Discovery and study of *Leishmania turanica* for the first time in China*

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Reported are the results of multidisciplinary studies on *Leishmania turanica*, which was isolated from the auricular tissues of naturally infected great gerbils in Xinjiang, China. Discussed are the biology of the parasite, its molecular biology, its pathogenicity in rodents and humans and its vectors. This was the first time that *L. turanica* had been reported in China. *L. turanica* is highly pathogenic in BALB/c mice, with the resulting systemic infection being lethal, and it causes dermal lesions in Meriones unguiculatus. *L. turanica* parasitizes the macrophages in the interstitium of the testes of Crictetus barabensis, and entirely destroys the Leydig's cells of severely infected animals. Inoculation of *L. turanica* can induce simian and human cutaneous leishmaniasis.

The cell membrane and flagella of the promastigotes of *L. turanica* have rather active ACPase. The major vectors of *L. turanica* were Phlebotomus mongoisensis and *P. andrejevi* being its major vectors.

In 1988, we detected a species of *Leishmania* in the auricular subcutaneous tissue of great gerbils captured in Karamay, Xinjiang; later, eight human cases of cutaneous leishmaniasis were confirmed parasitologically (1). Over the period 1989–92, multidisciplinary studies of the *Leishmania* species were carried out to characterize it and to determine its pathogenicity in humans. This is the first identification of *Leishmania turanica* to be reported in China. *L. turanica* was found to be pathogenic in both monkeys and humans, with *Phlebotomus mongolisens* and *P. andrejevi* being its major vectors.

**Results**

**Natural infections in great gerbils**

Over the period 1988–90, superficial examinations and necropsies were performed on 27 great gerbils (*Rhombomys opimus*) that had been captured from Xiaoguai Farm and Baijiantan, oil-production areas in Karamay. Of the animals examined, 17 (63.0%) exhibited *Leishmania* infection in auricular tissues, and the isolated promastigotes grew well and replicated in NNN culture; whereas visceral smears and cultivation gave negative results (1). Although macroscopically there was no visible dermal lesion on the auricles of the infected great gerbils, hyperaemia and diffuse infiltration of inflammatory cells in the dermis of the auricles were found upon histological examination—some with necrotic lesions showing the parasites in the macrophages and prickle-cell layer. Also, the parasites were present in the cytoplasm of the epithelial cells of hair follicles (2).

*Leishmania* parasites were not detected upon microscopic examination of specimens of auricular tissue and visceral smears taken from 46 other rodents including *Meriones tamariscinus* (15 animals) *M. meridianus* (11), *Euchoreutes naso* (10), *Dipus sagitta* (4), *Mus musculus* (3), *M. erythrophus* (2), *Cricetulus migratorius* (1) and five hedgehogs: all the animals examined were caught at or close to the site where the infected great gerbils were found (Guan Li-Ren et al, unpublished data, 1992).

**Morphological study**

Microscopically, the amastigotes of *L. turanica* were 4.71 ± 0.71 μm in length and 2.35 ± 0.44 μm in width, the size index being 11.09 ± 2.92 μm (3); these parameters were significantly different from those of *L. major* and *L. gerbilli* isolated from the auricular tissues of great gerbils in the Old World (4, 5).

An ultrastructural study revealed that the average perimeter of the *L. turanica* amastigotes was...
9.98 ± 2.01 μm and the mean number of subpellicular microtubules, 92 ± 13 (6). Interestingly, 20.6% of the flagellae of the amastigotes collected from some vertebrates extended from the flagella pockets beyond the main body of the organisms, the mean length of the extruded portion being 0.55 μm — approximately a third of the length of the parasite (7). The number of subpellicular microtubules in the L. turanica promastigotes was 80 ± 9, considerably fewer than the average number (113) for L. gerbilli (8, 9).

Identification of the Leishmania species

Monoclonal antibodies (McAbs) against L. donovani, L. major, L. tropica and the Leishmania species collected from the great gerbils in Karamay (KGL) were used in a dot enzyme-linked immunoassay (ELISA) to detect the antigens from 10 isolates of Leishmania from rodents in the pilot area. The antigens from the 10 isolates reacted only with the McAbs prepared from KGL (10). Typing the n-DNA gene of KGL and L. gerbilli using the gene gp 63 (1.8 kbase) as a probe proved that each retained its specific bands (11).

Isoenzymatic (ALAT, GPI, 6PGD, SOD) assays on the genic loci of eight Leishmania isolates from Xiaoguai Farm and an isolate from Baijiantan were carried out using isoenzyme electrophoresis. The eight isolates from Xiaoguai were identified as L. turanica and the one isolate from Baijiantan as L. gerbilli (D.A. Evans, unpublished reports 1992–93). This was the first time that L. turanica had been found in great gerbils from north Xinjiang, China.

