controls and from BT patients (Fig. 1). In nonreactional LL patients there was no chemiluminescence with PMA stimulation. In contrast, monocytes from LL patients with ENL showed activation with PMA. In patients with ENL reactions being treated with thalidomide, chemiluminescence induced by PMA was lower than that seen in patients with untreated ENL.

As expected, lymphocyte proliferation *in vitro* in response to *M. leprae* was positive in some of the healthy controls and BT patients, but was essentially negative in BL and LL patients (Fig. 2).

Although the number of patients studied in each group is small, the results suggest that during ENL the monocytes of LL patients can respond to PMA although their lymphocytes remain unresponsive to *M. leprae.*

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Can Sandflies Be the Vector for Leprosy?

To the Editor:

There are several biting arthropods existing in leprosy-endemic areas of which any one could theoretically act as a vector for the transmission of leprosy. Arthropods such as mosquitoes, bed bugs and a few others take up *Mycobacterium leprae* while feeding on lepromatous leprosy patients ([1, 3, 7]). Bacilli remain viable for many days in the body of these arthropods ([5, 6, 8]). In tropical and subtropical countries where leprosy is endemic, sandflies are also commonly found. However, their role as a vector in the transmission of leprosy has so far not been investigated.

Acid-fast bacilli in field-collected sandflies. As a preliminary approach to screen for acid-fast bacilli (AFB), sandflies were periodically collected from the mud houses of known leprosy patients as well as from houses where there were no cases of leprosy. The sandflies were identified and classified ([2, 9]). There are eight species of sandflies belonging to the genera *Phlebotomus* and *Sergentomyia* existing in these areas of Agra, India. *P. papatasi* is found to be the most

predominant species of this region and accounted for 75% of the collection. Forty-seven percent of the sandflies caught from the houses of leprosy patients were found to harbor AFB in their gut meal. The number of AFB in the gut of these wild-caught sandflies varied from 1 to 195. This finding prompted us to carry this study further to determine the role of sandflies in the transmission of leprosy.

AFB in experimentally fed sandflies. Four-day-old laboratory-reared *P. papatasi* ([4]), about 20 in number, were made to feed on untreated, active lepromatous leprosy patients. The patients' bacterial index (BI) ranged from 4+ to 5+ with the morphological index (MI) between 1.2% and 3%. All were bacteremic with a load of 1 to 6 × 10^5 bacilli per ml. After allowing the sandflies to have a full bloodmeal, groups of 10 sandflies were sacrificed immediately by chilling. The remaining sandflies were sacrificed periodically thereafter at daily intervals up to 10 days. The sandflies were dissected, and a uniform smear was made of all the teased-out components of the proboscis, gut, and fecal deposits on the slide and stained for AFB. The results of periodic screening of the proboscis, gut, and fecal deposits showed that the number of AFB present in these sites declined over time. Occasional AFB were still present in the proboscis on the 8th day; the gut content and fecal deposit did not show any AFB by the 8th day. The maximum number of AFB found was 290 in the proboscis, 595 in the gut meal, and 437 in the fecal deposits.

Inoculating mice with gut meals of sandflies. Sandflies fed on lepromatous leprosy patients were placed in groups of 10 and 20 for each experiment. Each day, from day 0 to day 10, sandflies were sacrificed by chilling, dissected, and the gut contents were pooled. It was observed that a small proportion of AFB were present in these pooled gut suspensions only up to day 7. These suspensions were inoculated into the foot pads of mice, and foot pad harvests at 12 months postinoculation revealed the presence of an insignificant number of bacilli in only one mouse each in two groups of sandflies. In both of these groups mice were inoculated with day 0 suspensions. In all other mice, including the controls, the harvests were negative for AFB.

Feeding half-fed sandflies on foot pads of mice. Sandflies half fed on lepromatous leprosy patients were allowed to refeed on a mouse to determine whether *M. leprae* could be carried mechanically through their contaminated proboscis. The sandflies were made to refeed on the hind foot pads of mice from day 0 to day 8 in batches of 1, 6, and 10. The mouse foot pads were harvested immediately and also at the end of the 12-month post-refeeding. Only a single mouse in each group, harvested immediately after refeeding, showed a few AFB.

From these experiments it is evident that while taking a bloodmeal sandflies pick up AFB through their proboscis. Some of these organisms are ingested and subsequently excreted. However, very few bacilli were carried mechanically by the contaminated proboscis. The decline in the number of AFB in the proboscis, gut and fecal deposits with the passage of time and the failure to multiply significantly in the foot pads of mice indicate that the organisms are nonviable. The mere presence of AFB in the proboscis, gut, and fecal deposits may not make the sandfly a vector for the transmission of leprosy.

In conclusion, the present study indicates that sandflies do not seem to have any epidemiological significance in the transmission of leprosy.

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Comparison of PGL-I Level with AFB Numbers in Foot Pad Suspension

To the Editor:

Since the time that phenolic glycolipid-I (PGL-I) was first isolated and characterized as a *Mycobacterium leprae*-specific product ([4, 5]), it has been widely used in serological tests for leprosy. Besides its use for purposes of detecting antibodies to the antigen, PGL-I has been found in various clinical specimens, such as serum ([1–3, 10]), urine ([6]), and tissues ([8, 9]), etc. However, PGL-I has never been assayed in tissues of mouse foot pads inoculated with *M. leprae* where it could prove to be a useful surrogate of acid-fast bacilli (AFB) numbers. Therefore, we attempted to measure the levels of PGL-I in a mouse foot pad suspension using the dot enzyme-linked immunosorbent assay (ELISA) described previously ([2, 3]). Briefly, foot pad suspensions (1.0–1.7 ml) were lyophilized, and the lipids were extracted with chloroform : methanol (2:1) solution. After application to a florisil column, the chloroform : methanol (19:1) elute was examined for the presence of PGL-I by dot-ELISA using rabbit anti-*M. leprae* antiserum containing anti-PGL-I antibodies. A series of normal mouse foot pad suspensions containing different amounts of the standard PGL-I were processed using the same procedures to determine the test parameters for

The Table. *Detection of PGL-I in foot pad suspensions.*

AFB numbers counted	No. assayed	PGL-I-positive[a]	PGL-I level (ng)
		No. (%)	Mean ± S.D.[b]
$<7.22 \times 10^3$ or $<1.77 \times 10^4$	14	1 (7.1)	7.0
7.22×10^3 –9.4×10^4	15	8 (53.3)	34.7 ± 39.6
1.0×10^5 –1.0×10^6	15	15 (100)	98.0 ± 89.7
$>1.0 \times 10^6$	14	14 (100)	353.4 ± 428.2

[a] Determined by dot-ELISA.
[b] Calculated based on the suspensions containing PGL-I.