A COMMON MAJOR SURFACE ANTIGEN ON AMASTIGOTES AND PROMASTIGOTES OF *LEISHMANIA* SPECIES

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The pathogenic *Leishmania* cause a wide range of human disease, from cutaneous lesions to generalized visceral involvement. This heterogeneity in clinical expression has led to attempts at classifying *Leishmania* species with more precision. Recent biochemical technologies employing isoenzyme patterns (1), DNA buoyant density (2), restriction endonuclease patterns of kinetoplast DNA (3), as well as immunological methods using monoclonal antibodies (mAb)[1] (4–6), have been described. As yet, none of these have been completely successful, used in isolation, in defining species specificity, and considerable crossreactivity is often seen. Presently, species determination is performed on the basis of three or more of the above methods, in addition to the clinical characteristics of the disease produced in man and the biological behavior of the specific isolate in experimental animals.

We have recently reported (7) that the surface profile of a number of *L. donovani* isolates with worldwide distribution displayed as a major surface component a glycoprotein (gp) of M_r 65,000. It was the major antigen recognized by the sera of patients with visceral Leishmaniasis, and was also immunoprecipitated by the sera of patients with other clinical forms of Leishmaniasis (7, 9). This is in keeping with the presence of major shared antigen(s) involved in protection to different forms of Leishmaniasis. The many instances in which crossimmunity between Leishmanial species have been described serologically and clinically substantiate this hypothesis, both by the extensive serological crossreactivity observed in patients' sera, by the presence of natural crossprotection (9–12), and more recently, by the striking results with experimental crossimmunization with irradiated promastigotes (13–15).

[1] *Abbreviations used in this paper:* CSDM, Complete Schneider's Drosophila medium; gp, glycoprotein; HBSS, Hank's balanced salt solution; HIFBS, heat-inactivated fetal bovine serum; mAb, monoclonal antibody; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

TABLE I

Leishmania Species Used in This Study

Leishmania species	Human host	Strain	Reference	Abbreviation
donovani	SU	Khartoum	19	Ld
mexicana amazonensis	BR	Josefa	20	Lma
mexicana mexicana	PA	1 VLM	21	Lmm
major	SN	MHOM/SN/00/DK 106	22	Ltm
braziliensis guyanensis	SR	MHOM/SR/80/CUMC 1	*	Lbg
braziliensis guyanensis	SR	Cloned from above	*	Lbg
braziliensis braziliensis	PE	MHOM/PE/83/CUMC 2	‡	Lbb

* Galvao-Quintao et al.[2]

‡ Lbb was isolated by J. Keithly from a 50-yr-old male ornithologist who presented with two cutaneous pustules on W3 right leg after collecting on the Andean slopes in Peru in 1983. Promastigotes were isolated from the lesion in blood agar cultures overlaid with HBSS, and in CSDM isoenzyme. mAb and kinetocore DNA typing (R. D. Kreutzer, Youngstown State Univ., OH; D. MacMahon Pratt and D. Wirth, Harvard Univ., Cambridge, MA, respectively) confirmed the isolate to be Lbb. Initial cultures and those subsequent to a full course of antimony treatment (Pentostain; Burroughs-Wellcome, Beckenham, United Kingdom) were positive. The patient was cured with Amphotericin B. Additional clinical information on the patient is available.

In addition, it has been reported recently (16) that mAb and rabbit sera raised against promastigotes of one *Leishmania* species recognized similar surface determinants in others.

Herein, we extend our initial observations on the 65,000 M_r glycoprotein, and show that it is a major surface determinant on both promastigotes and amastigotes of *L. donovani* (Ld), *L. mexicana amazonensis* (Lma), *L. mexicana mexicana* (Lmm), *L. major* (Ltm), *L. braziliensis guyanensis* (Lbg), and *L. braziliensis braziliensis* (Lbb). Patients convalescing from both cutaneous and visceral forms of the disease contain antibodies that react with this determinant. Finally, the glycoproteins from various species exhibit strong homologies, as demonstrated by Cleveland mapping, physicochemical properties, and immunorecognition.

Materials and Methods

Parasites.   Nine different isolates of *Leishmania* were used in this study. Of these, seven have been characterized by at least three of the following methods: isoenzymes (1), kinetoplast DNA buoyant density (2), mAb typing (4), and growth in phlebotomid sandflies (17). All have been characterized both by growth in blood agar (18) overlaid with Hanks' balanced salt solution (HBSS), and in complete Schneider's drosophila medium (CSDM) (Gibco Laboratories, Grand Island, New York) with 15% (vol/vol) heat-inactivated fetal bovine serum (HIFBS) (Hyclone, Sterile Systems Inc., Logan, UT) plus 100 U penicillin and 100 μg streptomycin per milliliter, in outbred golden hamsters (LVG:LAK, Lakeview Farms, Wilmington, MA), and inbred BALB/c/ByJ mice (The Jackson Laboratory, Bar Harbor, ME), and have been maintained as frozen stabilates in liquid nitrogen. The seven strains are listed in Table I. The Chinese strain of Ld (*L. donovani infantum*) was kindly provided by Dr. K. P. Chang, Dept. of Microbiology, Chicago Medical School, North Chicago, IL. The Brazilian strain of Ld (HB1) was isolated from a human case in Rio de Janeiro, and was obtained from Dr. Mauro Marzochi (Fundacao Oswaldo Cruz, Rio de Janeiro, Brazil) (23). Promastigotes of each species were cultured to stationary phase in CSDM (5–7 d), and were harvested by centrifugation at 500 *g* for 10 min at 4°C.

