INFECTION AND IMMUNITY, Sept. 1991, p. 3163–3168
0019-9567/91/093163-06$02.00/0

Vol. 59, No. 9

The T-Cell-Stimulating 17-Kilodalton Protein of *Francisella tularensis* LVS Is a Lipoprotein

A. SJÖSTEDT,[1]* A. TÄRNVIK,[2] AND G. SANDSTRÖM[1]

Department of Microbiology, National Defence Research Establishment, S-901 82 Umeå,[1] and Department of Infectious Diseases, University of Umeå, S-901 85 Umeå,[2] Sweden

Received 21 February 1991/Accepted 30 May 1991

A T-cell-stimulating, membrane-located 17-kDa protein of the live vaccine strain *Francisella tularensis* LVS has previously been cloned and sequenced. In the present study, it is shown to be a lipoprotein. When *F. tularensis* was grown in the presence of [^{3}H]palmitate, several proteins of the organism, including a 17-kDa protein, were radiolabeled. The labeled 17-kDa protein was found by Western blot (immunoblot) analysis to be identical to the cloned protein. It was located in the detergent phase after partitioning with the nonionic detergent Triton X-114, thereby behaving like a hydrophobic integral membrane protein. The protein was predominantly hydrophilic and contained no putative transmembrane domain. The presence of fatty acids is therefore the probable explanation of the membrane location of the 17-kDa protein. The amino acid sequence of the 17-kDa protein contains the tetrapeptide Leu-Ala-Ser-Cys, which is a recognition sequence of the lipoprotein signal peptidase. Globomycin, a specific inhibitor of the peptidase, inhibited maturation of the 17-kDa lipoprotein. The protein incorporated [^{3}H]palmitate also when expressed by *Escherichia coli*. The 17-kDa lipoprotein was recognized not only by T cells but also by serum antibodies of *F. tularensis*-primed individuals.

Tularemia is caused by the highly virulent, facultative intracellular coccobacillus *Francisella tularensis*. The organism is endowed with a capsule which confers resistance to the lytic effect of serum (19). Moreover, virulent bacteria resist the antibacterial effects of professional phagocytes (15). The bacterial invasion of mononuclear phagocytes results in a strong T-cell response by the host, a response which eventually evades the infection and will protect effectively against reinfection. Protection against tularemia in humans can also be afforded by immunization with the live vaccine strain *F. tularensis* LVS. This strain is less resistant to the bacteriocidal effect of phagocytes (15) and is not allowed to proliferate into numbers sufficient to cause disease before a cell-mediated response can be evoked in the host.

Virtually all data available regarding the structure of the cell wall of this potent pathogen originate from studies on T-cell-stimulating membrane proteins (20, 22, 27). Several membrane proteins of *F. tularensis* LVS induce a response in T cells from vaccinated or naturally infected individuals (22, 27). One major membrane protein is a 17-kDa protein, which has been cloned and mapped for T-cell epitopes (24, 25). The protein, denoted TUL4, contains as amino acids 17 to 20 the sequence Leu-Ala-Ser-Cys. This sequence is homologous to the consensus tetrapeptide Leu-X-Y-Cys of bacterial lipoproteins, where X and Y denote small, neutral amino acids. The tetrapeptide is a recognition sequence for the addition of diacylglycerol and fatty acids to the cysteine residue and for cleavage of the prolipoprotein (31). The present study showed that the 17-kDa protein of *F. tularensis* LVS fulfills criteria for being a lipoprotein. This may be of special interest because lipid modification has been suggested to endow proteins with immunogenic properties (5, 14). The results also imply that mechanisms for processing of lipoproteins found in members of the family *Enterobacteriaceae* are present in *F. tularensis*.

* Corresponding author.

MATERIALS AND METHODS

Chemicals. Triton X-114 was from Boehringer, Mannheim, Ingelheim, West Germany, and 9.10(*n*)-[^{3}H]palmitate (hexadecanoic) was from Amersham International, Amersham, England. Globomycin was a kind gift from M. Arai of Sankyo Co. Ltd., Tokyo, Japan. Other chemicals were purchased from Boehringer Mannheim and from Pharmacia AB, Uppsala, Sweden.

Plasmid and bacterial strains. *Escherichia coli* JM105 (16) and plasmid pTUL4-9 (24) have been previously described. pTUL4-9 is a recombinant of pUC18 that contains a 1.2-kb fragment encoding the 17-kDa protein TUL4 of *F. tularensis* LVS. *F. tularensis* LVS was supplied by the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md. *E. coli* was cultivated in Luria-Bertani broth (1) supplemented with 100 μg of ampicillin per ml, and *F. tularensis* LVS was cultivated on modified Thayer-Martin agar containing Gc-medium base (21) or, alternatively, in Proteose Peptone broth (28).