Pathogenicity of L. turanica and pathology in infected rodents, monkeys and humans

Subcutaneous inoculation of L. turanica into the footpads of in-bred BALB/c mice induced ulcerative and necrotic lesions and resulted in metastasis at several sites. There was no tendency for spontaneous healing. Some of the parasites invaded the viscera of the mice. A large number of Leishmania were found in Kupffer’s cells and the reticuloendothelial cells in the lymphatic sinus of the spleens of the infected mice. Eventually all the mice with systemic infection died (3).

Histopathological studies on mouse skin over a period of 1 year revealed that at the preliminary stage of infection, hyperaemia, oedema and infiltration of inflammatory cells (largely neutrophils and eosinophils) occurred at the inoculation site; many Leishmania were visible in the macrophages, and some in the neutrophils. At the abscess stage, the parasites were detected in the prickle-cell layer, and degeneration and necrosis of muscle cells occurred. Infiltration of lymphocytes and plasma cells, and to a lesser extent neutrophils, occurred in lesions of the dermis; concurrently, an increase in the number of fibroblasts and a marked decrease in collagenous and reticular fibres occurred. Degeneration and necrosis of peripheral nerve fibres occurred 5–6 months after infection. Infiltration of lymphocytes and plasma cells still persisted 7-months’ post-infection, while collagenous and reticular fibres had proliferated and encircled the parasites, causing reduction or even disappearance of Leishmania in the lesion; nevertheless, metastatic lesions were still present on the skin and cartilage of the auricle, nose, and tail, which exhibited identical histopathological changes to those on the primary lesion (12). The dermal histopathological changes in L. turanica-infected BALB/c mice were the same as those in cases of human cutaneous leishmaniiasis (13, 14).

In outbred Kunmin-strain mice, L. turanica only caused dermal swelling at the inoculated site; although the parasites could be detected, there was no evidence of visceral invasion (3).

Subcutaneous inoculation of L. turanica into the auricles of Meriones unguiculatus induced the formation of small ulcers and scabs. Tissue sections revealed a large number of Leishmania that had been engulfed by macrophages in the dermal layer and abscess formation in the prickle-cell layer; focal necrosis was present in some animals. There was no evidence of spontaneous healing over a 14-month observation period (3).

Intraperitoneal inoculation of L. turanica into Cricetulus barabensis failed to establish infection; however, intratesticular inoculation of the parasites gave rise to local infection. L. turanica mainly parasitized the macrophages in the interstitium, some parasites being found in the giant cells and a few in Sertoli’s cells in the convoluted seminiferous tubules; many of the lymphocytes that surrounded the parasites had been invaded; hyperaemia and plasma retention occurred in the interstitium, all the Leydig’s cells were destroyed, and abscesses of different sizes were formed. Despite the severity of the pathological changes in the testes, no ulceration was observed (3).

Subcutaneous inoculation of L. turanica on the face and forearm of a monkey produced cutaneous tubercles and ulcers which spontaneously healed after 3–4 months. Upon autopsy 1 year later, a few parasites were still present in lesions, lymphocytes in the vicinity of parasites had been invaded, while visceral smears and cultures were negative (15).

Two healthy volunteers were subcutaneously inoculated with L. turanica on the flexion side of the forearm. A dermal reaction similar to that resulting
from leishmanin injection occurred 48 hours later in one of the volunteers; a reddish swelling developed with central dermal necrosis and lymphangitis, which disappeared 1 week later with the development of induration. This individual complained of severe pain at the inoculation site. Microscopically, ulceration with parasites surrounded by infiltrating lymphocytes was observed on the 128th day; ulceration and necrosis were seen in some sections and parasites were detected in all the prickle-cells and the epithelial cells of hair follicles that were viewed. A swelling measuring 5.0 cm × 7.0 cm was formed on the forearm of the second volunteer, which gradually decreased in size to reach 0.5 cm in diameter on the 18th day; no ulceration was visible (15).

These results illustrate the high pathogenicity of L. turanica in BALB/c mice, being equivalent to the lethal systemic infection produced by inoculating such mice with L. major isolated from the auricular tissue of great gerbils in the former Soviet Union and the Islamic Republic of Iran (16–18). The size of the dermal ulcers that were formed in monkeys and humans who had been inoculated with L. turanica, was, however, smaller than that caused by L. major (15). Also, the dermal lesions induced by L. gerbilli in BALB/c mice were confined to the inoculation site and the number of parasites decreased and in some instances vanished with time. In C. baraben-sis, L. gerbilli parasitizes mainly the Sertoli’s cells of convoluted seminiferous tubules; L. gerbilli is not pathogenic in humans (5, 19, 20).

