INFECTION AND IMMUNITY, Oct. 1985, p. 136–141
0019-9567/85/100136-06$02.00/0

Vol. 50, No. 1

Subspecies-Specific Surface Antigens of Promastigotes of the *Leishmania donovani* Complex

JEAN-LOUP LEMESRE,[1] FARRUKH S. RIZVI,[1] DANIEL AFCHAIN,[1] MOYSES SADIGURSKY,[2] ANDRE CAPRON,[1] AND FERRUCIO SANTORO[1]*

Centre d'Immunologie et de Biologie Parasitaire, Unité Mixte INSERM 167-CNRS 624, Institut Pasteur, 59019 Lille Cédex, France,[1] and Centro de Pesquisas Gonçalo Moniz, FIOCRUZ-UFBA, 40000 Salvador, Bahia, Brazil[2]

Received 1 May 1985/Accepted 28 June 1985

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of proteins and externally exposed labeled surface constituents were analyzed in promastigotes of three etiological agents of kala azar (*Leishmania donovani*, HS70 strain from India; *L. chagasi*, Imperatriz strain from Brazil; *L. infantum*, ITMPA K263 strain from Morocco and MO strain from France). Coomassie blue-stained gels showed similar protein patterns for *L. donovani* and *L. chagasi* and a more distinct one for *L. infantum*. Surface radioiodination with two different methods, lactoperoxidase and IODO-GEN, gave identical autoradiographic patterns for each parasite. Four major labeled proteins with apparent M_r values of 65,000, 60,000, 50,000, and 26,000 were detected in both *L. chagasi* and *L. donovani*. However, the radioiodinated polypeptide pattern of *L. infantum* only showed two major bands with an apparent M_r of 62,000 and a doublet of 26,000 to 23,000. Immunoprecipitation of detergent extracts of labeled promastigote subspecies with immune sera from rabbits immunized with either *L. chagasi* or *L. infantum* and from patients and mice infected with these two parasites, as well as with a monoclonal antibody against the surface of *L. donovani* promastigotes, demonstrated that the surface antigenic expression of *L. infantum* is different from that noticed in the two other subspecies, which are similar. Immunofluorescence experiments with some of these antibodies confirmed these results. The present findings should be considered in taxonomic and immunological studies in visceral leishmaniasis.

Parasites from the genus *Leishmania* are protozoan hemoflagellates which cause a wide range of disease states in animals and humans in many parts of the world. The life cycle of the *Leishmania* parasite involves an extracellular motile promastigote in the sand fly vector and an intracellular nonmotile amastigote within the mononuclear phagocytes of their vertebrate host. In cell-free culture medium, at 26 to 28°C, the parasites grow and multiply in a similar promastigote form (3) which remains infective when injected into susceptible hosts (12).

In humans, parasites from the *Leishmania donovani* complex cause visceral leishmaniasis or kala azar (24). The classic kala azar of Indian type, due to *L. donovani*, especially affects adults and induces post-kala azar dermal lesions. In Africa, *L. donovani* also infects adults but frequently presents a high resistance to antimonial treatment. Mediterranean kala azar by *L. infantum* especially affects children, and South American visceral leishmaniasis caused by *L. chagasi* is a disease of both adults and children. These geographical and clinical data have been used in the past to classify the organisms causing the visceral disease. Furthermore, immunological (19, 20, 22) and biochemical (1, 4, 15; P. Desjeux, F. LePont, S. Mollinedo, and M. Tibayrenc, Ann. Parasitol, in press) methods have proved to be particularly useful in the taxonomic classification of *Leishmania* spp. Attempts to correlate these biological characteristics and the different clinical and epidemiological manifestations of the various species of these parasites have led to numerous classification schemes (3).

Recently, the analysis of surface antigens with either polyvalent antisera or monoclonal antibodies (mAb) has been used to differentiate species, subspecies, and strains of *Leishmania* (9, 19, 20). Moreover, these immunological reagents have also shown that different leishmanial stocks present several common or cross-reactive antigens (8, 9). In a preliminary investigation on the surface antigen profiles of promastigotes from an *L. donovani* complex isolated in Brazil and Africa, no difference has been observed (17). In addition, a glycoprotein of 65,000 molecular weight, recognized by human immune sera from different geographical areas, was the main component in both subspecies (17). More recently, this glycoprotein has been found as the major promastigote surface antigen of different stocks of *Leishmania* spp. (8). In the present study, we report the comparative analysis of surface polypeptides and antigenic components, immunoprecipitated with both polyvalent antisera and mAb, of the three main etiological agents of visceral leishmaniasis (*L. donovani*, *L. chagasi*, and *L. infantum*).

MATERIALS AND METHODS

Parasite strains and culture. Promastigotes from *L. donovani* (HS70 strain from India), *L. infantum* (ITMPA K263 strain from Morocco and MO strain from South of France), and *L. chagasi* (Imperatriz strain from Brazil) were cultured at 26°C in a cellular monophasic GLSH medium (18) supplemented with 10% (vol/vol) heat-inactivated fetal calf serum.

