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STUDIES ON THE RELATIONSHIP BETWEEN LECTIN BINDING CARBOHYDRATES AND DIFFERENT STRAINS OF *LEISHMANIA* FROM THE NEW WORLD

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The culture forms of L. mexicana pifanoi *(LRC L-90),* L. mexicana mexicana *(LRC L-94, M-379);* L. braziliensis braziliensis *(LRC L-77, L-1, M-2903, H-LSS) and* L. mexicana amazonensis *(H-JMMO, M-JOF, H-21, H-PLL, M-1696) were tested with the following lectins:* Canavalia ensiformis, Ricinus communis-*120,* Axinella polypoides, Phaseolus vulgaris, Evonymus europaeus, Lotus tetragonolobus, Dolichos biflorus, Aaptos papillata *II,* Laburnum alpinum, Ulex europaeus, Arachis hypogaea *and* Soja hispida.

All examined strains of Leishmania were agglutinated by C. ensiformis, R. communis-*120 and* A. popypoides. *No agglutination reactions were observed with* P. vulgaris, D. biflorus, A. papillata *II,* E. europaeus *and* L. tetragonolobus. *Only* L. m. pifanoi *and the* L. m. amazonensis *strains H-JMMO and MJOF showed agglutination reactions with* S. hispida, U. europaeus, L. alpinum *and* A. hypogaea, *while* L. m. mexicana *(LRC L-94; M-379) strains,* L. b. braziliensis *H. LSS, LRC L-77; L-1; M-2903 and the* L. m. amazonensis *strains, H-PLL, H-21, M-1696 showed no agglutination reactions with these four lectins.*

Polysaccharides, glycoproteins and lipopolysaccharides are components of cell membrane-surfaces. The agglutination test with lectins is a method to demonstrate membrane-fixed carbohydrates (Cohen, 1974; Gold & Balding, 1975; Goldstein & Hayes, 1978). In *Salmonella* characterization every serotype corresponds to a chemotype which is defined by specific carbohydrate compositions (Uhlenbruck, 1971). Martinez-Palomo, A.; Gonzales-Robles, A. & Torre de la, M. (1973) could differentiate between pathogenic

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and non pathogenic strains of *Entamoeba histolytica* using *C. ensiformis* (Con A). Dawidowicz, K.; Hernandez, A. G. & Infante, R. B. (1975) were able to differentiate between pathogenic and non pathogenic strains *L. braziliensis* using lectins. The characterization of *Leishmania* strains was possible whith a carbohydrate-rich excretion factor (EF) excreted by leishmania in vivo and in vitro (Schnur, L. F.; Zuckerman, A. & Greenblatt, Ch. L., 1972; Schnur, 1974; Schnur & Zuckerman, 1977; Decker & Janovy, 1974). Alves & Colli (1974) found differences between the blood forms and culture forms of *T. cruzi* using Con A. Pereira et al (1980) found differences between trypomastigote, epimastigote and amastigote stages of *T. cruzi* using lectins. Mühlpfordt & Schottelius (1977) and Bretting & Schottelius (1978) were able to point out differences between *T. rangeli, T. conorhini, T. cruzi,* and *T. cruzi*-like strains using the lectin of the marine sponge *Aaptos papillata.*

Lectins have been used to a great extent for the intra and interspecific differentiation of leishmania strains. In this work we tested 12 leishmania strains from the New World using 12 lectins.

MATERIAL AND METHODS

The leishmania strains tested in this study are listed in Table I. The parasites were cultivated in a diphasic medium of brain infusion agar (Difco) with 10 percent difibrinated rabbit blood at 26°C and saline (0.9% NaCl) as liquid phase. In order to get a homogeneous parasite culture three passages of four days were made. Cells were centrifuged (1400g, 5 min) and washed once in saline. The culture forms were resuspended in saline to a concentration of 1×10^8 cell/ml. Autoagglutinations were not observed. 50 μl of parasite suspension and 50 μl of lectins were incubated together for 15 min. at 26°C. Subsequently the cells were mixed several times using a pipette. The agglutination reactions were controlled under a light microscope using a slide with a cover glass.