SDS-PAGE, Western blot analysis, and autoradiography. Bacteria or bacterial preparations were suspended in sample buffer (62.5 mM Tris [pH 6.8], 1% sodium dodecyl sulfate [SDS], 20% β-mercaptoethanol, 10% glycerol). The samples were boiled for 5 min, and proteins (10 to 15 μg per sample) were separated by SDS-polyacrylamide gel electrophoresis (PAGE) (13) on gels containing a linear gradient of acrylamide (10 to 17.5%). When [^{3}H]palmitate-labeled bacterial preparations were used, a sample containing approximately 10^6 cpm was loaded on each lane. For Western blot (immunoblot) analysis, proteins were transferred to Immobilon sheets (Millipore Corp., Bedford, Mass.) and probed as previously described (29). An alkaline phosphatase-conjugated secondary-antibody system was used. Molecular masses of identified proteins were determined by the inclusion of standard proteins (electrophoresis calibration kit, Pharmacia AB) in the gel. After Western blot analysis, autoradiography was performed by use of X-ray film (Hyperfilm-[^{3}H]; Amersham) at −70°C.

Labeling of bacterial proteins with [^{3}H]palmitate. Over-

night cultures of *F. tularensis* LVS in Proteose Peptone broth were diluted 1/10 and reincubated. When the optical density at 540 nm of the cultures reached 0.3, 250 μCi of [^{3}H] palmitic acid dissolved in ethanol was added per ml of medium (final ethanol concentration <1.0%). Incubation was continued until the optical density at 540 nm reached 0.8, after which the bacteria were pelleted by centrifugation at 13,000 × *g* for 10 min. After being washed twice in methanol-chloroform (2/1) and once in phosphate-buffered saline (PBS), pH 7.2, bacteria were suspended in sample buffer for separation of proteins by SDS-PAGE. Western blot analysis with a previously described (24) murine monoclonal antibody specific to TUL4 of *F. tularensis* LVS and autoradiography were thereafter performed. *E. coli* JM105 (pTUL4-9) was labeled and analyzed according to the same protocol.

Phase partitioning of *F. tularensis* proteins with Triton X-114. *F. tularensis* LVS (10^{10} cells) labeled with [^{3}H]palmitate was suspended in 1.0 ml of ice-cold 2% (vol/vol) Triton X-114 in PBS. After overnight incubation at 4°C with gentle agitation, the insoluble material was pelleted by centrifugation at 20,000 × *g* for 30 min at 4°C. The soluble material was partitioned into aqueous and detergent phases as described by Radolf et al. (17), using Triton X-114 at a concentration of 2%. The insoluble material and the materials of the two soluble phases were precipitated with acetone (20), and then each material was suspended in sample buffer. After SDS-PAGE, the materials were blotted to Immobilon sheets and analyzed either by Western blot analysis with the monoclonal antibody to TUL4 or by india ink staining. Autoradiography was also performed.

Hydrolysis of [^{3}H]palmitate-labeled TUL4. After SDS-PAGE of a lysate of [^{3}H]palmitate-labeled *F. tularensis* LVS, polypeptides were visualized by treating the gel with 0.25 M potassium chloride (20), and a gel piece containing TUL4 was excised by use of a scalpel. To determine background radioactivity, a separate gel piece containing an apparently nonlabeled 80-kDa protein was also excised. After extensive washing with 50% methanol until the washing solution contained no radioactivity, the two gel pieces were subjected to sequential alkaline and acid hydrolysis for extraction of fatty acids. First, each gel piece was incubated in 0.5 ml of 1.5 M NaOH under N_2 at 30°C for 3 h. Fatty acids were extracted by organic solvents as described by Bligh and Dyer (2). Thereafter, the gel pieces were acidified by the addition of 0.5 ml of 6 M HCl. Hydrolysis was carried out at 100°C for 4 h, and the extraction of fatty acids was repeated. The dissolved materials were dried under nitrogen and assayed by liquid scintillation counting. The radioactivity (disintegrations per minute) of the 80-kDa protein was less than 10% of the activity of TUL4.

Globomycin treatment of *F. tularensis* LVS. To an exponentially growing culture (optical density at 540 nm of 0.3) of *F. tularensis* LVS, globomycin dissolved in methanol was added to a final concentration of 160 μg/ml (final methanol concentration, <1.0%). Thirty minutes later, [^{3}H]palmitate was added. After 4 h of incubation, bacteria were chilled, washed in PBS, and suspended in sample buffer. Control cultures contained [^{3}H]palmitate but no globomycin. The presence of TUL4 in lysates of these bacteria was determined by Western blotting using the monoclonal antibody to TUL4. Thereafter, the blotted preparations were analyzed by autoradiography.