Polyclonal antisera. Rabbit antisera to *L. infantum* (ITMPA K263 strain) and *L. chagasi* were obtained in New Zealand White rabbits by repeated subcutaneous injections of 2 mg of the total soluble extract of culture promastigotes in complete Freund adjuvant as previously described (18). Preimmunization sera from the same rabbits were used as controls. Sera of BALB/c mice inoculated intraperitoneally with 2.5×10^7 viable promastigotes of either *L. infantum* (ITMPA K263 strain) or *L. chagasi* were also used. Normal

* Corresponding author.

mouse serum was used as a control. All of these sera were aliquoted and stored at −20°C before use.

Human immune sera. Immune sera from five Mediterranean patients with *L. infantum* kala azar were generously provided by D. Le Ray (Tropical Medicine Institute, Anvers, Belgium). Sera from five Brazilian patients with visceral leishmaniasis and a positive serology for *L. chagasi* and from normal European subjects were also used.

Hybridoma production and mAb. mAb against the surface of both *L. chagasi* and *L. donovani* 3-day-old promastigotes were generated by the method of Köhler and Milstein (14). Briefly, spleen cells from BALB/c mice injected once intraperitoneally and thrice subcutaneously at 1-week intervals with 0.1% glutaraldehyde-fixed promastigotes (10^7) and boosted intravenously 3 days before fusion with 10^6 fixed parasites were fused with SP2/0 myeloma cells (23) in the presence of polyethylene glycol-dimethyl sulfoxide (7). Antibody production was assessed by indirect immunofluorescence (IF) and radioimmunoassay, using glutaraldehyde-fixed promastigotes (9). Positive hybrid cell lines were cloned twice by limiting dilution and ascitic fluids were produced in Pristane-primed BALB/c mice. Two particular mAb were used in this investigation: one produced against *L. chagasi* (IIIB1/5.9.12) and another produced against *L. donovani* (VH1/19.2.6). Both were immunoglobulin M (IgM) isotypes as determined by double diffusion in agar gels.

IF. IF was carried out with 0.1% glutaraldehyde-fixed promastigotes of the *L. donovani* complex coated on IF slides (Institut Pasteur, Paris, France). These slides were air dried and kept at −20°C until used. Diluted mice antisera or mAb were applied to the slides for 30 min at 37°C. After two washes by immersion in phosphate-buffered saline (PBS), pH 7.3, the slides were incubated for 30 min with fluorescein-conjugated goat IgG anti-mouse immunoglobulin (IgG plus IgM plus IgA), heavy and light chain specific (Cappel Laboratories, Cochranville, Pa.), diluted 1:80 in PBS containing 0.01% Evans blue.

Surface radiolabeling with ^{125}I. Promastigotes of all *Leishmania* stocks were collected in their logarithmic growth phase (3-day-old culture) by centrifugation at 600 × *g* for 10 min at 4°C. The pellets were suspended and washed three times in Hanks balanced salt solution containing 100 U of aprotinin (Sigma Chemical Co., St. Louis, Mo.) per ml and once in PBS. Lactoperoxidase-catalyzed radioiodination was performed essentially as previously described (5). Briefly, 5 μg of lactoperoxidase (Sigma), 0.5 mCi of $Na^{125}I$ (Radiochemical Centre, Amersham, U.K.), and 5 μl of 0.01% (wt/vol) hydrogen peroxide were added sequentially to 10^8 parasites in 500 μl of PBS. After an incubation of 15 min in ice, the labeled promastigotes were washed three times by centrifugation (600 × *g*, 10 min, 4°C) with 1 ml of ice-cold PBS containing 1 mg of NaI per ml.

For IODO-GEN-mediated labeling, a modification of the method described by Howard et al. (11) was used. Briefly, 1 mg of IODO-GEN (Pierce Chemical Co., Rockford, Ill.) was dissolved in 10 ml of chloroform, and 625 μl of this solution was transferred to 5-ml glass tubes. The solvent was evaporated under a stream of N_2, leaving a coating of IODO-GEN bound to the tube. Subsequently, 10^8 promastigotes, washed as described above, in 500 μl of PBS and 0.5 mCi of $Na^{125}I$ (5 μl) were added to the glass tube containing dried IODO-GEN. After a 10-min contact in ice, this mixture was transferred to a 1.5-ml Microfuge (Beckman Instruments, Inc., Fullerton, Calif.) tube, and 1 ml of 10 mM NaI in PBS was added to the labeled parasites to quench the reaction. Final washes were as described above. After both radioiodinations, >95% of the promastigotes remained viable and actively motile and therefore were assumed to have retained intact surface membranes.

Solubilization of promastigotes. Labeled or unlabeled promastigotes were solubilized in a lysis buffer containing 10 mM Tris, 0.5% Nonidet P-40 (Bethesda Research Laboratories, Bethesda, Md.), and 100 U of aprotinin per ml and left overnight on agitation at 4°C. Detergent extract was then centrifuged at 5,000 × *g* for 20 min at 4°C to remove cell debris, and the supernatant was used.