**Location and content/activity of the histochemical components of L. turanica**

A histochemical study of L. turanica indicated that the DNA, RNA, protein-combined α-amino acids, protein-combined tyrosine, tryptophan, and histidine, as well as basic proteins, are mainly located in the nucleus and kinetoplast of the promastigotes. Glycogen is profuse in the cytoplasm of the parasite. In the nucleus and kinetoplast AKPase and ATPase are more active, while in the cell membrane and flagella ACAPase is comparatively active. The activity of ACAPase is comparable in L. major, L. turanica, and L. donovani, and is more evident than that in L. ger-billi (21). ACAPase protects the proliferation of promastigotes in the digestive tract of sandflies, and prevents the harmful action of neutrophils of the vertebrate host; its activity mirrors the pathogenicity of the parasite (22–24).

**Vectors of L. turanica**

The results of a series of surveys, conducted in accordance with the criteria stipulated by WHO for discriminating vectors of Leishmania, are described below.

**Population and habitats of sandflies.** A total of six species of sandflies were found in Karamay: P. mongolensis, P. major wui, P. andrejevi, P. caucasicus, Sergentomyia minutus sinkiangensis and S. arpaklen-sis. Being the principal species of the local sandflies, the first three constituted, respectively, 45.7% (4798/10 476), 28.2% (2956/10 476) and 13.1% (1375/10 476) of the total sandfly population, whereas the second three species represented only 0.3%, 7.3%, and 5.4%, respectively. P. mongolensis and P. andrejevi were closely associated with the ecology of great gerbils, the major species in the rodent burrows. The distribution of these two species of sandfly varied with the soil zone, P. mongolensis accounting for 67.3% of the sandflies in the burrows in the alluvial zone and P. andrejevi for 71.6% of those in the sandy zone. P. major wui was not an essential species in the great gerbil burrows, accounting, respectively, for 15.7% and 1.2% of the population in these two soil zones (25).

**Homophilic habit of sandflies.** P. mongolensis, P. major wui, and P. andrejevi are anthropophilic but also suck rodent blood. In contrast, the two species of Sergentomyia discussed above only feed on cold-blooded animals. At night in residential quarters the proportion of P. mongolensis and P. major wui in the sandfly population always surpassed 70%, while P. andrejevi was scarcely captured. Field surveys using human bait showed that P. mongolensis and P. major wui accounted for 94.4% of the captured sandflies.

**Multiplication and shift of L. turanica in the digestive tract of sandflies.** After sucking blood from the dermal lesions of experimental animals, engorged P. mongolensis, P. andrejevi and P. major wui had promastigote infection rates of 37.6%, 52.9% and 62.8%, respectively, while the rates in those with completely digested blood were 8.3%, 6.9% and 55.6%, respectively. A firm peritrophic membrane was formed to limit the further reproduction of promastigotes in P. mongolensis and P. andrejevi. Nevertheless, in about 25% of these two species this membrane had ruptured in the process of blood digestion, and the promastigotes were released into the stomach, where further multiplication and shift to anterior parts of the sandflies occurred. For P. major wui, the peritrophic membrane ruptured on the third day in all those that had sucked blood and there was no constraint on the multiplication and shift of promasti-gotes. The shift of the promastigotes to the oesophagus and pharynx occurred in all three of the above-mentioned Phlebotomus species. It was also demonstrated that when sucking blood for the second
time, the newly formed peritrophic membranes in *P. mongolensis* and *P. andrejevi* could not envelop all the previously ingested promastigotes in the stomach, which were able then to reproduce and shift. This finding indicates that once infected with *L. turanica*, these two species of sandflies carry the parasites permanently and can transmit them repeatedly (26).

**Natural infection in sandflies and identification of Leishmania species.** Naturally infected *P. mongolensis* and *P. major wui* were caught in field (man-bait, rodent burrows) and residential sites; naturally infected *P. andrejevi* could only be captured from rodent burrows. Promastigotes were detected in the pharynx of all the infected sandflies. The parasites isolated from *P. mongolensis* and *P. andrejevi* reproduced readily in NNN culture (27).

Promastigotes isolated from naturally infected *P. mongolensis* and *P. andrejevi* were inoculated subcutaneously into BALB/c mice or intratesticularly into *C. barabensis*; the induced lesions were the same as those caused by *L. turanica* (27).

The ultrastructure of the amastigotes in the experimental animals was also identical to that of *L. turanica* (28). In contrast, promastigotes isolated from naturally infected *P. major wui* did not induce dermal lesions in mice, but in both mice and *C. barabensis* exhibited visceral involvement; also, growth of the parasites in NNN culture was poor (27, 29), indicating that the species involved was different from *L. turanica*.