Assay of antibody response to TUL4. Bacteria were harvested at mid-logarithmic phase, pelleted by centrifugation, washed twice in PBS, suspended in sample buffer, and separated by SDS-PAGE. In some experiments, washed bacteria were resuspended in PBS containing 10 mg of proteinase K (Sigma, Poole, England) per ml and incubated overnight, after which an equal volume of phenol was added. The mixture was vigorously mixed, incubated at 68°C, and centrifuged at 30,000 × *g* for 30 min. Pelleted material was suspended in sample buffer at a concentration of 10 μg/ml and separated by SDS-PAGE. Serum samples from five healthy adults (aged 28 to 42 years) vaccinated with *F. tularensis* LVS, from five individuals (aged 7 to 46 years) who had suffered from tularemia, and from four healthy volunteers (aged 18 to 20 years) denying any exposure to *F. tularensis* were diluted 1/500 and analyzed by Western blotting.

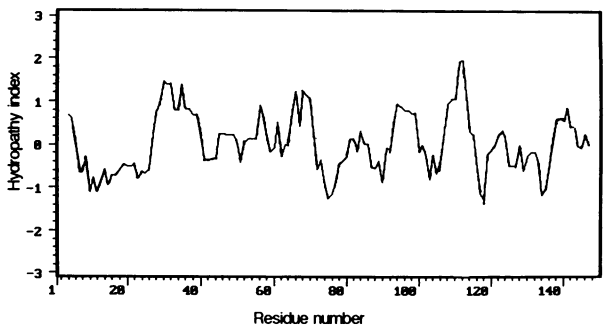

FIG. 1. Hydropathy analysis of the amino acid sequence, including the signal peptide, of TUL4 by the algorithm of Kyte and Doolittle (12). Positive values denote regions of relative hydrophilicity. The average window size was 6. The amino acid residues are numbered on the *x* axis.

Assay of lymphocyte response to TUL4. Blood samples were obtained from seven healthy adults (aged 25 to 42 years) vaccinated with *F. tularensis* LVS and from nine healthy adults (aged 27 to 58 years) who had suffered from tularemia. Samples were also obtained from 11 healthy adults (aged 18 to 45 years) who denied previous tularemia or tularemia vaccination. Peripheral blood mononuclear cells were prepared from heparinized blood by centrifugation on a Ficoll-metrizoate gradient (Lymphoprep; NYCOMED AS, Oslo, Norway), and cultures were established as described previously (24). As stimulating agent, a preparation of TUL4 (22) was added to a final concentration of 10 μg/ml. Cultures were incubated at 37°C for 6 days, pulsed for 6 h with 0.5 μCi of [^{3}H]thymidine, and harvested. Results were recorded as stimulatory indices (index = mean cpm of wells containing antigen/mean cpm of wells lacking antigen, where cpm is counts per minute). When control cultures of cells from primed individuals were stimulated with heat-killed *F. tularensis* LVS (10^6 bacteria per ml), indices were invariably >3.0.

RESULTS AND DISCUSSION

Analysis of amino acid sequence of TUL4. Hydropathy analysis (12) of the deduced amino acid sequence of TUL4 and the presumed signal peptide (amino acids 1 to 19) was performed (Fig. 1). The most probable transmembrane domain was the signal sequence. The remaining sequence appeared to be predominantly hydrophilic and showed no putative transmembrane domain.

It has previously been reported (25) that the amino acid sequence of TUL4 shows a significant similarity to a 21-kDa peptidoglycan-associated protein of *E. coli*, which is also a