Amastigotes were obtained from the macrophage cell line J774 1N-BALB/c (24), infected 4 d previously with stationary phase promastigotes at a 10:1 parasite/cell ratio, except for Ltm, where the cultures were infected with amastigotes obtained from lesions. These were purified from lesions at the base of the tail of BALB/c mice, which had been injected intradermally 8–12 wk previously with a 0.1 ml suspension containing 3×10^7 primary culture promastigotes from CSDM. Lesions were aseptically removed, weighed, and homogenized, as previously described (25). Amastigotes were separated from tissues by centrifugation at 300 g for 10 min at 4°C. The amastigote-containing supernatant was centrifuged at 1,000 g to pellet the cells. When necessary, erythrocytes were lysed by adding freshly prepared 0.83% NH_4Cl for 3 min at 4°C, washing by centrifuging three times with 40 ml HBSS at 1,000 g for 10 min. Amastigotes thus obtained were >85% viable, as measured by Erythrosin B dye exclusion (26). The macrophage cell line was cultured in RPMI 1640 containing 10% (vol/vol) HIFBS, 2 mM glutamine, 25 mM Hepes, under 5% CO_2 at 35°C, at an initial density of 10^6 parasites/ml. Amastigotes were harvested by cooling the infected macrophages at 4°C for 15 min, scraping them with a rubber policeman, and centrifuging at 1,000 g for 10 min. At this time, no promastigotes were present. Cells were then lysed by exposure to 2 ml of a hypotonic solution (42.5 mM KCl, 5 mM $MgCl_2$) (27), and rapidly passing them seven times through a 23- and a 25-gauge needle. Osmotic pressure was restored by immediately raising the volume to 40 ml HBSS. Amastigotes were separated from lysed and intact macrophages by centrifugation at 1,000 g for 10 min at 4°C. Parasites were resuspended in 5 ml HBSS and the total number of viable cells determined by Erythrosin dye exclusion in a Neubauer haemocytometer.

Human Immune Serum. Sera were obtained from eight patients hospitalized with Kala-azar (K) or cutaneous (tegumental) Leishmaniasis (T). These sera were the gift of Dr. Mauro Marzochi (Fundacao Oswaldo Cruz, Rio de Janeiro, Brazil). Patients were recruited from a local focus of infection (23), and clinical histories were available. Additional Kala-azar sera were obtained from six patients in East Africa (A), diagnosed as Kala-azar, but without detailed clinical documentation. Sera were heat-inactivated (56°C for 30 min) before incubation with live, biosynthetically labeled promastigotes.

Cell Surface Iodination. Promastigotes were washed twice in HBSS by centrifugation at 500 g for 10 min. Surface iodination was performed as previously described (28). Lactoperoxidase was purchased from Calbiochem-Behring Corp. (San Diego, CA); glucose oxidase (Type V) from Sigma Chemical Co. (St. Louis, MO); $Na^{125}I$ from New England Nuclear (Boston, MA). After iodination, >95% of the parasites remained viable and actively motile. Free ^{125}I was eliminated by washing in HBSS. Cells were lysed in phosphate-buffered saline (PBS) containing 0.5% Nonidet P-40 (Bethesda Research Laboratories, Gaithersburg, MD), 100 U/ml aprotinin, and 1 mM phenylmethylsulphonyl fluoride (Sigma Chemical Co.).

Biosynthetic Labeling. 2×10^8 promastigotes were washed as above in HBSS. Parasites were then resuspended in 4 ml of Schneider's methionine-free medium (Gibco Laboratories) supplemented with 15% heat-inactivated fetal bovine serum (previously dialysed against PBS), and 150 μCi of [^{35}S]methionine (500 μCi/mmol) (Amersham Corp., Arlington Heights, IL). After 12 h of incubation at 26°C, cells were washed in HBSS, aliquots of live ^{35}S-labeled parasites were removed for immunoprecipitation of surface components, and those remaining were lysed as described above.

Immunoprecipitation. Radiolabeled promastigote lysates were clarified by centrifugation at 23,000 g for 20 min, at 4°C. 50-μl samples of the supernatants were incubated with 5 μl of human sera for 1 h at 4°C, with constant agitation. Antigen-antibody complexes were precipitated with 20 μl of a 30% (vol/vol) solution of Protein A–Sepharose 4 B in PBS, as previously described (29, 30). Antigens were eluted in 50 μl of electrophoresis sample buffer (2% sodium dodecyl sulfate [SDS], 12% sucrose, 0.01% bromophenol blue, 50 mM dithiotreitol, and 50 mM Na_2CO_3 buffer, pH 8.6) and boiled for 2 min. Samples were stored at −70°C until used.

