Short communication

Characterization of *Leishmania* isolated from unhealed lesions caused by leishmanization

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خصائص الليشمانية المستفرَدة من الآفات غير الملتئمة الناجمة عن اللَّشْمَنَة

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الخلاصة: تم استفراد طفيليات الملاريا بعد 8 و13 عاماً من حدوث آفات غير ملتئمة في جنديين سَبَقَ تمنيعُهما ضد داء الليشمانيات أثناء الحرب بين العراق وجمهورية إيران الإسلامية. وقد تم إجراء التوصيف المرتكز على النظائر الإنزيمية لهذه المستفرَدات باستخدام أحَدَ عشر نظاماً إنزيمياً. وقُورِنَت النتائج مع البروفيلات الإنزيمية للمستفردَات الأصلية لليشمانية الكبيرة *L.major*، والتي استُخدمت في اللَّشْمَنَة (التمنيع بالليشمانيات). وقد لوحظ وجود اختلافات إنزيمية طفيفة في نازعة هيدروجين الغلوكوز – 6 – فُسفات وفي الفسفوغلوكوموتاز، ولم تلاحظ أية فروق أخرى بين الدُّرِّيتين في ماعدا ذلك.

ABSTRACT ***Leishmania*** parasites were isolated after 13 and 8 years from the unhealed lesions of 2 soldiers who had been immunized against leishmaniasis during the war between Iraq and the Islamic Republic of Iran. Isoenzyme characterization on these isolates using 11 enzyme systems was carried out and the results were compared with the enzyme profiles of the original isolates of ***L. major*** used for leishmanization. Minor enzymatic differences in glucose-6-phosphate dehyrognase and phosphoglucomutase were observed but otherwise the strains appeared unchanged.

Caractérisation des *Leishmania* isolés sur des lésions non cicatrisées causées par la leishmanisation

RÉSUMÉ Des parasites ***Leishmania*** ont été isolés après 13 et 8 années sur des lésions non cicatrisées de deux soldats qui avaient été vaccinés contre la leishmaniose durant la guerre entre l'Iraq et la République islamique d'Iran. On a procédé à la caractérisation isoenzymatique de ces isolats en utilisant 11 systèmes enzymatiques et comparé les résultats avec les profils enzymatiques des isolats originaux de ***L. major*** utilisés pour la leishmanisation. Des différences enzymatiques mineures ont été observées pour la glucose-6-phosphate-déshydrogénase et la phosphoglucomutase, sinon les souches paraissaient inchangées.

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Very sadly, Professor Ardehali passed away shortly after preparation of this report.

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Introduction

Leishmanization with live virulent promastigotes has at times been carried out to protect high-risk people against cutaneous leishmaniasis in endemic areas. During the war between Iraq and the Islamic Republic of Iran, immunization against leishmaniasis was carried out for more than 2 million Iranian soldiers over several years. Soldiers were inoculated in the left deltoid muscle with $2–3 \times 10^5$ live *Leishmania major* promastigotes. Follow-up studies on individuals who had received the immunization indicated that 2%–3% had a lesion for over a year, and half of those did not heal after many years even with treatment [*1,2*].

During a countrywide cutaneous leishmaniasis survey in the Islamic Republic of Iran from 1998 to 1999 on 407 patients [*3,4*], we had opportunity to characterize isolates (isoenzymatically and with monoclonal antibodies) from the long-term lesions of 2 individuals who had been immunized during the war. We were interested to find out the probable phenotypic changes in the parasite during its long presence in a human host.

Methods

The first case from whom *Leishmania* was isolated was a 30-year-old man from the north of the country who had resided in the war region (Khouzestan) for 1 year after had he had been immunized. He had a single lesion at the inoculation site which had not healed in spite of extensive treatment for 13 years; otherwise he was an apparently healthy individual. No *Leishmania* amastigotes were found in the direct smear of the lesion, but *Leishmania* promastigotes were recovered in NNN medium, 3 days after inoculation in the medium.

