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Delayed-Type Hypersensitivity Activity of the Brucella L7/L12 Ribosomal Protein Depends on Posttranslational Modification

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The ribosomal protein L7/L12 isolated from *Brucella melitensis* induces a delayed-type hypersensitivity (DTH) reaction in brucella-sensitized guinea pigs. Surprisingly, the recombinant brucella L7/L12 protein expressed in *Escherichia coli* as a fusion protein with a six-histidine tag cannot elicit such a reaction. The six histidines tagged to the recombinant L7/L12 protein were removed enzymatically, but the resulting protein did not induce a DTH reaction in sensitized animals. Incubation of the recombinant L7/L12 fusion protein in a *B. melitensis* lysate endowed the recombinant protein with a DTH activity, suggesting that the recombinant protein was modified by this treatment. Glycosylation or phosphorylation of the recombinant L7/L12 protein could not be detected. On the other hand, radiolabeled palmitic acid was found to be incorporated to the recombinant protein during its incubation in the brucella lysate. This incorporation was specific for the brucella L7/L12 protein and was inhibited when the brucella lysate was frozen and thawed before the incubation. The data reported here indicate that posttranslational modification of L7/L12 protein comprising at least an acylation step is required for the brucella L7/L12 DTH activity.

Vaccines based on ribosomal preparations have been described for the last 30 years (17, 32, 33). Their use has been controversial, mainly due to the lack of a common mechanism explaining the protection achieved by the various ribosomal vaccines (17). Nevertheless, ribosomal preparations of intracellular pathogens were shown to be able to induce a delayed-type hypersensitivity (DTH) reaction in animals sensitized with the pathogen (6, 10, 23, 27). The involvement of the protein fraction of these ribosomal extracts in the DTH reaction has been also controversial (17, 35). However, lately the allergenic immunogenicity (DTH) of the ribosomal proteins L7/L12 purified from mycobacteria and brucellae has been definitively established (5, 30). Tantimavanich et al. (30) found that among all the ribosomal proteins, L7/L12 was the only protein able to reveal a DTH reaction in guinea pigs sensitized by live BCG or killed *Mycobacterium tuberculosis*. Bachrach et al. (5) found that the brucella L7/L12 ribosomal protein was the main immunogenic protein in the DTH activity of the commercial protein preparation Brucellergen injected in guinea pigs sensitized with live *Brucella melitensis* Rev1 strain. Ribosomal proteins L7 and L12 are two proteins encoded by the same gene (*rplL*) and differ from each other only by an acetylic posttranslational modification that occurs at the L12 N terminus converting it to L7 (24, 31). Each ribosome contains two L7/L12 dimers. Being the only proteins present in the ribosome in more then one copy (24), L7/L12 may become very abundant in multiplying bacteria (1).

The native brucella ribosomal L7/L12 protein purified by antibody affinity column was shown to induce a strong DTH skin reaction in brucella-sensitized guinea pigs (5). The *rplL* gene encoding L7/L12 of *B. melitensis* was cloned, sequenced (4), and expressed in *Escherichia coli* as a fusion protein with a six-histidine tag facilitating its purification (5). Like the native brucella L7/L12 protein, the recombinant L7/L12 protein comprised both L12 and the acetylated L7 (5a). Surprisingly, the recombinant brucella L7/L12 protein did not elicit a DTH reaction (5). Since it has been unequivocally demonstrated that the allergenic response is caused by the native protein itself and not because of brucella contaminating components (5), there are at least two possibilities to explain the inability of the recombinant protein to reveal a DTH reaction. The first is that the six-histidine tag added to the recombinant N-terminal amino acid sequence interferes with the protein DTH activity. The second is that besides acetylation, a specific posttranslational modification of L7/L12 which occurs in brucellae but not in *Escherichia coli* is necessary for the DTH activity of L7/L12.

In the present work, we demonstrate the following. (i) Incubation of the recombinant brucella L7/L12 protein in *B. melitensis* lysate followed by repurification of the protein to homogeneity endowed the protein with DTH activity. (ii) The L7/L12 recombinant protein was palmitoylated when incubated in *B. melitensis* lysate. (iii) The six-histidine tag at the amino-terminal end of the protein did not interfere with its allergenic activity.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *B. melitensis* Rev1 was grown on tryptic soy agar supplemented with serum-dextrose (2) from freeze-dried bacterial stock maintained at the Israeli Brucellosis Reference Center, Kimron Veterinary Institute. *E. coli* was grown on LB broth.