Immunoprecipitation. Immunoprecipitation was carried out in 1.5-ml Microfuge tubes by the method of Kessler (13), in which 40 μl of detergent extract of labeled promastigotes was diluted in 500 μl of 10 mM Tris–150 mM NaCl–2 mM EDTA–0.5% Nonidet P-40–100 U of aprotinin per ml and incubated with 10 μl of immune or normal serum (rabbit, mouse, or human) for 3 h at room temperature with constant agitation. Immune complexes were adsorbed with a 10% (vol/vol) suspension of protein A-Sepharose 4B (Pharmacia, Uppsala, Sweden) by rocking overnight at 4°C. For immunoprecipitation with mAb, 5 μl of ascites was added to the precleared leishmanial extract and the complexes were adsorbed with a suspension of protein A-Sepharose 4B previously incubated with a rabbit antiserum against mouse IgM, μ chain specific (Bionetics, Kensington, Md.). Adsorbed antigens were eluted by suspending washed Sepharose pellets in sodium dodecyl sulfate (SDS)-containing slab gel buffer (5) with 20% β-mercaptoethanol and boiled for 3 min. After centrifugation, the cleared supernatants were stored at −20°C for SDS-polyacrylamide gel electrophoresis (PAGE).

SDS-PAGE and autoradiography. SDS-PAGE of the immunoprecipitates was carried out in slabs, using a 10% polyacrylamide gel as previously described (16). Coomassie blue-stained gels were dried under vacuum. Labeled antigens were identified after autoradiography of the dried gels at −70°C, using Kodak X-Omat RP film (Eastman Kodak Co., Rochester, N.Y.) and Cronex intensifying screens (Du Pont de Nemours, Orsay, France).

RESULTS

Polypeptide profiles of promastigotes of *L. donovani*, *L. chagasi*, and *L. infantum*. Under reducing conditions, up to 40 polypeptide bands with molecular weights (M_r) ranging from 14,000 to 130,000 could be clearly distinguished by Coomassie blue staining of the SDS-PAGE analysis of the three subspecies detergent extracts (Fig. 1). The polypeptide profile observed for each parasite was reproducible in experiments performed over a period of several months. Protein patterns of *L. donovani* and *L. chagasi* appeared qualitatively similar (Fig. 1, lanes D and C). A minimum of 36 polypeptide chains were found to migrate with the same mobility. However, the protein pattern of *L. infantum* was clearly different from those of the other two (Fig. 1, lane I). It showed only 45 and 38% homology with the polypeptide profiles of *L. donovani* and *L. chagasi*, respectively.

Surface radioiodinated proteins of *L. donovani*, *L. chagasi*, and *L. infantum*. The specificity of both lactoperoxidase- and IODO-GEN-mediated labeling procedures for cell surface membranes has been demonstrated previously (8, 17). The autoradiographic patterns resulting from SDS-PAGE of the surface promastigotes of the three leishmania stocks, labeled by these two methods, are indicated in Fig. 2. A similar electrophoretic pattern was observed for each subspecies radiolabeled with both the lactoperoxidase and the IODO-GEN procedures. Nevertheless, the labeling yield with IODO-GEN was greater than that with lactoperoxidase (data

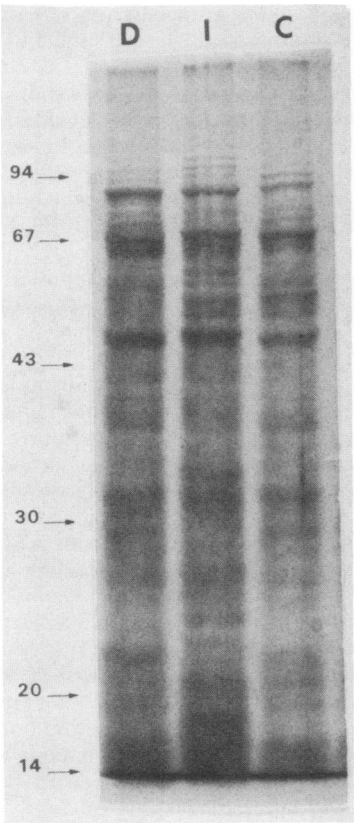

FIG. 1. Coomassie blue-stained patterns of promastigotes of *L. donovani* (lane D), *L. infantum* (lane I), and *L. chagasi* (lane C) separated by SDS-PAGE. The position of migration of marker proteins (M_r, $\times 10^{-3}$) are indicated.

not shown). For this reason, in subsequent experiments all parasites were surface labeled by the IODO-GEN method.

In Fig. 2 (lanes d and c) one can also notice that the patterns of ^{125}I-labeled surface proteins of promastigotes of *L. donovani* and *L. chagasi* are almost identical. Four major bands with apparent M_r of 65,000, 60,000, 50,000, and 26,000 were detected in both parasites. A small variation was sometimes observed in the mobility of the 50,000 and 26,000 M_r polypeptides. The protein profile of radioiodinated surface promastigotes of *L. infantum* was different from those of the other two and only showed two major labeled bands with apparent M_r of 62,000 and a doublet of 26,000 to 23,000 (Fig. 2, lane i). Only the 26,000 M_r labeled component seemed to be common to all three leishmanial subspecies. The labeled surface protein pattern of promastigotes of another strain of *L. infantum* (MO strain) was identical to that of the ITMPA K263 strain (data not shown).