TABLE I

Details of *Leishmania* Strains Employed in this Study

Species	*Strain*	*Clinical picture*	*Origin*	
L. m. pifanoi	LRC L-90	DCL	Venezuela	isolated by Dr. Pifano 1957 from a human case of DCL. We received this strain from Prof. Zuckerman, WHO Ref. Centre, Jerusalem, Israel.
L. m. amazonensis	H-21	DCL	Cametá Pará Brasil	isolated by Lainson and Shaw from R. Melo 1967. We received this strain from Dr. J. Menezes, Rio de Janeiro.
L. m. amazonensis	H-JMMO	DCL	Belém Pará Brasil	Montenegro test negative. Isolated by S.C.G. da Costa and N. Thomaz in the Dept. of Protozoology. Inst. Oswaldo Cruz, Rio de janeiro, 1975.

Details of *Leishmania* Strains Employed in this Study

Species	*Strain*	*Clinical picture*	*Origin*	
L. m. amazonensis	H-PPL	DCL	Rosário Maranhão Brasil	Montenegro test positive. Isolated by S.C.G. da Costa and N. Thomaz, in the Dept. of Protozoology, Inst. Oswaldo Cruz, Rio de Janeiro, 1977.
L. m amazonensis	M-1696	ACL	Altamira Pará Brasil	isolated by Lainson and Shaw from T. de Souza. Four month old lesions on the right leg.
L. m. amazonensis	M-JOF	ACL	Minas Gerais Brasil	isolated by Dr. P. Magalhães. Maintained by S.C.G. Costa in the Dept. of Protozoology, Inst. Oswaldo Cruz, Rio de Janeiro.
L. m. mexicana	LRC L-94	ACL	British Honduras	isolated by P.C.C. Garnham 1958. We received this strain from Prof. Zuckerman, WHO Ref. Centre, Jerusalem, Israel.
L. m. mexicana	M-379 (L-11)		British Honduras	isolated by Lainson from Vyctomys sumichrasti, 1962. We received this strain from Dr. Ch. de Souza, UFRJ, Rio de Janeiro, 1979.
L. b. braziliensis	LRC L-77	MCL	Ceará Brasil	isolated by Dr. M. Coelho from a human case of espundia, 1960. We received this strain from Prof. Zuckerman, WHO Ref. Centre, Jerusalem, Israel.
L. b. braziliensis	L-1	MCL	Ceará Brasil	identical with strain L-77. We received this strain from Dr. Lumsden, School of Hyg. and Trop. Med., London.
L. b. braziliensis	M-2903	ACL	Serra Norta Pará Brasil	isolated by Lainson 1975 from a patient with lesions in the face. Montenegro test positive.

Details of *Leishmania* Strains Employed in this Study

Species	*Strain*	*Clinical picture*	*Origin*	
L. b. braziliensis	H-LSS	ACL	Campo Grande Jameira R. de Janeiro Brasil	Montenegro test positive. Isolated by S.C.G. da Costa and N. Thomaz, Dept. of Protozoology, Inst. Oswaldo Cruz, Rio de Janeiro.

DCL = diff, cutan. leishm.
ACL = amer. cutan. leishm.
MCL = mucocutan. leishm.

The sugar-specific proteins which were used for the parasite-characterization are listed in Table II. The agglutination tests were controlled by inhibition tests using the following sugars: Concanavalin A 500 μg/ml plus 2% D-mannose (Merck), *R. communis*-120 1:10 plus 1% D-galactose (Merck), Phytohaemagglutinin-P 1% plus 5% N-acetyl-D-glucosamine (Fluka), Aaptos II 1:128 plus 1% N-acetyl-D-glucosamine (Merck), *U. europaeus* plus 1% L-fucose (Serva), *L. alpinum* plus 1% L-fucose (Serva), A. hypogaea 1:10 plus 1% D-galactose (Merck), *S. hispida* 1% N-acetyl-D-galactosamine (Fluka). As an additional control the parasites were incubated in a volume of saline equal to the volume of lectins to check for possible auto-agglutination.