For isolation of [^{35}S]methionine-labeled surface components, live radiolabeled promastigotes were resuspended in 0.5 ml of PBS containing 100 μl of human immune serum

and incubated for 30 min at 4°C, with gentle agitation. After washing by centrifugation (500 g for 10 min), lysates were prepared, clarified by centrifugation (23,000 g for 20 min), and immunocomplexes were isolated as above.

Polyacrylamide Gel Electrophoresis (PAGE). Radiolabeled proteins were electrophoresed in 1-mm thick, 7.5–11% polyacrylamide gradient slab gels (31). Gels of ^{35}S-labeled proteins were processed for fluorography (32) and exposed on prefogged Kodak X-Omat R-1 film at −70°C. ^{125}I gels were autoradiographed using Kodak XAR-5 film with Dupont Cronex Lightning Plus intensification screens (Dupont Instruments, Wilmington, DE).

Peptide Analysis. Quintuplet samples of ^{125}I-labeled promastigotes were electrophoresed as described above. A lane containing molecular weight standards was removed, stained briefly, and used as a reference to localize the region between standards of M_r 43,000–67,000 in the remaining gel. This section for each lane was cut into 1-mm slices, which were subsequently counted in a gamma counter. The slices containing the highest number of counts per minute were used in a modified Cleveland peptide map (34), as described by Piperno et al. (35). Briefly, the gel slices were placed in the sample wells of a second 7.5–15% polyacrylamide slab gel, without prior elution. The slices were previously quickly immersed in a buffered solution (1 mM EDTA, 0.1% SDS, and 0.05 M Tris/H$_2$SO$_4$, pH 5.8, with 0.0001% bromophenol blue), and pushed down the bottom of the sample wells. The wells were then overlaid with 10 μl of the same buffer containing 20% glycerol, and finally 10 μl buffer with 10% glycerol, to which appropriate amounts of *S. aureus* V8 protease (Miles Laboratories, Inc., Elkhart, IN) were added (0, 0.001, 0.01, 0.1, or 1 μg/well). Electrophoresis was started, interrupted for a 75-min incubation when the samples were focused at the interface between the stacking gel and the running gel, then continued until completion.

Monoclonal Antibody. mAb 6H12 (36) developed against *L. mexicana amazonensis* was kindly provided by Dr. K. P. Chang.

Results

Externally Disposed Polypeptides of Leishmania Promastigotes. Promastigotes were surface-iodinated at mid–log growth phase, and the labeled peptides were then subjected to 7.5–11% gradient SDS-PAGE, under reducing conditions. The resulting pattern as seen in Fig. 1 for Lbb (Fig. 1, *A 1*), *L. donovani* HB1 (*B, 1*), and Ltm (*C, 1*), shows that only a few surface components are labeled by this method. Among these, a peptide of M_r 65,000 is the most prominent band in all strains analyzed. This may occur in association with a band of M_r 59,000 (not seen in this case). A 50,000–55,000 M_r band is seen with variable intensities, and it will be discussed below. A similar profile is found in all other species examined. It can also be seen in Fig. 1 that, even when the gels are exposed for longer periods of time, only a few additional bands of lower intensity are observed (Fig. 1, *A 3, B 3*, and *C 3*) of M_r 45,000, 35,000, 32,000, and 20,000.

Immunoprecipitation by Convalescent Sera from Patients with Cutaneous and Visceral Disease. Immunoprecipitation patterns using sera from patients with Kala-azar (K), or cutaneous disease (T) reveal a consistent pattern, demonstrating the recognition of the 65,000 M_r gp by all immune sera. Some are represented in Fig. 1 (*A 2, B 2*, and *C 2*). The Kala-azar serum recognized the 65,000 M_r gp of Lbb less intensely than it did the homologous strain (Ld) or Ltm. This seems to be a consistent finding. This indicates the immunogenicity of the 65,000 M_r gp in various clinical forms of the disease and in a worldwide distribution.