Expression of brucella L7/L12 protein in *E. coli* and its purification. Recombinant brucella L7/L12 protein was expressed in *E. coli* harboring the pQE3012 plasmid (5) and purified by affinity chromatography using immobilized nickel chelate (Ni-NTA column; Hoffmann-La Roche) as described previously (5). Briefly, the primers p512 (GGGGGATCCGCTGATCTCGCAAAGATTGTTG AA) and p312 (CCAGGTACCTCCAAACTTACTTGAGTTCAACCT) were selected from the *B. melitensis rplL* sequence (4). These primers create a *Bam*HI site at the 5′ end (p512) of the gene and a *Kpn*I site at the 3′ end (p312). DNA encoding the L7/L12 protein was amplified from the *B. melitensis* 16M strain by PCR ($Vent_R$ DNA polymerase; New England Biolabs). The PCR product was digested simultaneously with *Kpn*I and *Bam*HI and ligated to the *E. coli* pQE30 plasmid expression vector (Qiagen) predigested by *Kpn*I and *Bam*HI creating the pQE3012 plasmid. The plasmid pQE3012 encodes for six histidines that are tagged to the NH_2 terminus of the expressed L7/L12 protein. The recombinant L7/L12 protein was produced by the pQE3012 plasmid in *E. coli* SG13009 and purified on a Ni-NTA column as specified by manufacturer's instructions (Diagen). The purified protein was dialyzed against saline and subjected to sodium

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dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (22) and Western blot analysis (28) with an antirecombinant L7/L12 protein hyperimmune rabbit serum at a dilution of 1/2,000 as described previously (5).

Removal of the six-histidine tag from the recombinant protein. In order to remove the six histidines tagged to the L7/L12 recombinant protein, an enterokinase endopeptidase cleavage site was introduced between the six histidines and the L7/L12 encoding gene. This was achieved by using the primer p312 (see above) and the primer p512R GCGGATCCGATCCGATGACGATGACAAA GCTGATCTCGCAAAGATTGTTGAA, which is almost identical to p512 but encodes the enterokinase site (KKKD) upstream of the L7/L12 5′ DNA sequence. The DNA fragment coding for the brucella L7/L12 protein was amplified, purified, and cloned as described above, giving rise to the pQE3012R plasmid. The recombinant protein was produced in *E. coli* SG13009 by the pQE3012R plasmid and purified as described above. After purification and dialysis against saline, enterokinase (Boehringer Mannheim Biochemica) was added to the recombinant protein in a ratio of 1:50 (wt/wt). Digestion was performed in a total volume of 1 ml at 37°C overnight. The peptide fraction containing the histidines was removed from the digested protein by purification on a Ni-NTA column.

L7/L12 posttranslational modification in brucella lysate. *B. melitensis* Rev1 cells (2×10^9 CFU) were harvested and washed in 25 mM HEPES, pH 8.0. The bacterial pellet was resuspended in 5 ml of HEPES buffer and sonicated (six pulses of 20 s each at maximal energy on a MSF sonicator). The brucella lysate was centrifuged for 20 min at 20,000 × *g* at 4°C, and the supernatant was collected. Phenylmethylsulfonyl fluoride at a final concentration of 5 mM was added to prevent proteolysis. The recombinant L7/L12 protein (0.3 to 20 μg expressed from pQE3012 plasmid) was added to 1.5 ml of the freshly prepared lysate, and the mixture was incubated for 20 min at 37°C. The whole reaction mixture was subjected to SDS-PAGE and stained with Coomassie blue (28) or alternatively, the recombinant L7/L12 protein was then reisolated from the lysate by using a Ni-NTA column. The lysate-treated recombinant L7/L12 protein was subjected to SDS-PAGE (14% polyacrylamide), and the specific band corresponding to the protein was further purified by electroelution followed by overnight dialysis against saline as described previously (5). Protein concentration was determined according to the Bradford procedure (8).