Identification of *L. donovani* subspecies-specific antigens by immunoprecipitation. Precipitates of Nonidet P-40 extracts of ^{125}I-surface-labeled promastigotes of *L. donovani*, *L. chagasi*, and *L. infantum* with rabbit antisera against either *L. chagasi* or *L. infantum* were analyzed in Fig. 3 (lanes J to O). The autoradiographic pattern obtained with *L. donovani* showed that the 65,000, 60,000, and 50,000 M_r proteins were strongly recognized by both rabbit antisera (Fig. 3, lanes J and M). Similar results were observed with *L. chagasi*, which showed an additional band of 43,000 M_r (lanes K and N). With the extract of *L. infantum*, both rabbit antisera precipitated only a major diffused band of 62,000 M_r (lanes L and O). Control precipitations with normal rabbit serum showed a minor nonspecific band in different leishmanial extracts (Fig. 3, lanes d, e, and f).

Immune sera from mice injected with viable promastigotes of either *L. chagasi* or *L. infantum* were also used to precipitate labeled extracts of the different *L. donovani* subspecies. Sera against *L. chagasi* revealed on both *L. chagasi* and *L. donovani* a single major protein of 50,000 M_r (Fig. 3, lanes A, B, D, and E). As noted above, the relative electrophoretic mobility of this band exhibited little variation with *L. donovani*. Nevertheless, the same sera revealed in *L. infantum* two other proteins of 43,000 and 34,000 M_r (Fig. 3, lane F). Mouse immune serum against *L. infantum* precipitated four radioiodinated surface antigens of 43,000, 38,000, 34,000, and 25,000 M_r in the homologous subspecies but failed to recognize the labeled surface proteins of *L. chagasi* and *L. donovani* (Fig. 3, lanes G to I). No radiolabeled proteins were precipitated by normal mouse serum in the different promastigote extracts (Fig. 3, lanes a, b, and c).

Immunoprecipitation of the three leishmanial stocks with human immune sera are also illustrated in Fig. 3 (lanes P to

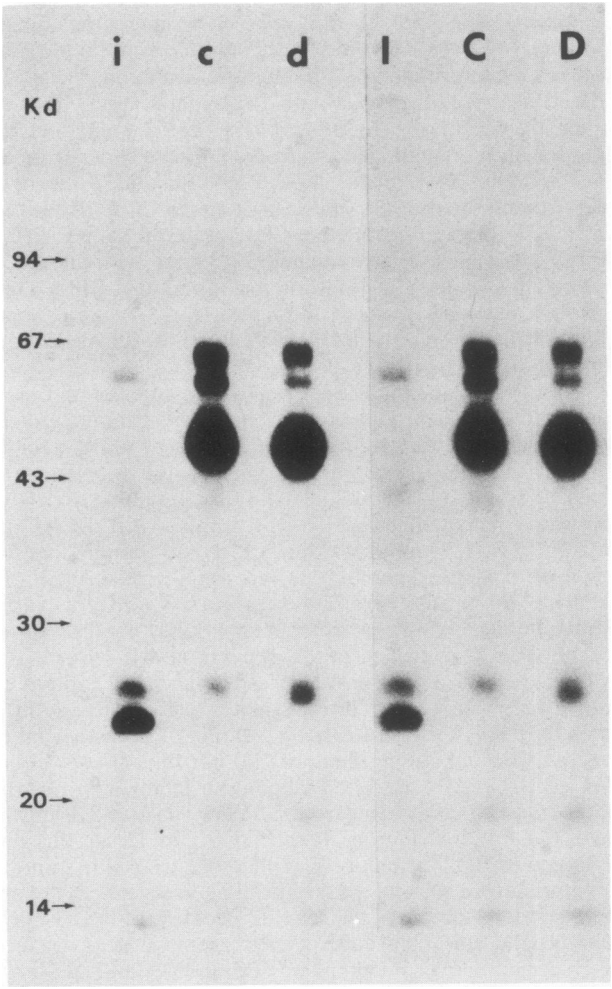

FIG. 2. Autoradiographic patterns of IODO-GEN-mediated ^{125}I-labeled *L. infantum* (lane i), *L. chagasi* (lane c), and *L. donovani* (lane d) promastigotes and respective lactoperoxidase-mediated ^{125}I-radiolabeled promastigotes (lanes I, C, and D) separated by SDS-PAGE. The molecular weights of marker proteins (M_r, $\times 10^{-3}$) are indicated.