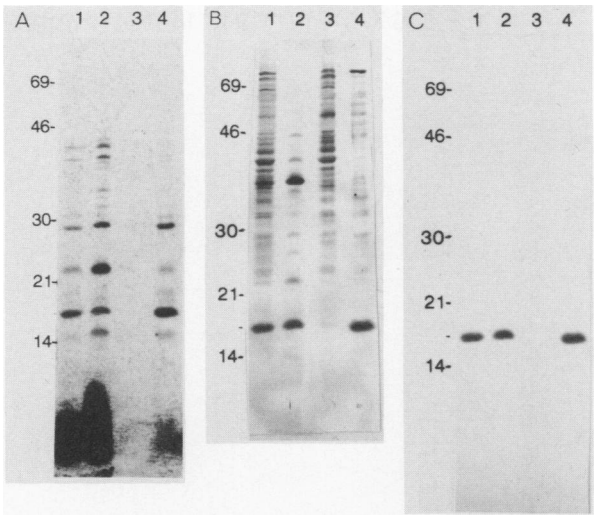

FIG. 2. [^{3}H]palmitate labeling of proteins of *F. tularensis* LVS and phase partitioning with Triton X-114. Triton X-114-insoluble material as well as the aqueous and detergent phases of the soluble material were separated by SDS-PAGE, blotted to Immobilon, and analyzed by autoradiography (A), india ink staining (B), and Western blot analysis using a monoclonal antibody to TUL4 (C). Lanes: 1, bacterial lysate; 2, Triton X-114-insoluble fraction; 3, aqueous phase; 4, detergent phase. Numbers on the left indicate molecular masses in kilodaltons.

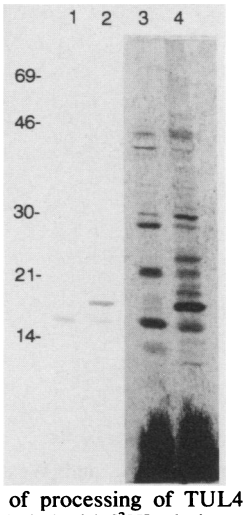

FIG. 3. Inhibition of processing of TUL4 by globomycin. *F. tularensis* LVS was labeled with [^{3}H]palmitate in the presence (lane 2 and 4) or absence (lane 1 and 3) of globomycin (160 μg/ml). Bacterial lysates were separated by SDS-PAGE and analyzed by Western blotting using a monoclonal antibody to TUL4 (lane 1 and 2) and autoradiography (lane 3 and 4). Numbers on the left indicate molecular masses in kilodaltons.

lipoprotein (7). A search in the present study of the updated versions of the National Biomedical Research Foundation protein data base (version 21.0) and the Swiss protein data base (version 15) failed to identify any significant homology to any other sequence. The 21-kDa protein is a major constituent of the bacterial cell wall, and attempts to obtain mutants lacking the protein have been unsuccessful (7). TUL4 may also be peptidoglycan-associated. The gene encoding TUL4 has been found to be conserved in various strains of *Francisella* spp. (23). This is to be expected for genes encoding essential cell wall constituents.

Identification of lipoproteins of *F. tularensis* LVS by radiolabeling. After labeling of *F. tularensis* LVS with [^{3}H]palmitate, bacterial lysates were separated by SDS-PAGE and analyzed by autoradiography. Six labeled proteins with apparent molecular masses of 43, 41, 31, 29, 23, and 17 kDa were identified (Fig. 2A, lane 1). The addition of [^{3}H]palmitate to the growth medium did not seem to affect the protein pattern of SDS-PAGE-separated lysates (data not shown).

Phase partitioning of proteins of *F. tularensis* LVS with Triton X-114. [^{3}H]palmitate-labeled *F. tularensis* LVS was treated with Triton X-114. Insoluble and soluble materials were separated, and the latter material was partitioned into aqueous and detergent phases. Hydrophobic proteins, such as integral membrane proteins, partition in the detergent phase (3). Of the radioactivity incorporated in *F. tularensis*, approximately 30% was recovered in the insoluble material. As shown by autoradiography of blotted SDS-PAGE gels, all six lipoproteins were present in this material (Fig. 2A, lane 2). When the soluble material was partitioned, 99% of the radioactivity was found in the detergent phase (Fig. 2A, lane 4). At least four lipoproteins (M_rs of 31, 29, 23, and 17) were identified in this phase, whereas none was identified in the aqueous phase (Fig. 2A, lane 3). According to india ink staining, both aqueous and detergent phases contained a considerable number of proteins (Fig. 2B).

The partitioning of TUL4 was determined by immunoblotting. TUL4 was identified in the insoluble material as well as in the detergent phase but not in the aqueous phase (Fig. 2C). The mobility of TUL4 was identical to that of the [^{3}H]palmitate-labeled 17-kDa protein.

Since the amino acid sequence of TUL4 is predominantly hydrophilic, lipid modification may obviously be important for its hydrophobicity. N-terminal fatty acids are known to confer hydrophobicity to lipoproteins and thereby allow proteins to be membrane integrated (31). Thus, the partitioning experiments afforded indirect evidence of membrane integration of TUL4 and other lipoproteins of *F. tularensis*. Many bacterial lipoproteins, including the well-characterized Braun's lipoprotein of *E. coli* (5, 9), are also membrane integrated.