To identify potential posttranslational modifications, [γ-^{32}P]ATP (3,000 Ci/mmol; 50 μCi per reaction) or [9,10(*n*)-^{3}H]palmitic acid (51 Ci/mmol; 1 mCi per reaction) (Amersham International plc) was added to the brucella lysate (1.5 ml) supplemented with the brucella recombinant L7/L12 protein and in some cases with the *M. bovis* L7/L12 protein (14). To identify glycosylation, Brucellergen (Rhone-Merieux), a commercial brucella protein preparation that contains the native L7/L12 protein (5), was subjected to SDS-PAGE and stained with the periodic acid-Schiff reagent (Sigma, St. Louis, Mo.), which reveals glycoproteins (18). Fetal calf serum was used as a positive control for the detection of glycosylated proteins.

DTH skin testing. Male Hartley guinea pigs (250 g at the beginning of the experiment) were sensitized by subcutaneous inoculation of 10^6 *B. melitensis* Rev1 organisms per animal (2) and were skin tested for DTH response 3 weeks later. Antigens were injected intradermally in a volume of 100 μl. Brucellergen was used as a positive control for DTH response. Diameters (in millimeters) of erythema were read 24 h after injection (21). The injected guinea pigs were checked for immediate nonspecific response after 4 h.

RESULTS

The six histidines added to the recombinant L7/L12 protein have no effect on its DTH activity. The DNA sequence coding for the enterokinase endopeptidase cleavage site (DDDK) was inserted between the sequences coding for the six histidines and the N terminus of the L7/L12 protein (plasmid pQE3012R described in Materials and Methods). After IPTG (isopropyl-β-D-thiogalactopyranoside) induction, the recombinant protein containing the enterokinase cleavage site was purified by using a Ni-NTA column and subjected to SDS-PAGE and Western blotting. This protein with a size of 16 kDa according to its migration (Fig. 1, lane a) was further digested by enterokinase. The digested recombinant protein was separated from the undigested protein by collecting the unbound protein after chromatography of the mixture on the Ni-NTA column. The resulting product was subjected to SDS-PAGE and Western blotting. Enterokinase digestion of this protein removed the six histidines as well as the four amino acids (M, R, G, and S) added by the vector and by the 5′ *Bam*HI restriction site sequence resulting in a 12-kDa protein (Fig. 1, lane b). Although some degradation of the protein is visible, the major band represents the recombinant L7/L12 with a size of 12 kDa,

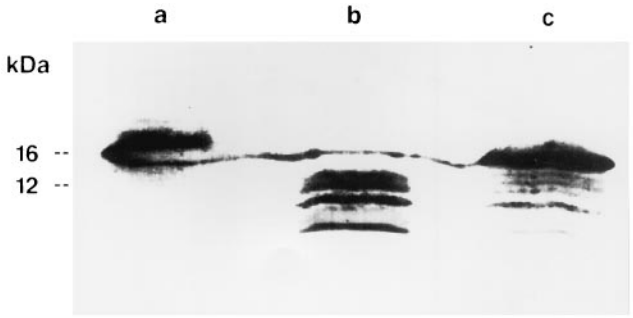

FIG. 1. Removal of the six histidines tagged to the recombinant L7/L12 protein. Plasmid pQE3012R (see Materials and Methods) encoding brucella L7/L12 protein and the enterokinase cleavage site was introduced into *E. coli* SG13009 cells. The recombinant protein was expressed as a fusion protein comprising six histidines and the enterokinase site. The recombinant L7/L12 product was purified on a Ni-NTA column and digested by enterokinase (see Materials and Methods). Cleaved and uncleaved proteins were separated by chromatography on a Ni-NTA column. Samples (10 μl) were subjected to SDS-PAGE followed by Western blotting and reacted with a rabbit anti-recombinant L7/L12 serum as described previously (4). Lanes: a, L7/L12 purified recombinant protein (16 kDa); b, enterokinase-digested L7/L12 protein which has the 12-kDa size of the native L7/L12 protein; c, undigested L7/L12 protein released from the Ni-NTA column.

which is identical to that of the native protein. The remaining uncleaved protein was released from the Ni-NTA column by adding 0.1 M EDTA and was also subjected to SDS-PAGE and Western blotting (Fig. 1, lane c). The enterokinase cleavage efficiency was close to 30%, as can be estimated from the data presented in Fig. 1.

No detectable DTH skin reaction could be seen in six brucella-sensitized guinea pigs injected with 1, 1.5, and 2 μg of the uncleaved or cleaved recombinant protein (Table 1). The same animals reacted with a typical DTH reaction to the Brucellergen injected into the contralateral side.