Externally Disposed Polypeptides of Leishmania Amastigotes; Surface Iodination Profile. Amastigotes obtained from J774-infected cultures were surface iodinated, and the labeled peptides were analyzed in 7.5–11% PAGE. Fig. 2 shows
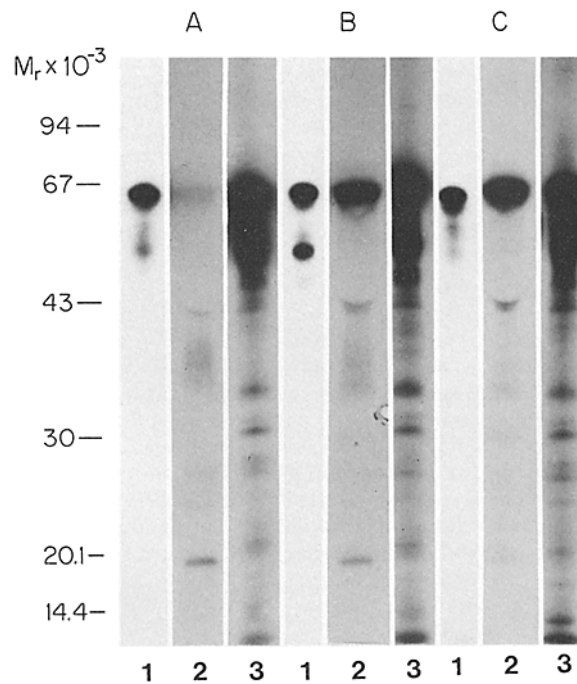
FIGURE 1.   Surface iodination and immunoprecipitation profiles of *Leishmania* species promastigotes under reduced conditions. *A*, Lbb; *B*, Ld HB1; *C*, Ltm. Lane 1, Autoradiographic pattern of whole cell lysate (6-h exposure); lane 2, immunoprecipitation of each strain with convalescent sera from Brazilian patients with visceral leishmaniasis; lane 3, as in 1, but with a 48-h autoradiographic exposure.

the resulting pattern for Lma (Fig. 2 *b*), Lmm (*c*), Lbg (*d*), and Ltm (*e*). As with promastigotes, the surface profile reveals relatively few surface components, and the major peptide displays an M_r of 65,000. The associated band at 59,000 M_r is seen with variable intensities in the different isolates, as is the 50,000 M_r. Fig. 2*a* shows the profile of an iodinated sample that consisted of a preparation obtained under the same experimental conditions, but prepared from an uninfected J774 culture. Fig. 2*f* shows the immunoprecipitation pattern of the sample of Fig. 2*e* (Ltm), when exposed to a serum obtained from one of the Kala-azar patients from East Africa (A_6).

Cleveland Map Profile of the 65,000 M_r gp in Promastigotes of Different Leishmania Species.   We further evaluated the seeming identity of the 65,000 M_r gp of the different *Leishmania* species by Cleveland maps. The proteins were cut from gels and digested with increasing concentrations of V8 protease. The peptides resulting from such digestion were analyzed by 7.5–15% SDS-PAGE and the patterns obtained are shown in Fig. 3. Under the experimental conditions used in the assay, enzyme concentrations of as little as 0.001 μg per sample initiate proteolysis, generating peptides of M_r 48,000, 33,000, and 22,000. A comparison of Fig. 3 *A–D* reveals the strong homology between the different *Leishmania* gp, where Fig. 3*A* is Lbg, *B* is Ltm, *C* is Ld (Khartoum), and Fig. 3*D* is Ld (HB1). Similar results were obtained for Lmm and Lbb (not shown). The striking homology patterns and the microheterogeneities observed among the different
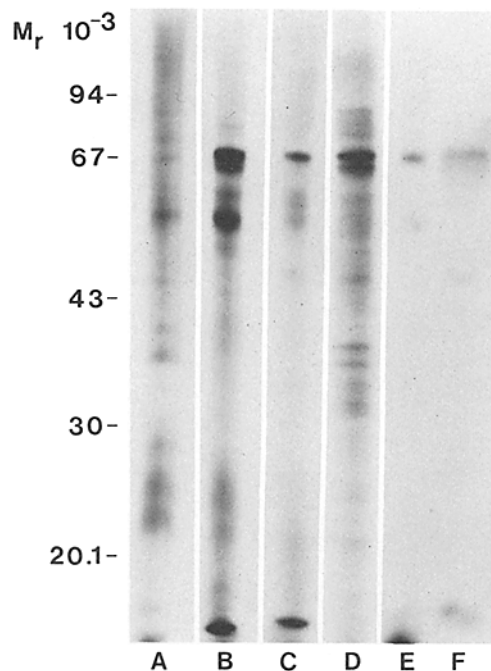
FIGURE 2. Surface iodination and immunoprecipitation profiles of *Leishmania* species amastigotes purified from infected J774 cells. *A*, Mock-purified amastigotes from uninfected J774 cells; *B*, amastigotes from Lma; *C*, Amastigotes from Lmm; *D*, Amastigotes from Lbg; *E*, Amastigotes from Ltm; *F*, Immunoprecipitation of *E* with an East African Kala-azar patient's immune serum.

species and isolates are suggestive of a basic peptide core and possible differences in glycosylation.

