show virtually no T-cell response at all to various membrane proteins of *F. tularensis* (38). However, a remarkably strong T-cell response to a membrane preparation of *E. coli* has been recorded for some individuals immunized with *F. tularensis* LVS (39). This may indicate that members of the family *Enterobacteriaceae* contain T-cell-reactive antigens in common with *F. tularensis*. The presence of conserved antigens among various gram-negative bacteria has been reported (18, 26, 46). In contrast, no protection was observed in similar studies when mice immunized with nonrecombinant salmonellae were challenged with plasmodia or leishmaniae (1, 45). In spite of the protection conferred by *S. typhimurium* χ4072 against *F. tularensis* LVS, the role of TUL4 in host protection against *F. tularensis* could still be evaluated when *S. typhimurium* χ4072 was used as a vector.

Adoptive transfer of a specific T-cell clone has been found to confer protection against tuberculosis only when quite a large number of T cells was used (21). Such numbers may not be obtained in vivo after immunization with one or a few T-cell-reactive proteins. In natural infection or vaccination with intracellular parasites, a large number of T cells may, however, be activated by the involvement of a multitude of antigens and contribute to protection (31). This would explain why a recombinant vector, such as *S. typhimurium* χ4072(pTUL4-15), with only a few relevant T-cell specificities, affords a much lower degree of host resistance to *F. tularensis* than does immunization with *F. tularensis* LVS.

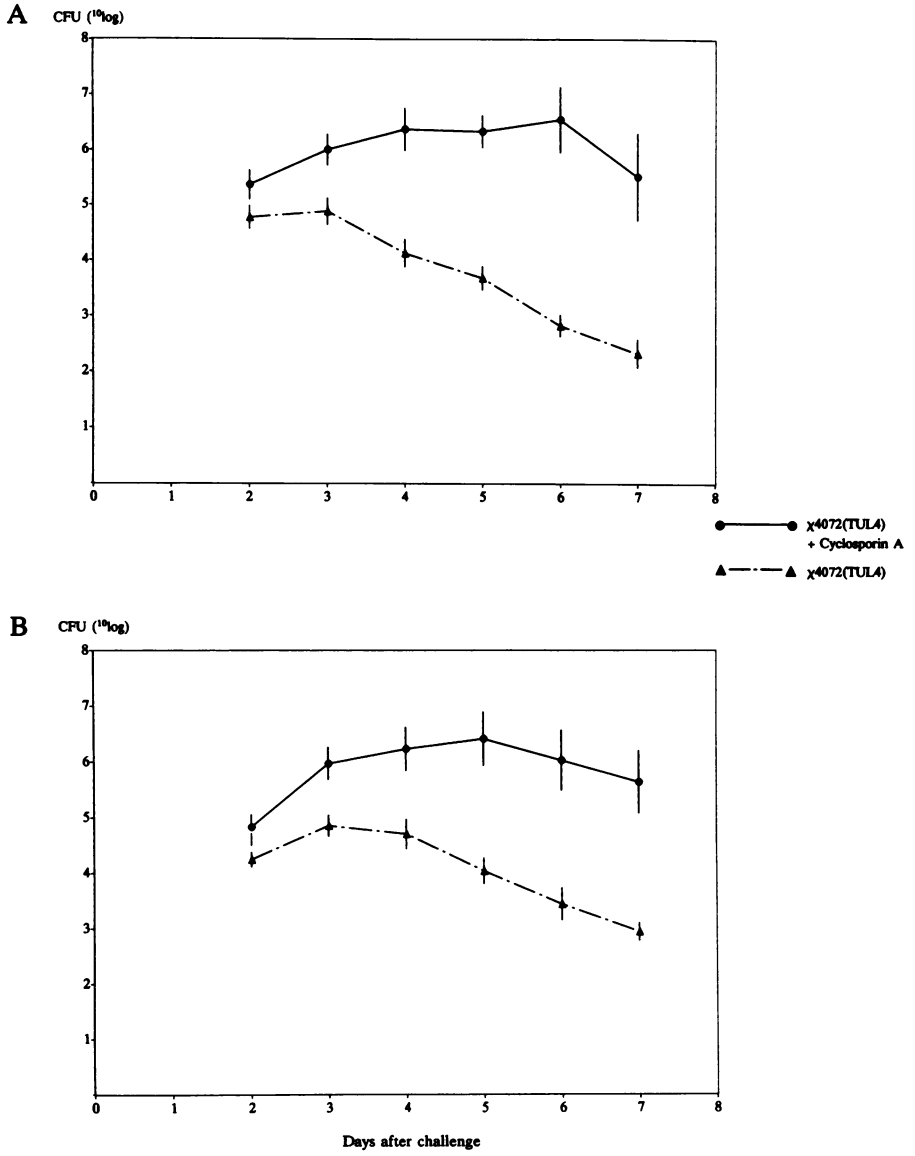

FIG. 7. Kinetics of bacterial growth of *F. tularensis* LVS in *S. typhimurium* χ4072(pTUL4-15)-immunized BALB/cJ mice, treated during challenge with cyclosporin A or olive oil. The animals were challenged with 230 bacteria of *F. tularensis* LVS 3 months after the completion of immunization. Beginning on day 2, mice were sacrificed and the numbers of CFU from livers (A) and spleens (B) were determined. The $\log_{10}$ number of CFU (mean ± SD) of five mice is indicated.

ACKNOWLEDGMENTS

We thank Thorsten Johansson and Kerstin Kuoppa for excellent technical assistance and Jan Carlsson for critical reading of the manuscript.

This work was supported by grants from the Swedish Medical Research Council (project no. 9485) and from the Medical Faculty, University of Umeå, Umeå, Sweden. It was also supported by grants from Magnus Bergvall Foundation; the Kempe Foundation; the Swedish Society for Medical Research Karolinska Institutet, Stockholm, Sweden; and the Swedish Society of Medicine.

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