DTH activity of the recombinant L7/L12 protein is restored after incubation in a brucella lysate. To test if the difference in DTH activity between the native and the recombinant L7/L12 proteins is caused by a posttranslational modification that occurs in brucellae, the recombinant L7/L12 protein (20 μg) was incubated in a *B. melitensis* cell lysate. After incubation, the

TABLE 1. DTH reaction induced by various preparations of L7/L12 ribosomal protein in brucella-sensitized guinea pigs

Nature of injected L7/L12[a]	DTH[b] reaction after injection of:		
	1 μg	1.5 μg	2 μg
Native	17	21	20
Recombinant	—[c]	—	—
Histidine-deleted recombinant	—	—	—
Recombinant (modified) incubated in a fresh brucella lysate	11	10	16
Recombinant incubated as described above and then purified by Ni-NTA column and electroelution	11	10	20
Recombinant incubated in a frozen-thawed brucella lysate	—	—	—

[a] Each protein preparation was injected intradermally in a volume of 100 μl in three male Hartley guinea pigs which were sensitized 3 weeks earlier by live *B. melitensis* Rev1; the data on the native protein are from Bachrach et al. (5).

[b] Numbers represent the mean diameter (in millimeters) of erythema measured 24 h after the injection.

[c] —, no detectable reaction.

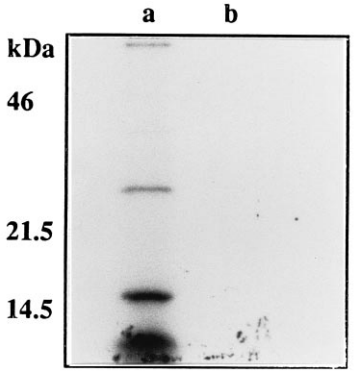

FIG. 2. Phosphorylation of *Brucella* proteins. The brucella recombinant L7/L12 protein (10 μg) was incubated in a *B. melitensis* Rev1 lysate in the presence of [γ-^{32}P]ATP (see Materials and Methods). After incubation, samples of the reaction mixture comprising the L7/L12 protein (0.5 μg, lane a) and the L7/L12 protein purified by Ni-NTA chromatography after incubation in the reaction mixture (0.3 μg, lane b) were subjected to SDS-PAGE (14% polyacrylamide) followed by fluorography. Four proteins present in the lysate but not the L7/L12 protein were phosphorylated.

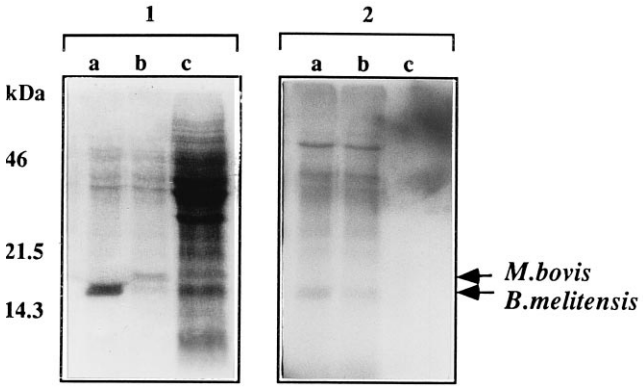

FIG. 3. Acylation of *Brucella* L7/L12 recombinant protein. The recombinant L7/L12 protein was incubated in a fresh or inactivated (frozen and thawed) lysate prepared from *B. melitensis* Rev1 strain in the presence of [9,10(*n*)-^{3}H] palmitic acid (reaction mixture) as described in Materials and Methods. The identification and the determination of the size of the L7/L12 proteins were done by Western immunoblotting (data not shown). (Panel 1) Coomassie blue-stained SDS-PAGE (14% polyacrylamide) of reaction mixtures containing recombinant brucella L7/L12 protein (0.5 μg) (lane a) and recombinant brucella L7/L12 protein (0.3 μg) and recombinant *M. bovis* L7/L12 protein (0.5 μg) (lane b) and an inactivated reaction mixture containing the recombinant brucella L7/L12 protein (0.5 μg) (lane c). (Panel 2) Autoradiogram of the gel shown in panel 1 (21 days of exposure). The recombinant brucella L7/L12 protein can be seen labeled in lanes a and b but not in lane c, whereas the *M. bovis* L7/L12 protein is not labeled (lane b). In lanes a and b but not in lane c (inactivated reaction mixture), additional labeled bands can be seen.

recombinant L7/L12 protein was repurified from the lysate by using the Ni-NTA column. A significant DTH reaction was produced by injecting 1, 1.5, and 2 μg of the purified lysate-treated recombinant L7/L12 protein. The erythema diameters obtained were 11, 10, and 16 mm, respectively (Table 1). No DTH reaction was detected in two naive control guinea pigs injected with 2 μg of this protein.