Surface Profile under Incomplete or Nonreducing Conditions, and Variability of Labeled Peptide Pattern. The surface profile for several *Leishmania* species described in previous reports (37–42) revealed considerable variability and complexity. These results could be explained by two of our findings. The first is the shift initially observed for the Ld 65,000 M_r gp to an M_r of 50,000 under nonreducing conditions (7). This is seen in Fig. 4, where under reduced conditions (Fig. 4A), the major iodinated surface component is seen as a 65,000 M_r band. Under nonreducing conditions (*C*), the same sample is seen as a 50,000 M_r band. However, under partially reduced conditions (i.e., reducing agent not added immediately before running the sample), a range of bands is observed between 65,000 and 50,000 M_r. It is also seen that the recognition by human immune serum (Kala-azar serum, K1) is preserved under all three conditions. This is further documented in Fig. 5 for other *Leishmania* species. Incomplete reduction leads to the appearance of a complex pattern of bands (Fig. 5, part *I*). This can be seen in *A* for Lmm, where most of the label is seen at 50,000 M_r, in *B* for Ld HB1, in *C* for Lbg (wild type) and in *D* for Lbg (clone 8-3). In the last three, a number of bands occur, ranging from 65,000 to 50,000 M_r. Fig. 5, part *II* illustrates the surface pattern of the same isolates under nonreducing conditions. In this case, all of the 65,000 M_r gp has shifted to M_r 50,000, and the
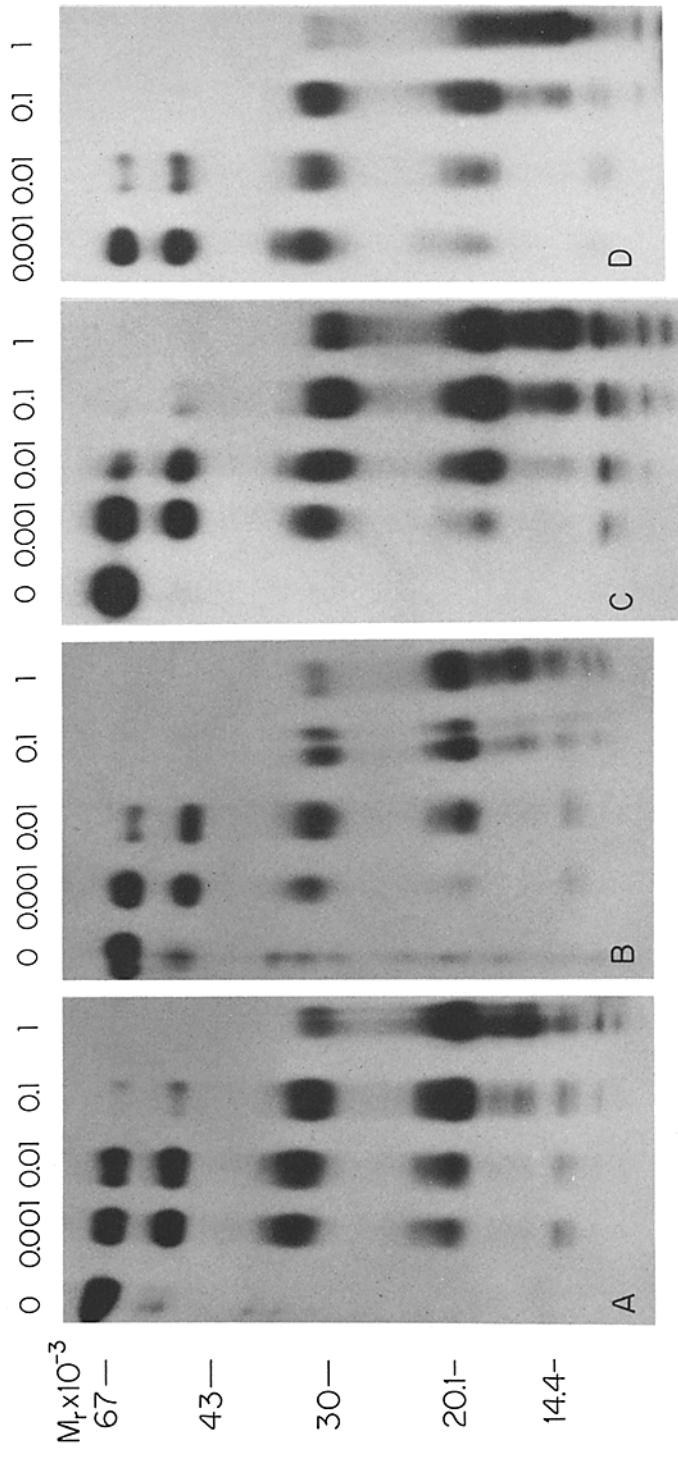
FIGURE 3. *S. aureus* V8 protease peptide profile of ^{125}I-labeled 65,000 M_r gp from *Leishmania* species promastigotes. *A*, Lbg; *B*, Ltm; *C*, Ld Khartoum; *D*, Ld HB1. Numbers on top correspond to concentration (in micrograms) of V8 protease added per lane.
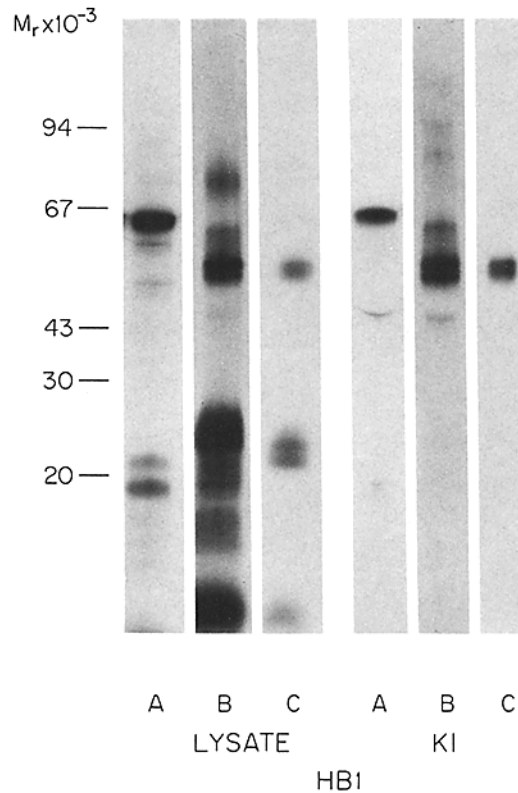
FIGURE 4. Surface iodination profile of Ld HB1 under completely reduced conditions (A), incompletely reduced conditions (B), and nonreducing conditions (C). The left panel shows the patterns for whole cell lysates; right panel shows the pattern for immunoprecipitates obtained with serum from a Brazilian Kala-azar patient (patient 1).