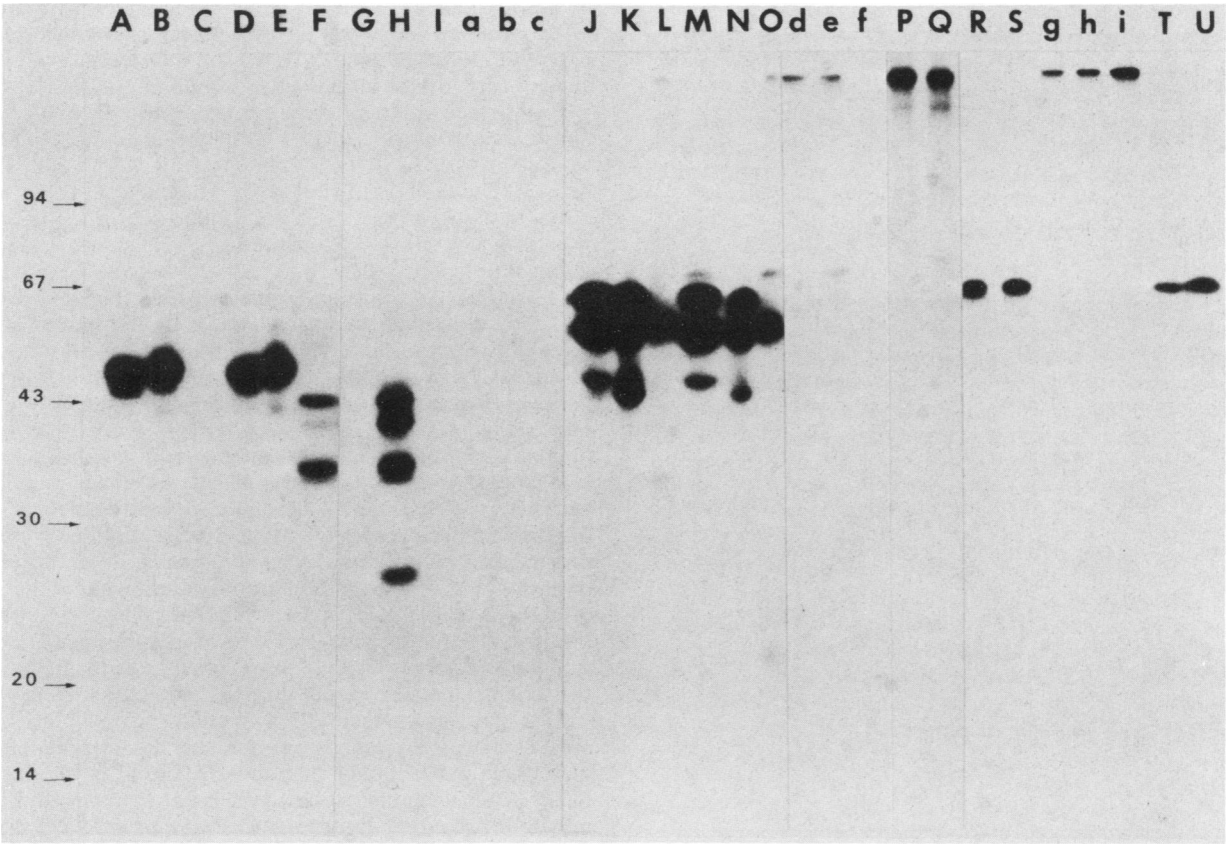

FIG. 3. Autoradiographic pattern of ^{125}I-labeled promastigote surface components precipitated from Nonidet P-40 extracts of different *L. donovani* subspecies with polyvalent immune sera after separation on SDS-PAGE. Precipitation with sera of mice infected after 43 days with *L. chagasi*: lane A, *L. donovani*; lane B, *L. chagasi*; lane C, *L. infantum*. Precipitation with sera of mice infected after 67 days with *L. chagasi*: lane D, *L. donovani*; lane E, *L. chagasi*; lane F, *L. infantum*. Precipitation with sera of mice infected after 67 days with *L. infantum*: lane G, *L. donovani*; lane H, *L. infantum*; lane I, *L. chagasi*. Precipitation with sera of a rabbit hyperimmunized with an antigenic extract of *L. chagasi*: lane J, *L. donovani*; lane K, *L. chagasi*; lane L, *L. infantum*. Precipitation with sera of a rabbit hyperimmunized with an antigenic extract of *L. infantum*: lane M, *L. donovani*; lane N, *L. chagasi*; lane O, *L. infantum*. Precipitation with sera of a patient infected with *L. chagasi*: lane P, *L. infantum*; lane R, *L. donovani*; lane T, *L. chagasi*. Precipitation with sera of a patient infected with *L. infantum*: lane Q, *L. infantum*; lane S, *L. donovani*; lane U, *L. chagasi*. Control precipitations of *L. donovani*, *L. chagasi*, and *L. infantum* with normal mouse (lanes a, b, c), rabbit (lanes d, e, f), and human (lanes g, h, i) sera, respectively.

U). All sera of patients infected with either *L. infantum* or *L. chagasi* recognized strongly the 65,000 M_r antigen in the extracts of *L. donovani* and *L. chagasi*. A demonstrative pattern with two of these sera is shown in Fig. 3 (lanes R, S, T, and U). This major band was absent in the pattern of *L. infantum* extract precipitated with the same human sera which revealed few minor bands (Fig. 3, lanes P and Q). Control precipitations with normal human sera were all negative (Fig. 3, lanes g, h, and i).

Further evidence for the presence of subspecies-specific surface-labeled antigens on promastigotes of the *L. donovani* complex was obtained with an mAb generated against the surface of *L. donovani*. This mAb precipitated only the 65,000 M_r major antigen of *L. donovani* and *L. chagasi* extracts (Fig. 4, lanes A and D). No band was revealed in the lysates of *L. infantum* (Fig. 4, lanes B and C). Another mAb produced against the surface of *L. chagasi* promastigotes precipitated a minor labeled antigen of 70,000 M_r in all subspecies studied (data not shown). Apparently minor nonspecific precipitation with an unrelated mAb occurred only in *L. chagasi* extract (Fig. 4, lanes a, b, c, and d).