To ensure that the recombinant modified protein was homogeneously pure, 10 μg of the lysate-treated repurified (Ni-NTA column) recombinant L7/L12 protein was subjected to SDS-PAGE followed by electroelution. After dialysis against saline, the electroeluted protein was injected (1, 1.5, and 2 μg) into three sensitized guinea pigs. All three guinea pigs reacted with a significant DTH reaction with erythema diameters of 11, 10, and 20 mm, respectively (Table 1).

Phosphorylation of brucella proteins. To test if the recombinant protein was phosphorylated during the incubation in the brucella lysate, 10 μg of the recombinant brucella L7/L12 protein was incubated in the brucella lysate (1.5 ml) in the presence of γ-^{32}P-labeled ATP. Figure 2 depicts the autoradiogram of the brucella lysate after SDS-PAGE (lane a) and of the recombinant L7/L12 protein after lysate treatment, Ni-NTA purification, and SDS-PAGE (lane b). As can be seen in Fig. 2 (lane b), the recombinant L7/L12 protein was not phosphorylated during the incubation in the brucella lysate. However, four unrelated proteins present in the lysate were phosphorylated under the same incubation conditions (Fig. 2, lane a).

Lack of glycosylation of native brucella proteins present in the Brucellergen. Twenty micrograms of Brucellergen that contains approximately 1 μg of L7/L12 native protein (5) was subjected to SDS-PAGE, followed by staining with the periodic acid-Schiff reagent. No detectable stained proteins could be identified. A strongly stained glycosylated protein band could be seen in a lane where 20 μl of fetal calf serum was subjected to SDS-PAGE and stained with the periodic acid-Schiff reagent as a positive control (data not shown).

Palmitoylation of the brucella L7/L12 protein. Figure 3 depicts the Coomassie blue-stained SDS-PAGE (panel 1) and the corresponding autoradiogram (panel 2) of brucella lysates containing recombinant brucella and *M. bovis* L7/L12 ribosomal proteins. The location and identification of the L7/L12 proteins was done by Western immunoblotting (data not shown). Tritium-labeled palmitic acid was incorporated into the brucella recombinant L7/L12 protein upon its incubation in brucella lysate (Fig. 3, panel 2, lane a). However, no palmitoylation could be detected when the brucella L7/L12 protein was incubated in a lysate that was frozen and thawed twice (Fig. 3, panel 2, lane c). Furthermore, no incorporation of labeled palmitic acid could be detected in the *M. bovis* L7/L12 recombinant protein (14) coincubated in the brucella lysate (Fig. 3, panel 2, lane b).

The palmitic acid-labeled L7/L12 recombinant protein (1 μg), repurified by Ni-NTA column followed by SDS-PAGE and electroelution purification, produced a DTH reaction (10-mm-diameter erythema) when injected in two different brucella-sensitized guinea pigs.

DISCUSSION

The native L7/L12 ribosomal protein extracted from brucella cells induces both a lymphocyte proliferation (6a, 9) and a DTH reaction in brucella-sensitized guinea pigs (5). In contrast, the brucella L7/L12 recombinant protein produced in *E. coli* is capable of inducing only lymphocyte proliferation (25) and lacks DTH activity (5). No DTH activity was observed even when up to 16 μg of the recombinant L7/L12 protein was injected intradermally in brucella-sensitized guinea pigs (5). Although it has been shown (3) that different epitopes of the *M. tuberculosis* 19-kDa protein can elicit either T-cell proliferation or DTH (some T-cell-eliciting epitopes are not able to elicit a DTH reaction), no data can be found on a whole protein which in its native form elicits both DTH and T-cell proliferation and in its recombinant form elicits only T-cell proliferation. Two hypotheses were raised to explain the loss of this immunological function in the recombinant protein.