Hydrolysis of fatty acids bound to 17-kDa protein. To investigate whether [^{3}H]palmitate was covalently bound to the 17-kDa protein, a lysate of labeled *F. tularensis* LVS was separated by SDS-PAGE and a gel piece containing the protein was subjected to subsequent alkaline and acid hydrolysis. These treatments are known to release fatty acids bound by ester and amide linkages, respectively. Alkali treatment of the 17-kDa protein released 10,960 dpm, whereas acid hydrolysis yielded 6,300 dpm. This gave a ratio of ester- to amide-bound fatty acids of TUL4 of 1.74, which is in reasonable agreement with the value of 2 reported for lipoproteins of the *Enterobacteriaceae* (31). Twenty percent of the radioactivity remained in the gel piece.

Effects of globomycin on maturation of TUL4. Residues 17 to 20 of TUL4 is the tetrapeptide Leu-Ala-Ser-Cys (25), which is a recognition sequence of signal peptidase II (31). After incubation of *F. tularensis* LVS in the presence of globomycin, a specific inhibitor of signal peptidase II (11), the incorporation of [^{3}H]palmitate in the 17-kDa protein was reduced (Fig. 3, lane 3 and 4). Instead, radioactivity appeared in a band of slightly higher molecular mass (Fig. 3, lane 4). Immunoblotting confirmed that the two labeled bands represented the mature and precursor forms of TUL4

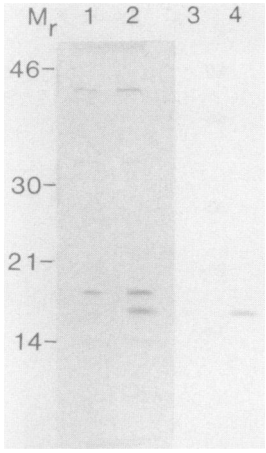

FIG. 4. [^{3}H]palmitate labeling and Western blot analysis of TUL4 expressed by *E. coli*. Lysates of [^{3}H]palmitate-labeled bacteria were subjected to SDS-PAGE for Western blot analysis using a monoclonal antibody to TUL4 and for autoradiography. Lanes: 1 and 3, lysate of *E. coli* JM105; 2 and 4, lysate of *E. coli* JM105(pTUL4-9); 1 and 2, autoradiography; lanes 3 and 4, Western blotting. Numbers on the left indicate molecular masses in kilodaltons.

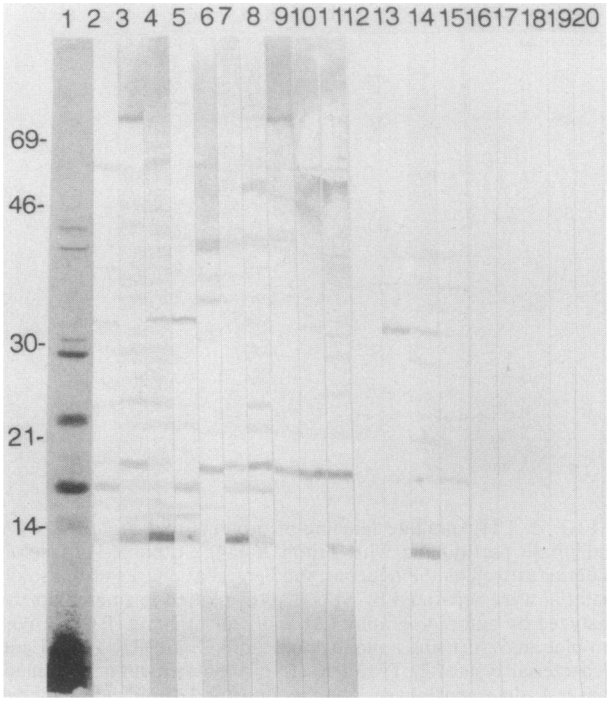

FIG. 5. Antibody responses of *F. tularensis*-primed individuals to proteins of *F. tularensis* LVS. Nontreated (lanes 1 to 15) and proteinase K-treated (lanes 16 to 20) lysates of *F. tularensis* LVS were separated by SDS-PAGE and transferred to Immobilon. Sera were diluted 1/500. Lanes: 1, [^{3}H]palmitate-labeled lysate of *F. tularensis* visualized by autoradiography; 2 to 6 and 16 to 20, sera from individuals vaccinated 5 to 18 months before sera were drawn; 7 to 11, sera from individuals who had had tularemia 8 to 24 months before sera were drawn; 12 to 15, sera from nonprimed individuals. Numbers on the left indicate molecular masses in kilodaltons.

(Fig. 3, lane 2). Thus, the lipoprotein nature of TUL4 was verified.