Detection of subspecies-specific surface antigens by immunofluorescence. An additional experiment was performed to evaluate by a classical immunological method the surface antigenic differences between *L. infantum* and the other two subspecies from the *L. donovani* complex. For this, polyvalent mouse antisera against glutaraldehyde-fixed promastigotes of *L. chagasi*, *L. infantum*, and *L. donovani* and mAb anti-*L. donovani* were allowed to react with the surface of the three leishmanial stocks by immunofluorescence (Table 1). Polyclonal immune sera against either *L. donovani* or *L. chagasi* presented a good cross-reactivity but failed to react efficiently with promastigotes of *L. infantum*. Furthermore, mouse antisera to *L. infantum* reacted only with the homologous surface. Finally, the mAb against *L. donovani* only recognized the surfaces of *L. donovani* and *L. chagasi*.

DISCUSSION

The present results demonstrate that the surface proteins of promastigotes of *L. infantum* are different from those of the two other subspecies of the *L. donovani* complex, *L. chagasi* and *L. donovani*, which are very similar. This finding was obtained by different technical approaches to protein analysis.

Coomassie blue staining after SDS-PAGE allowed the identification of the predominant proteins present in deter-

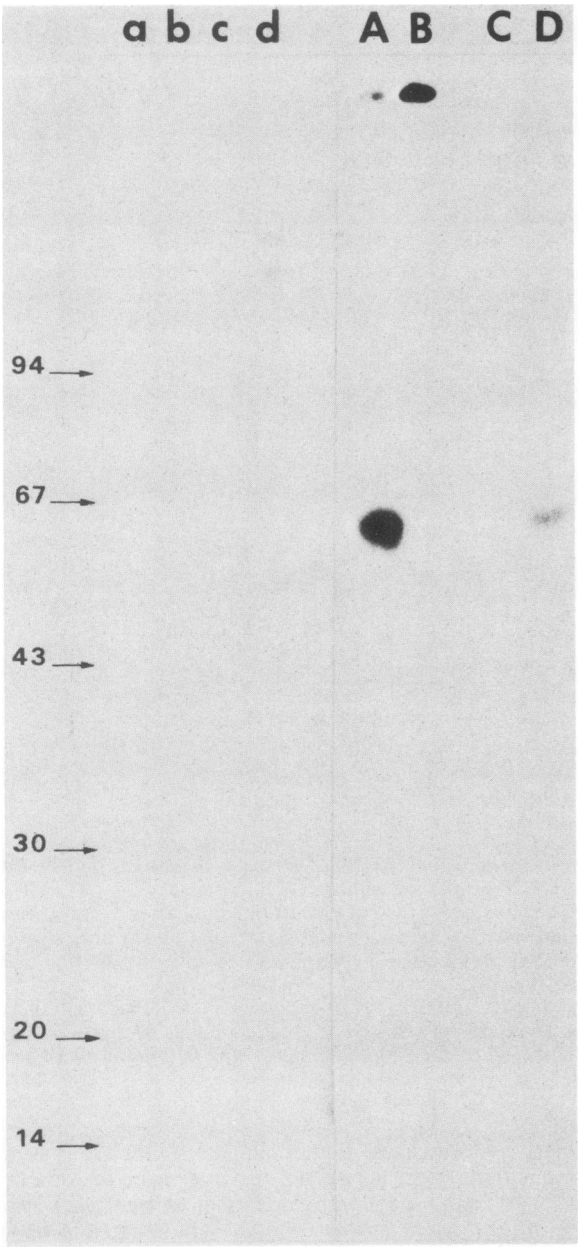

FIG. 4. Autoradiographic patterns of ^{125}I-labeled promastigote surface extracts precipitated with an unrelated mAb (small letters) or with the mAb produced against *L. donovani* (capital letters) after separation by SDS-PAGE. Lane A, *L. donovani*; lane B, *L. infantum* (ITMPA K263 strain); lane C, *L. infantum* (MO strain); lane D, *L. chagasi*.

gent extracts of the parasites. Although these polypeptide chains represent only a small fraction of the total number of promastigote proteins, their electrophoretic analysis already showed homologies between *L. chagasi* and *L. donovani* and strong differences between *L. infantum* and the two others. These preliminary data were further confirmed by analysis of surface-radiolabeled proteins of the three leishmanial subspecies. In fact, by using two different methods of radioiodination (lactoperoxidase and IODO-GEN) which gave identical profiles for each parasite (Fig. 2), the electrophoretic patterns of surface-labeled proteins of *L. donovani* and *L. chagasi* were closely similar and different from those of *L. infantum*. Analysis of both proteins and surface proteins has been previously used to differentiate not only species (9) but also strains of *Leishmania* (9, 10, 21).

The surface polypeptide patterns presented by *L. chagasi* and *L. donovani* were qualitatively similar to those reported by others with *L. donovani* strains from Africa and Brazil (17, 21). Only the doublet of lower M_r, showed previously in different *L. donovani* strains (17) and here in *L. infantum*, was not completely exhibited by *L. chagasi* and *L. donovani*. In our study, only a band with 26,000 M_r was revealed, which could be common to all subspecies of *L. donovani*. Although similarities in M_r cannot be considered as definitive proof of structural identity, the great homology observed between the patterns of *L. chagasi* and *L. donovani* suggests structural relationships among their proteins.