The first hypothesis was that the six histidines tagged to the N terminus of the recombinant protein, which facilitate the extraction and the purification of the recombinant L7/L12 protein expressed in the *E. coli* host, might interfere with the DTH

response. The six-histidine tag could change the protein conformation in a way that the antigenic epitope which induces the DTH reaction is masked or is not processed and presented correctly by antigen-presenting cells. After deletion of the six-histidine tag, the recombinant L7/L12 protein, identical in size to the native L7/L12 (Fig. 1), did not show any DTH activity (Table 1). Therefore, the presence of these amino acids cannot be the cause for the recombinant protein's inability to reveal a DTH reaction in sensitized animals.

The second hypothesis was that the difference in immunological function between the native and the recombinant L7/L12 protein is due to a specific posttranslational modification of the protein which occurs in *B. melitensis* and not in *E. coli*. To test this hypothesis, the recombinant brucella L7/L12 protein produced in *E. coli* was incubated in an extract prepared from brucella cells. Following incubation, the protein acquired a DTH activity. The DTH activity of the recombinant protein obtained after immersion in a brucella lysate might have resulted from the presence of small contaminants carried over during the Ni-NTA column purification. Consequently, the in vitro modified protein isolated by affinity Ni-NTA column was further purified by SDS-PAGE followed by electroelution of the protein band. As shown in Table 1, this preparation retained its DTH activity, thus ruling out that adsorbed contaminants are responsible for the DTH activity and suggesting a modification involving covalent bonds. In addition, the presence of a contaminating native L7/L12 protein was ruled out by the fact that the eluted band had the size of the recombinant protein with six histidines and not the size of the native L7/L12 protein (5). A kinetic analysis of this reaction showed that within 1 min, 50% of the recombinant protein became DTH active and 100% of the protein was active after 15 min (data not shown). The recombinant protein did not acquire a DTH activity when it was incubated in a brucella lysate inactivated by freezing and thawing (Table 1), suggesting that an active enzymatic mechanism produced the modification.

The recombinant preparation, like the native protein preparation, comprises L12 and the acetylated L7 (5a), so an additional modification had to be searched for. The nature of this modification was tested by adding labeled precursors of phosphorylation or acylation into the reaction mixture. Palmitic acid was the only compound found to be incorporated into the recombinant L7/L12 when incubated in a freshly prepared brucella lysate (Fig. 3). The incorporation of labeled palmitate was shown to be specific to brucella L7/L12, as the mycobacterial recombinant L7/L12 protein that was coincubated with the brucella protein was not labeled (Fig. 3). The results presented in this paper clearly demonstrate that acylation of the L7/L12 protein occurs in brucellae. The N-terminal myristoylation of glycine or acylation of cysteine through thioester bonds is not likely, since glycine is not present in the N terminus of the protein and there is no cysteine in the sequence (29). Palmitoylation through amidic bonds in internal glycines, serines, or threonines is probable (29). The exact nature of this modification and its location remain to be determined.

The acylation of the ribosomal protein L7/L12 has not been described before, and it is not yet clear if it is unique to brucellae. Posttranslational modifications (glycosylation and acylation) of bacterial proteins have been described before, mainly in the case of membrane-associated, surface, or excreted proteins (12–14, 16, 20), and play a crucial role in protein and cellular regulation in both eucaryotes and procaryotes (29). In these systems, the function of the acylation is related to protein-protein or protein-membrane interactions through lipophilic interactions. In addition, immunological properties related to acylation have been shown by analysis of the gram-negative lipoprotein (8, 20). Using proteolytic fragments and synthetic acyl peptides, it was demonstrated that the acyl moiety is involved in determining the immunogenicity of the lipoprotein and its activity as a B-cell activator (7). In the data presented here, the acylation did not involve a membrane-bound protein but a ribosomal protein. The L7/L12 protein is not membrane associated but is known to form quatromers (24). Therefore, it is possible that acylation is involved in protein-protein interactions. In any case our results clearly suggest that the acylation of the ribosomal protein L7/L12 is also associated with the DTH activity of L7/L12. The DTH reaction is an immunocellular reaction (11, 26), and cellular recognition is known for its sensitivity (34). We suggest that the modification of the brucella L7/L12 protein is crucial for the protein's recognition by the host cells triggering the DTH reaction. This finding can contribute to the understanding of the cellular and molecular basis of the DTH reaction. In addition, our findings can lead to the production of modified recombinant L7/L12 protein which could be used in a diagnostic skin test specific for brucellosis. This approach could be also applied to other diseases such as tuberculosis.