These data, in addition to the results of the hydrolysis experiments, strongly indicated that the palmitic acid of TUL4 was covalently bound, because signal peptidase II has an absolute requirement for the covalent addition of diacylglycerol prior to cleavage of the signal peptide (11, 30, 31). Covalent binding of the fatty acids was furthermore supported by the finding that TUL4 remained radiolabeled after being boiled in SDS. Thus, TUL4 seems to be a typical bacterial lipoprotein. The prolipoprotein is covalently linked at the cysteine residue to diacylglycerol, which contains two esterfied fatty acids. Subsequently, signal peptidase II cleaves the molecule, and the N-terminal cysteine is linked to another fatty acid by means of amide linkage. As in other bacterial lipoproteins (31), two fatty acids would thereby be covalently ester linked and one fatty acid would be amide linked to each molecule of TUL4.

Globomycin also seemed to inhibit the maturation of the lipoproteins with molecular masses of 29 and 23 kDa, as indicated by the appearance of proteins with approximate molecular masses of 31 and 25 kDa (Fig. 3, lane 4). The possible appearance of precursor forms of the 43-, 41-, and 31-kDa proteins could not be unambiguously determined.

The results imply that *F. tularensis* possesses mechanisms similar to those of the *Enterobacteriaceae* (31) for processing of lipoproteins. It should be remarked that globomycin, although used at a concentration as high as 160 μg/ml, did not completely inhibit the processing (Fig. 3, lane 2), indicating that these mechanisms of *F. tularensis* are relatively insensitive to globomycin.

Acylation of TUL4 by *E. coli*. A TUL4-expressing strain of *E. coli* JM105 was grown in the presence of [^{3}H]palmitate. Autoradiography of the lysate after SDS-PAGE disclosed one radioactively labeled band besides those present in JM105 (Fig. 4). Western blot analysis with a monoclonal antibody confirmed that this band represented TUL4 (Fig. 4). The result afforded strong evidence of the lipoprotein nature of TUL4 and confirmed a previous report indicating that processing of TUL4 in *E. coli* was inhibited by globomycin (25). Other labeled proteins corresponded in apparent molecular masses to previously described lipoproteins of *E. coli* (10).

Altogether, the evidence was convincing that TUL4 is a lipoprotein. Globomycin inhibited maturation of the protein, and it was, as expected for a lipoprotein, located in the detergent phase after Triton X-114 partitioning. Finally, TUL4 was found to incorporate [^{3}H]palmitate not only when expressed by *F. tularensis* but also when expressed by *E. coli*.

Immunogenicity of TUL4. Studies on *Treponema pallidum* and *Borrelia burgdorferi* have suggested that lipoproteins may be effective inducers of antibody responses in mammals (4, 6). To identify antibodies to lipoproteins of *F. tularensis* LVS, sera from primed individuals were analyzed by Western blotting (Fig. 5, lane 2 to 20), and bands were compared with those disclosed by autoradiography of lysates of [^{3}H] palmitate-labeled bacteria (Fig. 5, lane 1). Each serum exhibited a unique pattern of recognition. Serum from 4 of 10 primed individuals reacted with a protein whose mobility was identical to that of the radiolabeled 17-kDa protein. Apart from this band, Western blot analysis showed no distinct bands corresponding to the putative lipoproteins of *F. tularensis* LVS. The 19- and 13-kDa bands, on the other hand, were recognized by antibodies from several primed individuals. Sera from nonprimed individuals displayed some immunoreactivity (Fig. 5, lanes 12 to 15) but not with

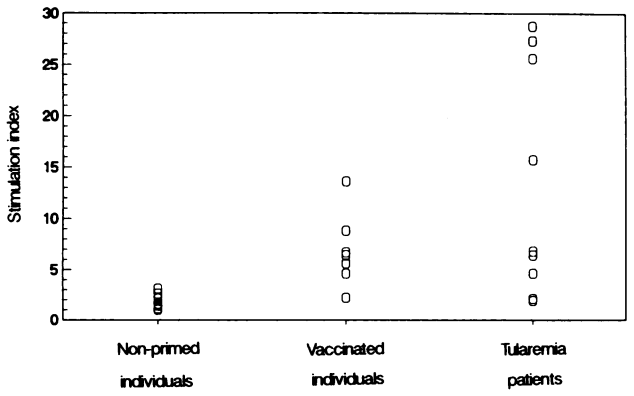

FIG. 6. Responses of peripheral blood mononuclear cells to TUL4. Cells from 7 individuals vaccinated 5 to 18 months before testing, 9 individuals who had suffered from tularemia 7 to 9 years before testing, and 11 nonprimed individuals were assayed for reactivity to TUL4 (10 μg/ml). The stimulatory index was calculated as mean cpm of five cultures containing TUL4/mean cpm of five cultures lacking antigen, where cpm is counts per minute. Mean counts per minute of cultures lacking antigen ranged from 900 to 4,700. Standard deviations were less than 17% of the means.

the 17-kDa protein. Proteinase K treatment of the bacterial lysates abolished all reactions with sera from vaccinated individuals, indicating that the antigens recognized were proteins (Fig. 5, lanes 16 to 20). According to these results, lipoproteins of *F. tularensis* LVS other than TUL4 were not convincingly recognized by sera from *F. tularensis*-primed individuals.