Immunoprecipitation experiments with polyvalent antisera and mAb also showed surface antigenic homologies between *L. chagasi* and *L. donovani* and differences with *L. infantum*. Major cross-reactivities were noticed with immune sera of rabbits hyperimmunized with either *L. infantum* or *L. chagasi*. Both antisera precipitated the same proteins in *L. chagasi* and *L. donovani* and the 62,000 M_r protein of *L. infantum*. This was probably because these rabbits, which received different subcutaneous injections of the total aqueous extract of promastigotes for several weeks before bleeding, presented an immune response no longer restricted to the epitopes exposed on the external surface membrane but including also those exposed on the internal face. When immune sera from both patients and mice infected with either *L. chagasi* or *L. infantum* were used to precipitate the three labeled subspecies of *Leishmania*, the surface antigenic differences between *L. infantum* and the two others were accentuated and only a few cross-reactions were observed. Moreover, an mAb directed against the 65,000 M_r surface protein of *L. donovani* recognized the same molecule on *L. chagasi* but failed to react with *L. infantum*. The possibility of a similar 65,000 M_r surface antigen in *L. infantum* being inaccessible for labeling with ^{125}I was excluded by the immunofluorescence experiments with mouse polyvalent antisera and mAb, which confirmed the surface antigenic relationships between the promastigotes of *L. donovani* and *L. chagasi* and the differences from those of *L. infantum*.

The surface-labeled promastigote antigens precipitated by sera of patients with kala azar were different from those

TABLE 1. Detection of subspecies-specific surface antigens in promastigotes of the *L. donovani* complex by IF, using polyclonal antibodies and mAB

Mouse antibodies against promastigotes	IF titer[a]		
	L. chagasi	*L. donovani*	*L. infantum* ITMPA K263
Polyclonal antisera to			
L. chagasi	1,280	640	40
L. donovani	640	640	80
L. infantum	(−)[b]	(−)	640
Monoclonal ascites to			
L. donovani	640	640	(−)

[a] Values represent the reciprocal titer of the last dilution giving a positive fluorescence.

[b] No positive reaction was observed at a 1:40 dilution.

revealed with sera of mice inoculated with viable promastigotes. It seems that the major antigenic molecule recognized by human antibodies in both *L. chagasi* and *L. donovani* (especially the 65,000 M_r one) is not immunogenic during murine infection and inversely with the 50,000 M_r antigen. This could be related to the susceptibility of these hosts to infection. In fact, the mouse is not an adequate model for visceral leishmaniasis. It is noteworthy that our mAb obtained from mice immunized with fixed promastigotes of *L. donovani* revealed, as did the sera from humans, the 65,000 M_r band.

In previous studies the 65,000 M_r antigen has been shown to be a glycoprotein (17) common to several species of *Leishmania* (8). More recently, Etges et al. (6) found a 63,000 M_r surface antigen in promastigotes from an *L. donovani* stock isolated in France (probably *L. infantum*) which should correspond to the 62,000 M_r antigen presently detected in two strains of *L. infantum*. Furthermore, it has been demonstrated that this protein is structurally related to a 63,000 M_r antigen of *L. major* but different from a component of similar M_r present in *L. tropica* (6). It seems clear that most *Leishmania* species have a major surface-labeled antigen of 62,000 to 65,000 M_r which expresses different levels of antigenic and structural relationships.

Using biochemical methods such as electrophoretic isoenzyme patterns (1, 15) and DNA buoyant density (4), several authors suggested that *L. chagasi* is more related to *L. infantum* than to *L. donovani*. In addition, Desjeux et al. (P. Desjeux, F. Le Pont, S. Mollinedo, and M. Tibayrenc, Ann. Parasitol, in press), investigating 13 isoenzyme patterns of the three *Leishmania* stocks studied here, also noticed a close homology between *L. infantum* and *L. chagasi* versus *L. donovani*. Nevertheless, the present results clearly demonstrate that *L. infantum* is different from the other two subspecies of the *L. donovani* complex as far as surface proteins are concerned. These findings indicate that the results obtained from isoenzyme investigations and from surface antigen studies are different. Both data are complementary and should be further considered in the taxonomy of *Leishmania*.

Finally, that the major surface antigens of *L. infantum* are different from those of *L. chagasi* and *L. donovani* can be related to previous studies suggesting that only species-specific antigens are involved in protective immunity against leishmaniasis (2). These findings imply a correct identification of subspecies-specific immunogens of most *Leishmania* species for immunization purposes.

ACKNOWLEDGMENTS

This work received financial support from the CEE [grant TSD-M-010-F (RS)], the Rockefeller Foundation, and UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

We are indebted to J. A. Rioux who generously provided the MO strain of *L. infantum*, to D. Le Ray who provided rabbit and human immune sera against *L. infantum*, and to Q. Z. Husain, who provided the HS70 strain of *L. donovani*. We also are thankful for the expert technical assistance of Marc Loyens, Didier Deslee, and Vera Vidal and the secretarial help of Claudine Colson and Marie-France Massard.