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REFERENCES

1. **Abshire, K. Z., and F. C. Neidhardt.** 1993. Growth rate paradox of *Salmonella typhimurium* within host macrophages. J. Bacteriol. **175:**3744–3748.
2. **Alton, G. G., L. M. Jones, R. D. Angus, and J. M. Verger (ed.).** 1988. Techniques for the brucellosis laboratory. INRA, Paris, France.
3. **Ashbridge, K. R., B. T. Backstrom, H. X. Liu, T. Vikerfors, D. R. Englebresten, D. R. Harding, and J. D. Watson.** 1992. Mapping of T helper cell epitopes by using peptides spanning the 19-kDa protein of *Mycobacterium tuberculosis*. Evidence for unique and shared epitopes in the stimulation of antibody and delayed-type hypersensitivity responses. J. Immunol. **148:**2248–2255.
4. **Bachrach, G., D. Bar-Nir, M. Banai, and H. Bercovier.** 1994. Identification and nucleotide sequence of *Brucella melitensis* L7/L12 ribosomal protein. FEMS Microbiol. Lett. **120:**237–240.
5. **Bachrach, G., M. Banai, S. Bardenstein, G. Hoida, A. Genizi, and H. Bercovier.** 1994. Brucella ribosomal protein L7/L12 is a major component in the antigenicity of Brucellin INRA for delayed-type hypersensitivity in brucella-sensitized guinea pigs. Infect. Immun. **62:**5361–5366.

5a. **Bachrach, G., and H. Bercovier.** Unpublished results.

6. **Baker, R. E., W. E. Hill, and C. L. Larson.** 1973. Ribosomes of acid-fast bacilli: immunogenicity, serology, and in vitro correlates of delayed hypersensitivity. Infect. Immun. **8:**236–244.

6a. **Banai, M.** Unpublished results.

7. **Bessler, W. G., M. Cox, A. Lex, B. Sehr, K. H. Weismuller, and G. Jung.** 1985. Synthetic lipopeptide analogs of bacterial lipoprotein are potent polyclonal activators for murine B lymphocytes. J. Immunol. **135:**1900–1905.
8. **Bradford, M. M.** 1976. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
9. **Brooks-Worrel, B. M., and G. A. Splitter.** 1992. Antigens of *Brucella abortus* S19 immunodominant for bovine lymphocytes as identified by one- and two-dimensional cellular immunoblotting. Infect. Immun. **60:**2459–2464.
10. **Corbel, M. J.** 1976. The immunogenic activity of ribosomal fractions derived from *Brucella abortus*. J. Hyg. Camb. **76:**65–74.
11. **Dannenberg, A. M.** 1994. Role of cytotoxic delayed-type hypersensitivity and macrophage-activating cell-mediated immunity in the pathogenesis of tuberculosis. Immunobiology **19:**461–473.
12. **Dobos, K. M., K. Swiderek, K. H. Khoo, P. J. Brennan, and J. T. Belisle.** 1995. Evidence for glycosylation sites on the 45-kilodalton glycoprotein of *Mycobacterium tuberculosis*. Infect. Immun. **63:**2846–2853.
13. **Espitia, C., and R. Mancilla.** 1989. Identification, isolation and partial characterization of *Mycobacterium tuberculosis* glycoprotein antigens. Clin. Exp. Immunol. **77:**378–383.