In vitro responses of lymphocytes from primed and nonprimed individuals to TUL4 were assayed (Fig. 6). Cells from nonprimed individuals showed little or no reactivity, the stimulatory indices being usually lower than 2.0. In contrast, most tularemia-vaccinated individuals and individuals having previously suffered from tularemia showed indices higher than 3.0. It should be noted that the patients had had tularemia 7 to 9 years before testing, indicating that the lymphocyte response to TUL4 may be long-lasting. These results confirm previous reports of a T-cell response to TUL4 in *F. tularensis*-primed individuals (20, 22, 25).

Studies on overlapping synthetic peptides encompassing the complete amino acid sequence of TUL4 have indicated that some part of the molecule, in addition to the epitope-containing sequence, is required to obtain an optimal T-cell response (25). In fact, the proliferative responses induced by relevant synthetic peptides were several times lower than those induced by the native protein. An adjuvant effect of some part of the molecule outside the epitope, such as the lipid moiety, is a possible explanation for the difference.

The fatty acylation of microbial proteins has been reported to constitute an adjuvant property for the humoral immune response (5, 14) and certain synthetic lipids like dimethyldioctadecyl-ammoniumbromide bound to proteins have been reported to strongly enhance T-cell responses to these proteins (26). The mechanism whereby the addition of fatty acid may enhance B- and T-cell responses is obscure. The presence of palmitic acid was found to be essential to the generation of an in vivo response by class I-restricted T cells to synthetic peptides of influenza virus (8). Deres et al. suggested that the lipid modification allowed for direct membrane incorporation of the peptides. Another possibility is that lipid modification facilitates the association of an epitope-containing peptide with the major histocompatibility complex molecule of the antigen-presenting cell, as reported in studies of class II-restricted T cells (18).

In conclusion, the results show that a 17-kDa protein of *F. tularensis* involved in the cell-mediated and humoral immune responses to the organism is a lipoprotein. It is also suggested that the organism possesses several other lipoproteins. The latter lipoproteins were evidently not recognized by serum from *F. tularensis*-primed individuals. Their possible role in T-cell recognition remains to be studied.

ACKNOWLEDGMENTS

We thank T. Johansson and K. Kuoppa for excellent technical assistance. We also thank M. Arai for providing globomycin, B. Lilliehöök for providing the monoclonal antibody, 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; from the Swedish Society for Medical Research, Karolinska Institutet, Stockholm, Sweden; and from the Swedish Society of Medicine.

REFERENCES

1. **Bertani, G.** 1951. Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. J. Bacteriol. **62**:293–300.
2. **Bligh, E. G., and W. J. Dyer.** 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**:911–917.
3. **Bordier, C.** 1981. Phase separation of integral membrane proteins in Triton X-114 solution. J. Biol. Chem. **256**:1604–1607.
4. **Brandt, M. E., B. S. Riley, J. D. Radolf, and M. V. Norgard.** 1990. Immunogenic integral membrane proteins of *Borrelia burgdorferi* are lipoproteins. Infect. Immun. **58**:983–991.
5. **Braun, V.** 1974. Covalent lipoprotein from the outer membrane of *Escherichia coli*. Biochim. Biophys. Acta **415**:335–377.
6. **Chamberlain, N. R., M. E. Brandt, A. L. Erwin, J. D. Radolf, and M. V. Norgard.** 1989. Major integral protein immunogens of *Treponema pallidum* are proteolipids. Infect. Immun. **57**:2872–2877.
7. **Chen, R., and U. Henning.** 1987. Nucleotide sequence of the gene for the peptidoglycan-associated lipoprotein of *Escherichia coli* K12. Eur. J. Biochem. **163**:73–77.
8. **Deres, K., H. Schild, K.-H. Wiesmuller, G. Jung, and H.-G. Rammensee.** 1989. *In vivo* priming of virus-specific cytotoxic T lymphocytes with synthetic lipopeptide vaccine. Nature (London) **342**:561–564.
9. **Hantke, K., and V. Braun.** 1973. Covalent binding of lipid to protein: diglyceride and amide-linked fatty acid at the N-terminal end of the murein-lipoprotein of the *Escherichia coli* outer membrane. Eur. J. Biochem. **34**:284–296.
10. **Ichihara, S., M. Hussain, and S. Mizushima.** 1981. Characterization of new membrane lipoproteins and their precursors of *Escherichia coli*. J. Biol. Chem. **256**:3125–3129.
11. **Inukai, M., M. Takeuchi, K. Shimuzu, and M. Arai.** 1978. Mechanism of action of globomycin. J. Antibiot. **31**:1203.
12. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
13. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
14. **Lex, A., K.-H. Weissmuller, G. Jung, and W. G. Bessler.** 1986. A synthetic analogue of *Escherichia coli* lipoprotein tripalmitoyl pentapeptide constitutes a potent immune adjuvant. J. Immunol. **137**:2676–2681.
15. **Löfgren, S., A. Tärnvik, G. D. Bloom, and W. Sjöberg.** 1983. Phagocytosis and killing of *Francisella tularensis* by human polymorphonuclear leukocytes. Infect. Immun. **39**:715–720.
16. **Norrander, J., T. Kempe, and J. Messing.** 1983. Improved M13