surface-labeled profile is much simpler. The reason for these differences is not yet understood. Fig. 6 shows for other *Leishmania* species that recognition by human immune serum (K and T) is not affected by the nonreducing conditions. Identity of the 50,000 M_r gp with the 65,000 M_r gp is further supported by its recognition using mAb 6H12 which recognizes a glycopeptide triplet of M_r 65,000–68,000 in Lma (36) (Fig. 6 M). Interestingly, the same mAb strongly recognizes the 50,000 M_r component in *L. donovani infantum*, under nonreducing conditions.

The second of our findings, which could explain some of the complex patterns described in previous reports (36–41), refers to the different cellular fragility of the several *Leishmania* species. Mechanical damage during the course of centrifugation leads to endoproteolysis of the M_r 65,000 gp, and optimal experimental conditions vary from one species to another. In Fig. 6, the immunoprecipitates show the recognition of components of M_r 20,000, 32,000, and 35,000, which may be represented at variable intensities from experiment to experiment. These seem to result from various degrees of endoproteolysis, on the basis of homologies found by peptide map analysis (data not shown).
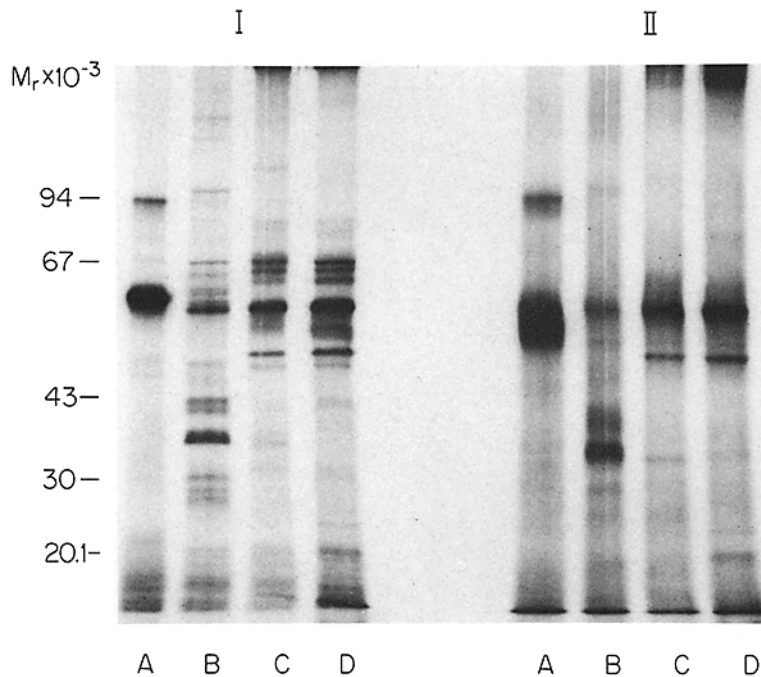
FIGURE 5.   Surface iodination profile of *Leishmania* species promastigotes under conditions of incomplete reduction (*I*) or nonreducing conditions (*II*). *A*, Lmm; *B*, Ld HB1; *C*, Lbg (wild type); *D*, Lbg clone 8-3.

Biosynthetic Labeling of Promastigotes of Leishmania Species.   To establish the parasite origin of the major iodinated polypeptide, and to confirm its external disposition on the parasite surface, promastigotes of the different *Leishmania* isolates were labeled with [^{35}S]methionine. The live, labeled organisms were then incubated with human immune serum at 4°C, washed to remove unbound antibodies, lysed, and the immunocomplexes were isolated and analyzed in SDS-PAGE. Fig. 7 highlights these results for Lbg. The incorporation of label in the total lysates is seen in Fig. 7 *C*. This contrasts both with the immunoprecipitation by a human immune serum from an African Kala-azar patient, which binds to only a few of these labeled components, in particular a band of M_r 65,000 (Fig. 7 *B*), and with normal human serum, which precipitates no bands (*A*). Identical results were obtained with all other species of *Leishmania* tested (not shown). These data agree with previous results for Ld (7).

