Ten years of kala-azar in West Bengal, Part I. Did post-kala-azar dermal leishmaniasis initiate the outbreak in 24-Parganas?*

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Following resurgence in Bihar of epidemic kala-azar, outbreaks of the disease were identified simultaneously in two separate foci about 500 km apart in West Bengal in 1980. While the outbreak in one of these foci, in northern West Bengal, was the result of a direct extension of the Bihar epidemic, the source of parasite in the other (in the village of Bandipur in the south of West Bengal) remained unknown until a case of nodulo-ulcerative post-kala-azar dermal leishmaniasis (PKDL) was located in the village. The continued presence of this case in the village from a time long before the outbreak aroused strong suspicion about its causal role.

Laboratory-bred female Phlebotomus argentipes sandflies were allowed to feed on four cases of PKDL, including the case from Bandipur, to determine the transmission potential of these cases. Of a total of 400 flies in the experiment, 104 (26%) fed on the cases, and of these flies 44 died (31 within 48 hours of feeding). Of the surviving 60 flies, 32 developed promastigotes in the mid-gut. In view of the presence of the case of PKDL in the village prior to the outbreak and the availability of a susceptible human population and vectors, it is possible that the patient with PKDL was a source of Leishmania donovani parasites for the local focus. Furthermore, PKDL may act as a reservoir of parasites during inter-epidemic periods.

Introduction

Unlike other areas where kala-azar is endemic, in India it appears to be anthropoontic (1, 2) and the evidence available so far, although incomplete, is not consistent with the existence of a nonhuman reservoir for Leishmania donovani. Post-kala-azar dermal leishmaniasis (PKDL) has long been suspected to have the potential of a reservoir (2–4), particularly in view of the occurrence of numerous parasites in the skin of individuals with nodular PKDL. Moreover, there are uncertainties about the role played by cases of PKDL in maintaining L. donovani in nature during inter-epidemic periods and also their role in the origin of fresh outbreaks. Apart from a few serological investigations (1, 5), there have been no systematic attempts to implicate animals as reservoirs of L. donovani in India.

After kala-azar had virtually disappeared from India for about 20 years, following the outbreak in Bihar State in 1970–71, the disease was identified simultaneously in epidemic form in 1980 in two distinct foci about 500 km apart in West Bengal. One of these foci was in Malda district, in northern West Bengal, which shares a common border with the kala-azar-affected district of Katihar in Bihar, and the other was in the district of 24-Parganas, in southern West Bengal (Fig. 1) (6–9). The source of infection for the focus in Malda was traced to the adjacent district of Katihar in Bihar, with the mode of entry being population movement (8). The situation in 24-Parganas was more complex since here the population appeared not to have any communication with known kala-azar foci in either West Bengal or Bihar. It was therefore of importance to identify the source of L. donovani in this isolated district, into which a few Bengalis had immigrated from Bangladesh 20–30 years ago. A thorough house-to-house survey revealed a possible source of infection—an individual with nodulo-ulcerative PKDL in the village of Bandipur in 24-Parganas.

We therefore carried out a study to ascertain the role of this individual in initiating the kala-azar outbreak in Bandipur. Three more cases of PKDL from other unrelated areas were also included in the study.

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Materials and methods

A total of four patients with PKDL (one nodular and three nodulo-ulcerative type) were studied. Patient B.D. (Fig. 1) was from the village of Bandipur in 24-Parganas; two patients were from Bangladesh, who came to Calcutta for medical advice; and the fourth patient came originally from Calcutta and had suffered from kala-azar during the late 1940s.

All the patients were confirmed parasitologically by detection of L. donovani in smears taken from the nodules and in cultures in NNN-medium.

Sandfly feeding experiment

Phlebotomus argentipes sandflies were reared in the laboratory, as described by Addy.\(^b\)

On several occasions the sandflies were allowed to feed on each patient, who was advised not to use soap or oil for 24 hours prior to the experiment. Also the patients were warned about the pain associated with the bites and were instructed not to disturb the flies during feeding. The flies were starved for 20 hours before each feeding. Each patient was fed upon by 100 female P. argentipes after 21h 00 for 1 hour by inserting a hand with nodular lesions into the cages containing the flies. After feeding, the blood-engorged flies were separated, counted, and after being transferred to new cages, were incubated at 22 °C with water-soaked resins and cotton wool soaked in a 25% glucose solution as food. The cages were checked twice daily, and the dead flies were removed, counted and dissected. From the third post-feeding day, randomly selected flies were dissected, examined by bright-field microscopy, and photographed. The positive specimens with motile promastigotes were transferred to NNN-medium containing penicillin and streptomycin at a concentration of 100 IU/ml and 100 μg/ml, respectively. Also, every day some of the randomly selected flies were embedded in paraffin for histological examination.