vectors using oligonucleotide-directed mutagenesis. Gene **26:** 101–106.

17. **Radolf, J. D., N. R. Chamberlain, A. Clausell, and M. V. Norgard.** 1988. Identification and localization of integral membrane proteins of virulent *Treponema pallidum* subsp. *pallidum* by phase partitioning with the nonionic detergent Triton X-114. Infect. Immun. **56:**490–498.
18. **Roof, R. W., I. F. Luescher, and E. R. Unanue.** 1990. Phospholipids enhance the binding of peptides to class II major histocompatibility molecules. Proc. Natl. Acad. Sci. USA **87:**1735–1739.
19. **Sandström, G., S. Löfgren, and A. Tärnvik.** 1988. A capsule-deficient mutant of *Francisella tularensis* LVS exhibits enhanced sensitivity to killing by serum but diminished sensitivity to killing by polymorphonuclear leukocytes. Infect. Immun. **56:**1194–1202.
20. **Sandström, G., A. Tärnvik, and H. Wolf-Watz.** 1987. Immunospecific T-lymphocyte stimulation by membrane proteins from *Francisella tularensis*. J. Clin. Microbiol. **25:**641–644.
21. **Sandström, G., A. Tärnvik, H. Wolf-Watz, and S. Löfgren.** 1984. Antigen from *Francisella tularensis*: nonidentity between determinants participating in cell-mediated and humoral reactions. Infect. Immun. **45:**101–106.
22. **Sjöstedt, A., G. Sandström, and A. Tärnvik.** 1990. Several membrane polypeptides of the live vaccine strain *Francisella tularensis* LVS stimulate T cells from naturally infected individuals. J. Clin. Microbiol. **28:**43–48.
23. **Sjöstedt, A., G. Sandström, and A. Tärnvik.** Unpublished data.
24. **Sjöstedt, A., G. Sandström, A. Tärnvik, and B. Jaurin.** 1989. Molecular cloning and expression of a T-cell stimulating membrane protein of *Francisella tularensis*. Microb. Pathog. **6:**403–411.
25. **Sjöstedt, A., G. Sandström, A. Tärnvik, and B. Jaurin.** 1990. Nucleotide sequence and T cell epitopes of a membrane protein of *Francisella tularensis*. J. Immunol. **143:**311–317.
26. **Smith, R. H., and B. Ziola.** 1985. CY and DDN immunopotentiate the DTH response to inactivated enveloped viruses. Immunology **58:**245–250.
27. **Surcel, H.-M.** 1990. Diversity of *Francisella tularensis* antigens recognized by human lymphocytes. Infect. Immun. **58:**2664–2668.
28. **Surcel, H.-M., M. Sarvas, I. M. Helander, and E. Herva.** 1989. Membrane proteins of *Francisella tularensis* LVS differ in ability to induce proliferation of lymphocytes from tularemia-vaccinated individuals. Microb. Pathog. **7:**411–419.
29. **Swanson, J., L. W. Mayer, and M. R. Tam.** 1982. Antigenicity of *Neisseria gonorrhoeae* outer membrane protein(s) III detected by immunoprecipitation and Western blot transfer with a monoclonal antibody. Infect. Immun. **38:**668–672.
30. **Tokunaga, M., H. Tokunaga, and H. C. Wu.** 1982. Post-translational modification and processing of *Escherichia coli* prolipoprotein *in vitro*. Proc. Natl. Acad. Sci. USA **79:**2255–2259.
31. **Wu, H. C., and M. Tokunaga.** 1986. Biogenesis of lipoproteins in bacteria. Curr. Top. Microbiol. Immunol. **125:**127–156.