The second case was a 30-year-old man from Karaj, a suburb of Tehran, who had also resided in a war area (Khouzestan) for 1 year after immunization. He had developed a lesion at the site of inoculation 8 years before this study, which had not healed even after various treatments. The patient was apparently healthy on physical examination carried out by a physician. In direct smear preparation, no *Leishmania* amastigotes were found, but promastigotes were obtained in NNN culture media 3 days after inoculation in the medium.

The isolates recovered from NNN medium were further propagated in RPMI 1640 (Sigma, United Kingdom) supplemented with 15% fetal calf serum (Gibco-RBL, United Kingdom), penicillin/streptomycin and L-glutamine. The methods and techniques for further cultivation, preparation of cell extracts and enzyme staining were those described by Evans [*5*]. Electrophoresis on polyacrylamide gel was carried out according to the methods described by Davis [*6*].

The World Health Organization reference strains were *L. major* (MHOM/SU/73/5ASKH), *L. tropica* (MHOM/SU/71/K27), *L. donovani* (MHOM/IN/80/DD8), and *L. infantum* (MHOM/TN/80/IPTI). *L. major* (MHOM/IR/72/Nadim5), the strain used for leishmanization, was also applied.

In order to determine if the original strain was still pathogenic, the organisms recovered from cryopreservation were propagated in RPMI 1640 medium supplemented with 15% fetal calf serum and L-glutamine. During the stationary growth phase, the parasites were harvested. Then 2 million promastigotes were inoculated into Balb/c mice at the base tail. Three weeks after inoculation, nodules appeared which ulcerated a few days later indicating the continued pathogenicity of the original

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strain. Amastigotes from the lesions that developed were cultured on NNN medium and further propagation was performed in RPMI 1640 as above.

The enzymes studied were malate dehydrogenase (EC 1.1.1.37), malic enzyme (EC 1.1.1.40), phosphoglucomutase (EC 4.5.2.2), glucose-6-phosphate dehydrogenase (G6PD) (EC 1.1.1.49), glucose phosphate isomerase (EC 5.3.1.9), nucleoside hydrolase (EC 3.2.2.1), isocitrate dehydrogenase (EC 1.1.1.42), superoxide dismutase (EC 1.1.5.1.1), estrase (EC 3.1.1.1) and alanine aminotransferase (EC 2.6.1.2). The monoclonal antibodies used were XLVI-5B80 (T-1) anti *L. major*, IS2-2B4-All anti *L. tropica* and LXXV III-2ES-A8 (D-2) anti *L. infantum* which were kindly supplied to us by Dr F. Modabber from WHO, Geneva. Freshly prepared whole promastigotes of *Leishmania* isolates were used as the source of antigen in the enzyme-linked immunosorbent assay (ELISA) test system [*7,8*].

Results

The zymodeme of the case 1 isolate was the same as original strain used for leishmanization, except for the G6PD enzyme system for which the isolate had an extra band with an Rf value of 0.36. The zymogram of this enzyme system for the strain used for leishmanization showed 3 bands with Rf 0.06, 0.12 and 0.3 respectively. In ELISA this isolate reacted only with monoclonal antibody specific for *L. major*, i.e. XLVI – 5B80 (T-1).

The zymodeme of the case 2 isolate was also the same as the original strain used for leishmanization, except for the PGM enzyme system in which a band with an Rf value of 0.62 was absent in the isolate compared with the original strain. In ELISA, this isolate also only reacted with monoclonal antibody specific for *L. major*, i.e. XLVI – 5B80 (T-1).

Discussion

To our knowledge this is the first study attempting to characterize the isolates from individuals who have been immunized against leishmaniasis. The results obtained demonstrated the following points.

- The lesion that persisted in the 2 immunized cases was caused by the original strain used for leishmanization and was not due to concomitant infection with another wild type of parasite.
- The zymodeme of the original strain used for leishmanization had not changed greatly in spite of residing in host tissues for a long period of time.
- All immunized individuals received the same dose of the same parasites under similar conditions, but in some cases the lesion that developed tended to persist. This therefore suggests that host factors play a role in the development and establishment of lesions. However, more studies are necessary to investigate the effect of host factors.

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