Discussion

This study was aimed at comparing the polypeptide surface profile of amastigotes and promastigotes of several *Leishmania* species and to characterize those that were recognized by antibodies in sera from convalescent patients. Previous work from this laboratory (7) had provided evidence that the major surface glycoprotein of Ld promastigotes was recognized by sera from patients with clinical forms of Leishmaniasis other than Kala-azar, suggesting that this same antigen might be common to other *Leishmania* species.
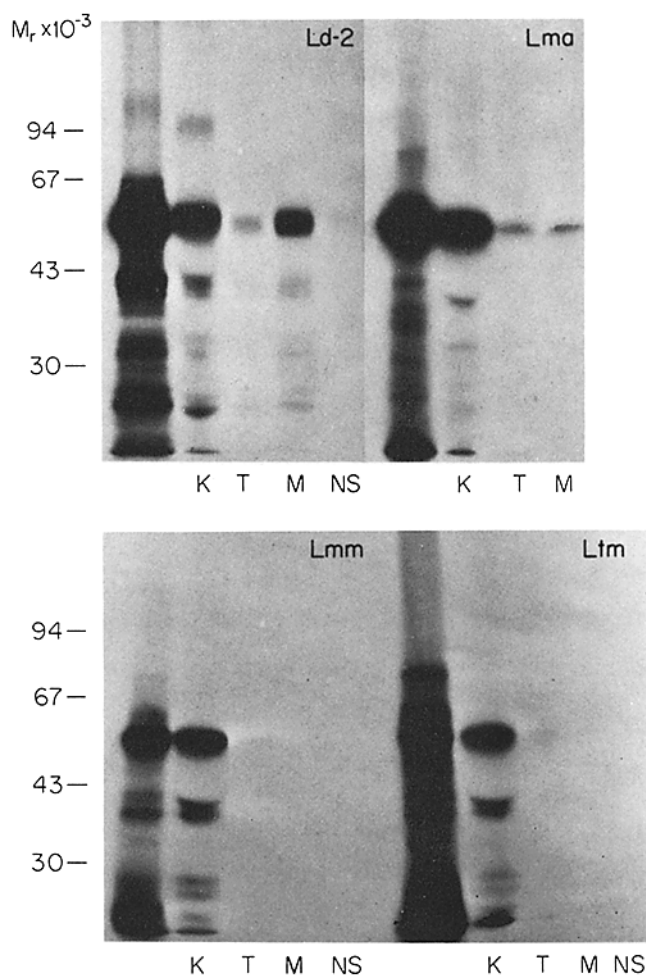
FIGURE 6.   Immunoprecipitation profiles of *Leishmania* species promastigotes under nonre-
ducing conditions. *Ld-2*, Ld *infantum*; K and T as described in Materials and Methods. NS,
normal human serum; M, monoclonal antibody 6H12.

Using surface radioiodination, we have now shown that the exteriorly disposed
glycopeptide profile of amastigotes and promastigotes of six different species of
Leishmania are remarkably similar, and display as the major determinant a
glycoprotein of M_r 65,000. This component was a potent immunogen in man
and was recognized by sera from patients with homologous as well as heterologous
infection. In addition, we have provided evidence that the major 65,000 M_r gp
in the different *Leishmania* species is not only similar in M_r and in displaying
crossreactive epitopes, but also homologous in molecular nature. The evidence
relies on two parameters: (*a*) behavior under reducing and nonreducing electro-
phoretic conditions, and (*b*) Cleveland mapping. Indeed, we have observed, in
all species and isolates examined, the same shift in the major immunogenic
determinant from 65,000 to 50,000 M_r under nonreducing conditions. The
presence of a sporadic band at M_r 50,000 in the reduced gels may represent
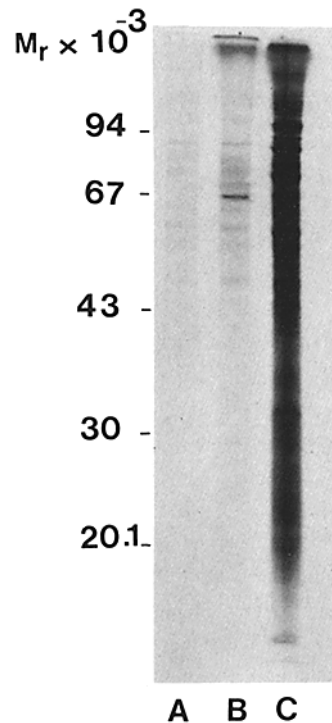
FIGURE 7. Biosynthetic labeling of promastigotes of Lbg with [^{35}S]methionine. *A* and *B*: Immunoprecipitation of live labeled parasites incubated with normal human serum (*A*) or with an East African Kala-azar patient's serum (patient A6) (*B*). *C*, Incorporation of label in the total parasite lysate.