TABLE II

Lectins Used in This Study

Lectins	*Origin*	*Conc. ml*	*Carbohydrate Specificities*	*References*	
Aaptos papillata II	Dr. Bretting, Univ. Hamburg	1:10	(β (1-4) DGluNAc$_3$ > $_2$ > (β (1-4) DGluNAc)	Bretting et al	(1976)
Laburnum alpinum	Dr. Fresenius K.G.	sol.com.	anti-H, L-fuc, (β (1 –4)DGuNAc)$_2$	Renkonen Gold & Balding	(1948) (1975)
Ulex europaeus	Dr. Fresenius, K.G.	sol.com.	anti-H, L-fuc, (β (1 –4)DG1uNAc)$_2$	Matsumoto & Osawa Gold & Balding	(1971) (1975)
Arachis hypogaea	Boehringer	1:10	anti-T DGal (β (1 – 4) DGluNAc, DGal (β (1 – 3) DGluNac DGal	Lotan et al Race & Sanger Pereira et al	(1975) (1975) (1976)
Soja hispida	Pharm. Fine Chen	1%	α, β DGalNAc DGal	Hammarström et al	(1977)
Canavalia ensiformis	Difco Lab	500 μg	DMan, DG1u, DG1uNAc	Bitting & Schnebli Sharon & Lis	(1976) (1972)
Ricinus communis-120	Miles Yeda	1:10	βDGal	Nicolson	(1974)
Axinella polypoides	Dr.Bretting, Univ. Hamburg	1%	β DGal (1 –6)	Gold et al Bretting & Kabat	(1974) (1976)
Phaseolus vulgaris	Difco Lab	1%	DGa1NAc	Lis & Sharon	(1973)
Evonymus europaeus	Dr. Bretting, Univ. Hamburg	10%	anti-(B+H), anti-A$_2$	Race & Sanger Pacak & Kocourek	(1975) (1975)
Lotus tetragonolobus	Sigma	500 μg	anti-H, αL-fuc	Yariv et al Kalb Goldstein & Hayes	(1967) (1968) (1978)
Dolichos biflorus	Dr. Fresenius, K.G.	sol.com.	anti-A, αDGa1NAc > βDGa1NAc	Race & Sanger Etzler & Kabat Hammarström et al	(1975) (1970) (1977)

RESULTS

All leishmania strains tested showed agglutination reactions with *C. ensiformis*, *R. communis*-120 and *A. polypoides*. No reactions were observed with *P. vulgaris*, *E. europaeus*, *L. tetragonolobus*, *A. papillata* and *D. biflorus* (Table III). The agglutination reaction with *C. ensiformis* and *R. communis*-120 could be inhibited with the specific sugars. No inhibition test was carried out with the extract from *A. polypoides*.

TABLE III

Lectin Typing of Different Strains of Leishmania from the New World

Lectins	*L. mexicana pifanoi*	*L. mexicana amazonensis*	*L. mexicana amazonensis*	*L. mexicana mexicana*	*L. brasiliensis brasiliensis*
C. ensiformis	▒	▒	▒	▒	▒
R. communis -120	▒	▒	▒	▒	▒
A. polypoides	▒	▒	▒	▒	▒
P. vulgaris	□	□	□	□	□
E. europaeus	□	□	□	□	□
L. tetragonolobus	□	□	□	□	□
D. biflorus	□	□	□	□	□
A. papillata II	□	□	□	□	□
S. hispida	▒	▒	□	□	□
U. europaeus	▒	▒	□	□	□
L. alpinum	▒	▒	□	□	□
A. hypogaea	▒	▒	□	□	□
Strains:	LRCL-90(B)	H-JMMO(B) M-JOF(A)	M-1696(A) H-21(B) H-PLL(B)	LRC L-94(A) M-379	LRC L-77(C) L-1 (C) M-2903 (A) H-LSS (A)

▒ = agglutination reaction □ = no agglutination reaction

A = simple cutaneous;
B = diffuse cutaneous leishmaniasis;
C = mucocutaneous leishmaniasis.