LITERATURE CITED

1. **Aljebori, T. T.** 1980. Leishmania spp. in Iraq. Electrophoretic isoenzyme patterns. I. Visceral leishmaniasis. Trans. R. Soc. Trop. Med. Hyg. **74**:169–177.
2. **Anderson, S., J. R. David, and D. McMahon-Pratt.** 1983. *In vivo* protection against *Leishmania mexicana* mediated by monoclonal antibodies. J. Immunol. **131**:1616–1618.
3. **Bray, R. S.** 1974. *Leishmania*. Annu. Rev. Microbiol. **28**: 189–217.
4. **Chance, M. L., W. Peters, and L. Shchory.** 1974. Biochemical taxonomy of *Leishmania*. I. Observations on DNA. Ann. Trop. Med. Parasitol. **68**:307–316.
5. **Dissous, C., C. Dissous, and A. Capron.** 1981. Isolation and characterization of surface antigen from *Schistosoma mansoni* schistosomula. Mol. Biochem. Parasitol. **3**:215–225.
6. **Etges, R. J., J. Bouvier, R. Hoffman, and C. Bordier.** 1985. Evidence that the major surface proteins of three *Leishmania* species are structurally related. Mol. Biochem. Parasitol. **14**:141–149.
7. **Galfre, G., S. C. Howe, C. Milstein, C. W. Butcher, and J. C. Howard.** 1977. Antibodies to major histocompatibility antigens produced by hybrid cell lines. Nature (London) **226**:250–252.
8. **Gardiner, P. R., C. L. Jaffe, and D. M. Dwyer.** 1984. Identification of cross-reactive promastigote cell surface antigens of some leishmanial stocks by ^{125}I labeling and immunoprecipitation. Infect. Immun. **43**:637–643.
9. **Handman, E., and R. E. Hocking.** 1982. Stage-specific, strain-specific, and cross-reactive antigens of *Leishmania* identified by monoclonal antibodies. Infect. Immun. **37**:28–33.
10. **Handman, E., G. F. Mitchell, and J. W. Goding.** 1981. Identification and characterization of protein antigens of *Leishmania tropica* isolates. J. Immunol. **126**:508–512.
11. **Howard, R. J., D. C. Kaushal, and R. Carter.** 1982. Radioiodination of parasite antigens with 1,3,4,6-tetrachloro-3 α, 6 α-diphenyl-glycoluril (IODOGEN): studies with zygotes of *Plasmodium gallinacium*. J. Protozool. **29**:114–117.
12. **Keilhly, J. S.** 1976. Infectivity of *Leishmania donovani* amastigotes and promastigotes for golden hamsters. J. Protozool. **23**:244–245.
13. **Kessler, S. W.** 1975. Rapid isolation of antigens from cells with a staphylococcal protein A-antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol. **115**:1617–1624.
14. **Köhler, G., and C. Milstein.** 1975. Continuous culture of fused cells secreting antibody of predefined specificity. Nature (London) **256**:495–497.
15. **Kreutzer, R. D., and H. A. Christensen.** 1980. Characterization of *Leishmania* species by isoenzyme electrophoresis. Am. J. Trop. Med. Hyg. **29**:199–208.
16. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature (London) **227**:680–685.
17. **Lepay, D. A., N. Nogueira, and Z. Cohn.** 1983. Surface antigens of *Leishmania donovani* promastigotes. J. Exp. Med. **157**: 1562–1572.
18. **Le Ray, D., D. Afchain, J. Jadin, A. Capron, S. Yasarol, G. Lanotte, and L. Fameree.** 1973. Diagnostic immunoélectrophorétique de la leishmaniose viscérale à l'aide d'un extrait antigénique hydrosoluble de *Leishmania donovani*. Résultats préliminaires. Ann. Soc. Belge Méd. Trop. **53**:31–41.
19. **McMahon-Pratt, D., E. Bennet, and J. R. David.** 1982. Monoclonal antibodies that distinguish subspecies of *Leishmania braziliensis*. J. Immunol. **129**:926–927.
20. **McMahon-Pratt, D., and J. R. David.** 1981. Monoclonal antibodies that distinguish between New World species of Leishmania. Nature (London) **291**:581–583.
21. **Ramasamy, R., H. Jamnadas, and M. J. Mutinga.** 1981. Proteins and surface proteins of *Leishmania* promastigotes and their possible relevance to the characterization of strains. Int. J. Parasitol. **11**:387–390.
22. **Schnur, L. F., A. Zuckerman, and C. L. Greenblatt.** 1972. Leishmanial serotypes as distinguished by the gel diffusion of factors excreted *in vitro* and *in vivo*. Isr. J. Med. Sci. **8**:932–942.
23. **Shulman, M., H. Nilde, and G. Köhler.** 1978. A better cell-line for making hybridomas secreting specific antibodies. Nature (London) **276**:269–272.
24. **Zuckerman, A., and R. Lainson.** 1977. Leishmania, p. 57–133. *In* J. P. Kreier (ed.), Parasitic protozoa, vol. 1. Academic Press, Inc., New York.