incomplete reduction of sulfhydryl groups or, as will be discussed below, the result of some degree of endoproteolysis in the samples. V8 protease Cleveland maps of the major iodinated band revealed a nearly identical peptide pattern for all *Leishmania* species, indicating the homologies in the molecular nature of the 65,000 M_r gp. The major peptides generated by partial V8 proteolysis (48,000, 33,000, and 22,000 M_r) are frequently observed in the surface iodination profiles when care is not taken to avoid proteolysis. This is in keeping with some divergent results in the literature, which display quite complex and distinct surface profiles for different *Leishmania* species (36–40). In sample handling, therefore, several points should be stressed: (*a*) the fragility of the cells during the washing steps, which will result in cell damage, release of lysosomal enzymes and endoproteolysis, with some *Leishmania* species being more susceptible to mechanical damage than others, (*b*) the necessity of keeping the samples at 4°C in the presence of protease inhibitors, and (*c*) the need to insure proper reduction of the samples.

Our data are apparently contrary to most of the available evidence and concept in terms of *Leishmania* surface antigens, their species uniqueness, and role in producing disease and/or immunity. On the other hand, it can be well reconciled with the majority of the available data (6, 36–41) when analyzed in the light of the above findings. A major surface protein so conserved among *Leishmania* species must fulfill an important biological role in the host-parasite interaction.

The different biological behavior of different *Leishmania* species and isolates, as well as their precise immunological specificities, could still be adequately explained by different glycosylation of the same core peptide.

Biosynthetic labeling with [^{35}S]methionine and immunoprecipitation of the live organisms with human immune sera revealed that, among the predictably high number of proteins labeled, the major protein accessible to the sera and, therefore, on the surface, was the 65,000 M_r gp. The glycoprotein nature of this polypeptide has been determined by us (7, 8) and others (36, 39, 41) on the basis of its specific binding to Con A.

Undoubtedly, there are surface components characteristic for each species and even isolates of *Leishmania*, but those seem to represent minor determinants (4–6). The striking feature demonstrated here is the similarity, rather than differences, among all the *Leishmania* species examined. These are all human pathogens isolated from worldwide cases of human disease. The recognition of the major surface component by sera from patients with all forms of Leishmaniasis indicates its role as the immunodominant antigen in *Leishmania* species.

The presence of crossreactive determinants in *Leishmania* stocks has long been known. Serological crossreactivities have been observed by many immunological techniques, including the generation of mAb (4, 5, 42, 43). In the latter, the majority of antibodies obtained crossreact with most species, rather than showing species specificity. Crossprotection, although not the rule, has been extensively demonstrated both in human and experimental infections (9–15). Of particular relevance are the results of Howard and coworkers (13–15), where prophylactic immunization of mice with irradiated promastigotes of Ltm induced protection and long-lasting immunity effective against both promastigote and amastigote challenges, and detectable not only against Ltm but also Lmm, Lma, and *L. braziliensis panamensis*. In addition, substantial protection could also be induced by irradiated promastigotes of Ld. These results are in keeping with the existence of common major surface determinant(s) present in promastigotes and amastigotes of all *Leishmania* species, which results in induction of protective immunity. Although other authors (13–15) have established that the resulting protective immunity is not mediated by antibodies, it is likely that the same immunogenic determinants are involved in induction of the protective cell-mediated responses. As we have previously suggested (7, 8), small differences in glycosylation could account for the differences in immunorecognition and biological behavior observed among *Leishmania* species. An assessment of the role of the 65,000 M_r gp in the induction of protective immunity to *Leishmania* infection is presently being investigated.

Summary

Enzymatic surface iodination and biosynthetic labeling with [^{35}S]methionine, combined with immunoprecipitation by sera from patients with different forms of Leishmaniasis revealed a 65,000 M_r glycoprotein as the immunodominant moiety in promastigotes and amastigotes of the nine *Leishmania* species and isolates examined. Sera from patients with one form of Leishmaniasis recognized this component strongly, not only in the homologous, but also in the heterologous species.

In addition to the crossreactivity displayed by immune sera, the 65,000 M_r glycoprotein (gp) common to all *Leishmania* species presented a characteristic shift to M_r 50,000 when samples were run in sodium dodecyl sulfate–polyacrylamide gel electrophoresis under nonreducing conditions. These results are in agreement with our previous studies (7), where a simple and similar profile was obtained for several geographic isolates of *L. donovani*, with a major surface glycoprotein of 65,000 M_r displaying the same characteristics described here.

The structural similarity of the major 65,000 M_r gp of the six *Leishmania* species was demonstrated by Cleveland mapping. It is suggested that immunological specificities may be contributed by minor differences in glycosylation of this molecule. In keeping with recent data (13–15), where strong crossprotection among different *Leishmania* species has been obtained by prophylactic immunization with irradiated whole promastigotes, this glycoprotein may be a good candidate for an antigen to be used for immunoprophylaxis of all forms of Leishmaniasis.

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