Only the lectins from *S. hispida, U. europaeus, L. alpinum* and *A. hyspogaea* could be used for differentiation. The leishmania strains could be divided into two groups: Group 1 included the strains of *L.m. pifanoi* L-90 and the *L. m. amazonensis* strains H-JMMO and M-JOF. These strains agglutinated with *S. hispida, U. europaeus, L. alpinum* and *A. hypogaea.* The reactions with *S. hispida* and *A. hypogaea* could be inhibited

with specific sugars. The reaction with *U. europaeus* and *L. alpinum* could not be inhibited with *L. fucose.* Group 2 included the strains of *L. m. mexicana* LRC L-94; M-379, *L. b. braziliensis* LRC L-77; L-1; M-2903, H-LSS and the *L. m. amazonensis* strains M-1969, H-21 and H-PLL. None of these strains reacted with the lectins typical for group 1 (Table III).

DISCUSSION

The results demonstrate that instraspecific variants of *L. m. amazonensis* can be differentiated by lectins (Table III). It is significant that the *L. m. amazonensis* strains H-JMMO and M-JOF can be not distinguished from *L. m. pifanoi* L-90; according to Lainson & Shaw (1978) these subspecies are closely related. The agglutination reaction of these strains with *S. hispida* and *A. hypogaea* could be inhibited by N-acetyl-D-galactosamine and D-galactose respectively. In contrast the reactions with *U. europaeus* and *L. alpinum* could not be inhibited by L-fucose. Therefore di-N-acetyl-chitobiose as membrane-component is responsible for the agglutination reactions with *Ulex* and *Laburnum* since both lectins react with this carbohydrate too (Gold & Balding, 1975). Since no reactions with *L. tetragonolobus* were detected it can be concluded that receptors for the blood group H do not exist. The lectins *C. ensiformis, R. communis*-120 and *A. polypoides* can not be used for differentiations since they showed positive reactions with all the *Leishmania* strains tested. *L. m. amazonensis* (M-1696, H21, H-PLL), *L. m. mexicana* and *L. b. braziliensis* did not agglutinate with *U. europaeus, L. alpinum, S. hispida* and *A. hypogaea.* Consequently they have a different receptor topography from *L. m. pifanoi* and *L. m. amazonensis.* The non-differentiation of *L. m. pifanoi* from defined strains of *L. m. amazonensis* (Table III) support the results of Convit, Pinardi & Rondon (1972) that an immune defect of the host rather than a special strain of leishmania is responsible for diffuse cutaneous leishmaniasis (DCL).

It was not possible to distinguish strains of *L. m. amazonensis* isolated from patients with simple cutaneous and DCL. Our results agree with those of Gardener, Chance & Peters (1974) who found that isolates of *L. m. amazonensis* from cases of DCL and from single cutaneous lesions cannot be differentiated. Different strains from DCL react differently with lectins (Table III) as well. *L. b. braziliensis* strains isolated from patients with simple cutaneous leishmaniasis or espundia react uniformly. So it is not possible to find a correlation between the agglutination test of the strain and the clinical syndrome of patients.

Though the development of DCL depends on immunological idiosyncracies of the host, apparently only certain strains of leishmania have the potential to cause the diffuse form as well as simple lesions, for example, in Brazil DCL has been associated exclusively with *L. m. amazonensis* infections. One objective of the present study was to determine if the potential to cause DCL could be related with a certain pattern of surface receptors. Our results demonstrate that no single carbohydrate surface topography detectable with the 12 lectins used by us is associated with DCL producing organisms. Since these lectins react with terminal sugars, the possibility cannot be excluded that a typical pattern of internal saccharide sequences may occur. Such patterns could only be determined by other biochemical methods.

The lectins used were not capable of differenting between *L. m. mexicana* and *L. b. braziliensis* (Table III), as are other methods such isozyme characterization (Ebert, 1974, Gardener, Change & Peters, 1974) and isoelectrofucusing (Momen, Grimaldi & Soares, 1980), or by bouyant density of DNA in a caesium chloride gradient (Chance, Peters & Shchory, 1974) but we can separate strains of both *L. m. amazonensis* and *L. b. braziliensis* into two sub-groups by lectin agglutination tests.