Results

As shown in Table 1, 104 out of the 400 flies (26%) had a blood meal from four patients and were incubated until the sixth post-feeding day. A total of 44 of these fed flies died, 31 within 48 hours of feeding. None of the dead flies exhibited parasite development. However, promastigotes were demonstrated in 32 out of 60 (53%) of the flies that were dissected or embedded in paraffin (data for smears and histological examinations combined). While the wet preparation of the mid-gut showed profuse growth of motile promastigotes (Fig. 2a), details of the flagellated parasites could be distinguished in the histological sections (Fig. 2b). All the NNN-culture tubes were contaminated with fungal growths.

The sequence of events in the development of the skin lesions on patient B.D., and the causal relationship with the outbreak of kala-azar in his village, are outlined below:

- in 1949 B.D. had kala-azar;
- in 1976 he developed hypopigmented skin lesions on the face and extremities;
- in mid-1978 the lesions, in addition to spreading to other parts of the body, transformed to the nodular type and some ulcerated;
- in July 1980 the outbreak of kala-azar was identified in the village of Bandipur; and
- the longest duration of illness in the group of patients in the focus was 18 months, i.e., the outbreak commenced early in 1979.

Discussion

In India, kala-azar appears to occur in epidemic waves that are interspersed with inter-epidemic periods of various durations \((1, 2)\). In the absence of established animal reservoirs of *L. donovani* the origin of each epidemic, with regard to the initial source of the parasite, is a cause for concern. Because of the ready availability of the parasites in the skin, particularly in patients with the nodular type of the disease \((10)\), cases of PKDL have been suspected to have the greatest potential for maintaining *L. donovani* during inter-epidemic periods \((2)\). Except for a study by Shortt et al. \((11)\), which included cases of PKDL, all other studies of the transmission of kala-azar in India have involved active untreated or drug-failure cases \((12–14)\). Shortt et al. investigated whether there were differences in the biological behaviour in the sandfly vectors of isolates from patients with kala-azar and PKDL \((11)\). While demonstration of the transmission potential of visceral leishmaniasis has relevance to the spread of kala-azar in India during kala-azar epidemics, it would be more important to identify the reservoir during inter-epidemic periods. Although it has been discussed, there has been no attempt to implicate cases of PKDL as the initial source of parasite for a particular outbreak of kala-azar.

The current epidemic of kala-azar was first observed in districts in north Bihar early in 1970, and gradually spread to adjacent districts as well as to Katihar and Purnia districts in south Bihar. The epidemic spread continuously as well as discontinuously through population migrations. Although the source of the parasite in the first outbreak in north Bihar in 1970 is still unknown, the transmission during the epidemic period was probably person-to-person from active cases of kala-azar.

Two independent foci of kala-azar were identified in West Bengal in mid-1980. The outbreak at Harishchandrapur in Malda district was a result of a direct extension from Katihar in Bihar \((8)\). However, a meticulous investigation of the outbreak in 24-Parganas failed to yield any evidence of spread from known active kala-azar foci. The continuous presence of a particular case of PKDL in the village dating from long before the outbreak then aroused the suspicion that this individual may have played a role in the local kala-azar outbreak. Our findings confirmed the ability of cases of PKDL to infect sandflies artificially, indicating that similar infection could occur under natural conditions. Clearly this evidence does not prove that the case of PKDL in Bandipur (B.D.) was the source of the *L. donovani* parasites for the outbreak in 24-Parganas. The following evidence, however, strongly suggests involvement of a local source of PKDL in initiating the outbreak: the presence of numerous amastigotes in skin nodules; the infectivity of the case; the availability of *P. argentipes* in the village \((15)\); the presence of the patient in the same village prior to the outbreak; and the absence of population exchange with other ongoing epidemic foci. Although the outbreak was first confirmed in 1980, the clinical manifestations dated back to early 1979. Since PKDL is at its maximum infectivity at the nodular stage \((which\ B.D.\ developed\ in\ mid-1978)\, the incubation period of the focus was well within the accepted limit.

The serological similarity between the strains of visceral leishmaniasis and PKDL indicated that the parasite causing the original visceral infection was also responsible for PKDL \((16)\). Isozyme characterization of isolates from Indian cases of PKDL, obtained from the Calcutta School of Tropical Medicine, were indistinguishable from those of Indian kala-azar (zymodeme, LON-41) \((17)\). Furthermore, inoculations of parasites isolated from the lesions of cases of PKDL have the ability to cause kala-azar, as indicated by the occurrence of visceral leishmaniasis in model experiments involving hamsters and mice \((18–21)\).

That population exchange played a decisive role in the intra-epidemic spread of kala-azar was clearly evident from the subsequent extension of the focus to other areas inhabited by the local tribal community in the same as well as in several other districts \((9)\). It is extremely difficult to prove definitively that a given case of PKDL was responsible for a particular outbreak in the absence of a follow-up study tracing cases of PKDL in subsequent outbreaks. The findings of a further prospective study (in preparation), i.e., the development and occurrence of PKDL between two outbreaks of visceral leishmaniasis, further strengthen the evidence implicating its role in initiating fresh transmission of kala-azar.

The high death rate of the blood-fed flies early in the post-feeding period is difficult to explain. Although some of the flies exhibited promastigotes on the third post-feeding day, the highest rate of infection occurred after the fourth post-feeding day.