McAbs against the antigens of *L. major*, *L. tropica*, *L. donovani* and *L. turanica* were prepared and used in dot ELISAs to detect 16 isolates from *P. mongolensis* and *P. andrejevi*. The results showed that all the isolates reacted only to the McAb to the *L. turanica* antigen prepared from local gerbil isolates.

*P. mongolensis* and *P. andrejevi* were established to be the vectors of *L. turanica* (27). The cloned 32P-labelled gene gp63 was used as a probe to detect the n-DNA of promastigotes isolated from naturally infected *P. major wui*; genic typing suggested that it was homogeneous with that of *L. infantum* (29).

Although both *P. mongolensis* and *P. andrejevi* were vectors for *L. turanica*, the latter sandfly seldom flew into residential sites, mainly restricting its activities to rodent burrows, and was thus assumed not to play an essential role in the propagation of *L. turanica* through a human–sandfly cycle (25). *P. major wui* can be naturally infected with *L. infantum*, but not with *L. turanica*; none the less, experimental infection studies revealed that it was an appropriate vector for *L. turanica* (26), and hence could have some significance in the transmission of the latter.

The *L. turanica* promastigote infection rates were rather low in both naturally and experimentally infected *P. mongolensis* and *P. andrejevi*, but the pregnant females often sucked their hosts repeatedly so that the frequency of blood feeding was higher than that of other species; hence the higher rate of transmission of *L. turanica* (30).

**Discussion**

Killina & Passova reported that of the *Leishmania* isolates collected from the auricular tissues of great gerbils in Uzbekistan, five displayed genic loci quite different from those of *L. major*, *L. tropica*, *L. gerbilli* and *L. donovani*, as shown by enzyme electrophoresis (31). It was suggested that the parasite responsible might be a new species of *Leishmania* (31). In 1990, Strelkova et al. proved, using enzyme electrophoresis and DNA hybridization with a cDNA probe prepared from *L. infantum*, that the *Leishmania* isolates from great gerbils in the former Soviet Union consisted of *L. major*, *L. gerbilli*, and a new species, *L. turanica*; and reported that this new species occurred in Turkmen SSR, Uzbek SSR, southern Kazakh SSR, as well as in Mongolia. Morphological studies using microscopy and experimental inoculations were also carried out. Dermal lesions without ulceration were detected in *R. opimus* and *Mesocricetus auratus* after inoculation with *L. turanica*, but in 2.5% of cases late ulceration was observed in *M. auratus*. *L. turanica* was not pathogenic to *Meriones libycus* and *Arvicanthus abyssinicus*. Of the three human volunteers inoculated with *L. turanica*, one exhibited a dermal tubercle that vanished after 14 days, while the other two exhibited small ulcers 1.5–2 months later that spontaneously healed after a further 2–2.5 months (32).

Almost concurrently, after carrying out parasitological and morphological studies on *Leishmania* isolated from great gerbils in Karamay, Xinjiang, we suggested that the parasite concerned might be a new species (3). This was subsequently identified as *L. turanica*, the first time it had been recorded in China.

Sufficient evidence has now accumulated to distinguish between *L. turanica*, *L. major*, and *L. gerbilli*, indicating that each is an individual species. Their differences have been characterized by enzyme electrophoresis (31, 32), gene typing (32, 11) and immunological assay (10), and by their natural host predisposition and pathogenicity. A broad range of natural hosts for *L. major* has been found, including *R. opimus* and several species of *Meriones*, *Allactaga*, *Mus*, *Spermophilus* (33), and *Psammomys*.
Humans (33). L. major also causes severe macroscopic dermal lesions in gerbils and acute dermal necrosis in humans (33). In contrast, for L. turanica and L. gerbilli, the only natural host identified is R. opimus (32, 35). Although no macroscopic dermal lesions were observed on the auricles of great gerbils infected with L. turanica, rather large necrotic areas of dermis were seen by microscopy (2) and small dermal ulcers were formed in humans (32, 15). Nevertheless, L. gerbilli did not induce visible dermal lesions in R. opimus or in humans (5, 20, 35).

Currently, three species of Leishmania, i.e., L. turanica, L. infantum and L. gerbilli, have been identified in rodents or sandflies in Karamay, Xinjiang. The pathogenicity of L. turanica in humans has now been verified (32, 15); human cutaneous leishmaniasis caused by L. infantum has been confirmed in eight countries in Europe, Asia and Africa. Studies on the possible role of L. infantum and L. turanica in the prevalence of human cutaneous leishmaniasis in Xinjiang should be carried out.

References
10. Xu Yong-xiang et al. [Leishmaniasis in Karamay...