We do not know yet the significance of this intraspecific variation in the leishmanias from cutaneous cases of the New World but immunological studies are being carried out by one of us (SCGC) to better understand this finding.

RESUMO

As formas de cultura de *L. mexicana pifanoi* (LRC L-90), *L. mexicana mexicana* (LRC L-94, M-379); *L. braziliensis braziliensis* (LRC L-77, L-1, M-2903, H-LSS) e *L. mexicana amazonensis* (H-JMMO, M-JOF, H-21, H-PLL, M-1696) foram testadas com as seguintes lectinas: *Canavalia ensiformis, Ricinus communis*-120, *Axinella polypoides, Phaseolus vulgaris, Evonymus europaeus, Lotus tetragonolobus, Dolichos biflorus, Aaptos papilata* II, *Laburnum alpinum, Ulex europaeus, Arachis hypogaea* and *Soja hispida.*

Todas as cepas de leishmania foram aglutinadas por *C. ensiformis, R. communis*-120 e *A. polypoides.* Nenhuma reação de aglutinação foi observada com *P. vulgaris, D. biflonus, A. papillata* II, *E. europaeus* e *L. tetragonolobus.* Apenas *L. m. pifanoi* e as cepas H-JMMO e M-JOF de *L. m. amazonensis* mostravam reações de aglutinação com *S. hispida, U. europaeus, L. alpinum* e *A. hypogaea* enquanto as cepas LRC L-94 e M-379 de *L. m. mexicana*; H-LSS, LRC L-77, L-1 e M-2903 de *L. b. braziliensis* bem como as cepas H-PLL, H-21, M-1696 não mostraram nenhuma reação de aglutinação com estas quatro lectinas.

Assim, as variações intra-específicas encontradas pelos testes de aglutinação por lectinas em cepas de leishmanias isoladas de casos de leishmaniose tegumentar não permitem estabelecer uma correlação entre formas clínicas e cepas isoladas. Por outro lado, estes testes mostram que a cepa de *L. mexicana pifanoi* apresenta reações idênticas às de duas cepas de *L. mexicana amazonensis,* não se podendo assim diferenciá-la por esta técnica.

Pelo teste de aglutinação por lectina, empregado neste trabalho, podemos agrupar as cepas estudadas em dois grupos, mas não sabemos até o presente momento qual o significado destas variações intra-específicas em leishmanias isoladas de casos de leishmaniose cutânea do novo mundo.

ZUSAMMENFASSUNG

Die Kulturformen von *L. mexicana pifanoi* (LRC L-90), *L. mexicana mexicana* (LRC L-94, M-369), *L. braziliensis braziliensis* (LRC L-77, L-1, M-2903, H-LSS) und *L. mexicana amazonensis* (H-JMMO, M-JOF, H-21, H-PLL, M-1696) wurden mit folgenden Lektinen untersucht: *Canavalia ensiformis, Ricinus communis*-120, *Axinella polypoides, Phaseolus vulgaris, Evonymus europaeus, Lotus tetragonolobus, Dolichos biflorus, Aaptos papillata* II, *Laburnum alpinum, Ulex europaeus, Arachis hypogaea* und *Soja hispida.*

Alle untersuchten Leismain Stämme agglutinierten mit *C. ensiformis, R. communis*-120 und *A. polypoides.* Keine agglutinationen wurden beobachtet mit *P. vulgaris, E. europaeus, L. tetragonolobus, D. biflorus* und *A. papillata* II. Mit *U. europaeus, L. alpinum, A. hipogaea* und *S. hispida* agglutinierten nur *L. mexicana pifanoi* und die *L. mexicana amazonensis* Stämme H-JMMO und M-JOF. Keine agglutinationen mit diesen vier Lektinen zeigten *L. mexicana amazonensis* Stamm (H-PLL, H-21, M-1696), *L. braziliensis braziliensis* Stamm (LRC L-77, L-1, M-2903, H-LSS) und *L. mexicana mexicana* Stamm (LRC L-94, M-379 (L-11).

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