It should be noted that despite their positive transmission potential and continued presence in Calcutta, the three other cases of PKDL in the study did not pose any problem, perhaps because of the absence of *P. argentipes* vectors in the city.

From the above discussion, it can be concluded that, under the epidemiological circumstances that prevail in India, cases of PKDL might play an important role as reservoirs of kala-azar, particularly in inter-epidemic periods, and given an appropriate

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See footnote a, p. 341.
Table 1: Parasite development in the laboratory-bred Phlebotomus argentipes fed on four patients with post-kala-azar dermal leishmaniasis on different occasions

<table>
<thead>
<tr>
<th>No. of days post-feeding</th>
<th>No. of flies:</th>
<th>Dissected + embedded in paraffin</th>
<th>No. with promastigotes:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed/used</td>
<td>Live, fed</td>
<td>Died</td>
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<tr>
<td>0</td>
<td>104/400</td>
<td>104</td>
<td>—</td>
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<tr>
<td>1</td>
<td>—</td>
<td>104</td>
<td>19</td>
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<td>2</td>
<td>—</td>
<td>85</td>
<td>12</td>
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<td>3</td>
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<td>3</td>
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<tr>
<td>6</td>
<td>—</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>400</td>
<td>104 (26)*</td>
<td>44 (42.3)</td>
</tr>
</tbody>
</table>

* Figures in parentheses are the percentages of the totals in the preceding column. For promastigotes, the figure in parentheses is the percentage of the total number of flies that were dissected and embedded in paraffin.

Fig. 2. Dissected mid-gut of an infected Phlebotomus argentipes sandfly, showing (a) clusters of refractile motile promastigotes (arrow) in a saline preparation (bright-field microscopy, X400); and (b) paraffin-embedded section (haematoxylin-eosin stained) of another infected P. argentipes showing an individual promastigote (arrow) with long flagellum (X 1000).

Acknowledgements

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Résumé

Dix ans de kala-azar au Bengale-Occidental, Partie I. La leishmaniose post-kala-azar a-t-elle déclenché l'épidémie dans le 24-Parganas?

Après une période silencieuse d'environ 20 ans, le kala-azar a fait sa réapparition en Inde sous forme épidermique dans le nord de l'Etat de Bihar en 1970, et s'est progressivement étendu non seulement aux autres parties de cet Etat, mais également, en 1980, au district de Malda, nord de l'Etat voisin du Bengale-Occidental. Simultanément, un autre foyer indépendant de
kala-azar a été reconnu en 1980 à Bandipur, village du district de 24-Parganas dans le sud du Bengale-Occidental. En l’absence de tout phénomène migratoire et de toute communication avec un foyer d’endémie connu, l’origine du parasite dans l’épidémie de Bandipur est restée obscure (de même que dans le cas de l’épidémie du nord du Bihar), jusqu’à la découverte d’un sujet atteint de leishmaniose cutanée post-kala-azar (PKDL) ayant longtemps résidé à Bandipur avant le déclenchement de l’épidémie.

En l’absence de réservoir animal connu de Leishmania donovani en Inde, on soupçonne depuis longtemps les cas de PKDL d’entretenir le parasite pendant les périodes interépidémiques et d’être à l’origine de la reprise de la transmission du kala-azar. On a donc réalisé une étude afin d’évaluer l’aptitude du sujet atteint de PKDL à transmettre L. donovani à des phlébotomes (Phlebotomus argentipes) élevés en laboratoire et, ainsi, à se comporter comme source locale d’infestation. L’étude comportait également trois cas de PKDL provenant d’autres régions.

Sur les 400 femelles de P. argentipes utilisées pour l’étude, 104 se sont nourries sur les malades; elles ont été incubées et 44 sont mortes dans les 48 heures suivant le repas de sang. Sur les 60 autres, 32 ont été trouvées infestées par L. donovani (présence de promastigotes dans le mésentéron). Ces résultats confirment donc l’aptitude des cas de PKDL, notamment de la variété nodulaire, à transmettre l’infestation à des phlébotomes dans des conditions expérimentales.

Au vu de ces résultats, le malade atteint de PKDL a été fortement soupçonné d’avoir servi de point de départ de l’épidémie de kala-azar à Bandipur. Cette hypothèse a été encore renforcée par les faits suivants: présence d’amastigotes en grand nombre dans les nodules cutanés du malade; infectiosité considérable du malade pour les phlébotomes; séjour continu du malade dans le village avant l’épidémie; absence de communication avec tout foyer endémique connu de kala-azar; similitude entre les parasites responsables du kala-azar et de la PKDL; existence de populations vectrices et humaines sensibles au moment de la transmission.

Ces résultats laissent à penser que dans la situation épidémiologique qui prévaut en Inde, les cas de PKDL pourraient jouer un rôle important dans le maintien du réservoir de L. donovani pendant les périodes interépidémiques et être à l’origine des flambées ultérieures de kala-azar. Pour éviter de nouvelles flambées dans le pays, il faudra exercer une surveillance appropriée afin de diagnostiquer et traiter les cas de PKDL, de façon à éliminer la source du parasite dans la communauté.

References