14. **Fishman, Y., G. Bachrach, and H. Bercovier.** 1995. Involvement of L7/L12 in DTH reaction in mycobacterial infections. J. Cell Biochem. Suppl. **19B:**77.
15. **Garbe, T., D. Harris, M. Vordermeier, R. Lathigra, J. Ivanyi, and D. Young.** 1993. Expression of the 19-kDa antigen in *Mycobacterium smegmatis*: immunological analysis and evidence of glycosylation. Infect. Immun. **61:**260–267.
16. **Gerard-Schahn, R., A. Bethe, T. Brennecke, M. Muhlenhoff, M. Eckhardt, S. Ziesing, F. Lottspeich, and M. Frosch.** 1995. Molecular cloning and functional expression of bacteriophage PK1E-encoded endoneuramidase Endo NE. Mol. Microbiol. **16:**441–450.
17. **Gregory, R. L.** 1986. Microbial ribosomal vaccine. Rev. Infect. Dis. **8:**208–217.
18. **Hamburger, J., and A. Zuckerman.** 1976. *Plasmodium berghei.* I. Immunochemical properties of fractions of a soluble extract. Exp. Parasitol. **39:**460–478.
19. **Hauschildt, S., P. Hoffman, H. U. Beuscher, G. Dufhues, K. H. Heinrich, K. H. Weismuller, G. Jung, and W. G. Bessler.** 1990. Activation of bone-marrow derived mouse macrophages by bacterial lipopeptide: cytokine production, phagocytosis, and Ia-expression. Eur. J. Immunol. **20:**63–68.
20. **Issartel, J. P., V. Koronakis, and C. Hughes.** 1991. Activation of *Escherichia coli* prohaemolysin to the mature toxin by acyl carrier protein-dependent fatty acylation. Nature (London) **351:**759–761.
21. **Jones, L. M., and D. T. Berman.** 1975. Antibody mediated and delayed type hypersensitivity reactions to *Brucella* skin test antigens in guinea pigs. Infect. Immun. **11:**360–364.
22. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
23. **Loge, R. V., W. E. Hill, R. E. Baker, and C. L. Larson.** 1974. Delayed hypersensitivity reactions provoked by ribosomes from acid-fast bacilli: physical characterization and immunological aspects of core ribosomal proteins from *Mycobacterium smegmatis*. Infect. Immun. **9:**489–495.
24. **Noller, H. F., and M. Nomura.** 1987. Ribosomes, p. 104–126. *In* F. C. Neidhardt et al. (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
25. **Oliveira, S. C., Y. Zhu, and G. A. Splitter.** 1994. Recombinant L7/L12 protein and gamma-irradiated *Brucella abortus* induce a T-helper 1 subset response from murine CD4+ T cells. Immunology **83:**659–664.
26. **Orme, I. M., E. S. Miller, A. D. Roberts, S. K. Furney, J. P. Griffin, D. Chi, B. Rivoire, and P. J. Brennan.** 1992. T lymphocytes mediating protection and cellular cytolysis during the course of *Mycobacterium tuberculosis* infection. Evidence for different kinetics and recognition of a wide spectrum of protein antigens. J. Immunol. **148:**189–196.
27. **Ortiz-Ortiz, L., E. B. Solarolo, and L. F. Bojalil.** 1971. Delayed hypersensitivity to ribosomal protein from BCG. J. Immunol. **107:**1023–1026.
28. **Sambrook, J., E. F. Fritsch, and T. Maniatis (ed.).** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
29. **Schmidt, M. F. G.** 1989. Fatty acylation of proteins. Biochem. Biophys. Acta **988:**411–426.
30. **Tantimavanich, S., S. Nagai, H. Nomaguchi, M. Kinomoto, N. Ohara, and T. Yamada.** 1993. Immunological properties of ribosomal proteins from *Mycobacterium bovis* BCG. Infect. Immun. **61:**4005–4007.
31. **Terhorst, C., B. Wittmann-Liebold, and W. Moller.** 1972. 50S ribosomal proteins. Peptide studies on two acidic proteins, A_1 and A_2, isolated from ribosomes of *Escherichia coli*. Eur. J. Biochem. **25:**13–19.
32. **Thompson, H. C., and I. S. Snyder.** 1971. Protection against pneumococcal infection by a ribosomal preparation. Infect. Immun. **3:**16–23.
33. **Venneman, M. R., and N. J. Bigley.** 1969. Isolation and partial characterization of an immunogenic moiety obtained from *Salmonella typhimurium*. J. Bacteriol. **100:**140–148.
34. **Wang, X. H., J. D. Ohmen, K. Uyemura, T. H. Rea, M. Kronenberg, and R. L. Modlin.** 1993. Selection of T lymphocytes bearing limited T-cell receptor beta chain in the response to a human pathogen. Proc. Natl. Acad. Sci. USA **90:**188–192.
35. **Youmans, A. S., and G. P. Youmans.** 1965. Immunogenic properties of a ribosomal fraction obtained from *Mycobacterium tuberculosis*. J. Bacteriol. **89:**1291–1298.

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