**Leishmania major** Promastigotes Inhibit Dendritic Cell Motility In Vivo

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Using an in vitro transwell migration assay, we have demonstrated that products secreted by **Leishmania major** promastigotes inhibit the motility of dendritic cells (DC) by up to 93%. Inhibition was dose dependent and reversible. By inhibiting DC migration in vivo, **L. major** may therefore subvert DC from their potentially protective role during leishmaniasis.

Resistance against **Leishmania** follows from the activation of Th1 CD4+ T-cell-mediated immunity (reviewed in reference 6). The production of interleukin 12 (IL-12), a cytokine that promotes the development of a Th1 response, is detectable in the early stages of experimental **Leishmania major** infection (5, 6, 8). Dendritic cells (DC) produce IL-12 following stimulation with **L. major** in vitro (8, 22) and can be found bearing parasites in lymph nodes draining the site of infection (11); these DC are able to stimulate **Leishmania**-specific T cells (8, 12). The initiation of a primary immune response requires the interaction of antigen-presenting DC with recirculating naive T cells in secondary lymphoid tissue. Therefore, movement of DC from the skin to the draining lymph nodes is a critical step in the initiation of a response to cutaneous **Leishmania** infection. Isolated DC are characteristically motile cells (7), but here we report that **L. major** is able to inhibit DC motility in vitro and suggest that this may be a further means by which the parasite can subvert the development of a protective immune response.

Spleen DC display high spontaneous motility. Partially enriched DC from 6- to 8-week-old BALB/c mice were isolated after overnight culture of spleen cells in complete tissue culture medium: Dutch modified RPMI 1640 (Gibco, Paisley, United Kingdom) supplemented with 10% (vol/vol) fetal calf serum (Gibco, Grand Island, N.Y.), 2 mM L-glutamine, 100 U of penicillin/ml, and 100 µg of streptomycin/ml. Metrizamide (13.7%) was used (Nygaard, Oslo, Norway) as previously described (8) to obtain low-density cells (LDC). Spontaneous migration of these cells (105/100 µl) from the upper to the lower chamber of 3-µm-pore-size Transwells (Costar, Cambridge, Mass.) was assayed after incubation for 1 h at 37°C in 5% CO2. Migrating cells were quantitated by one of two methods: (i) cytopsins were prepared from the contents of each lower chamber and were fixed and stained with modified Wright Giemsa (Sigma, Dorset, United Kingdom), and cells were counted microscopically, or (ii) cellular ATP was assayed in the lower chamber with a ViaLight HS ATP kit (LumiTech, Workingham, United Kingdom). Luminescence from the luciferase-catalyzed reaction between ATP and luciferin was measured with a Turner Designs luminometer (TD20/20) set up for a 3-s delay and a 10-s integration time. A linear relationship exists between ATP content and cell number; results obtained by this method are expressed as relative luminescence units (RLU).

When complete tissue culture medium was placed in the lower chamber, approximately 1.2% of the input cells migrated during the culture period. Immunomagnetic separation of the input cells with a Minimax column (labeling with anti-CD11c microbeads; Miltenyi, Bisley, United Kingdom) and FACScan analysis (labeling with fluorescein isothiocyanate-labeled anti-CD11c antibodies; Pharmingen, Oxford, United Kingdom) confirmed that CD11c+ DC but not contaminating CD11c- cells displayed spontaneous migration (Fig. 1).

**Leishmania inhibit DC motility.** **L. major** promastigote (JISH 118, originating in Saudia Arabia)-conditioned medium (PCM) was prepared as follows: parasites were cultured in conditioned medium, metacyclic parasites were recovered for use at the stationary phase of culture (approximately days 5 to 9), and the parasite-conditioned medium (PCM) was collected by centrifugation (600 × g, 5 min). When complete tissue culture medium was replaced by PCM in the lower chamber of the Transwell, DC migration was reduced by up to 93% (average over 5 experiments, 76% ± 5%) (Fig. 2A and Table 1). PCM in which residual dead parasites were present (killed by three rounds of freeze-thawing [F/Th-PCM]) was only marginally less inhibitory than untreated PCM (47% versus 66% inhibition of migration). Similarly, removal of live parasites by filtration (0.22-µm-pore-size filter) (F-PCM) had little effect on the inhibition of DC motility (73% inhibition). Together these findings demonstrate that the continued presence of viable promastigotes is not required for inhibition of DC motility. On the other hand, introduction of promastigotes into fresh medium in the lower chamber also inhibited DC migration by up to 24% (data not shown).

Inhibition of DC motility by PCM is dose dependent and reversible. To determine if **Leishmania**-mediated inhibition of DC motility is dose dependent, F-PCM was assayed neat or diluted 1/2, 1/10, or 1/30 (Fig. 2B). Neat F-PCM inhibited DC migration by 74%, which was reduced to 33% when F-PCM was diluted 1/2. Further dilution of the F-PCM ablated the ability of F-PCM to inhibit DC migration (Fig. 2B). The use of...
short-term culture media—media in which parasites were cultured for 1 h and then removed by centrifugation—was also inhibitory to DC motility in a dose-dependent manner, depending upon the amount of parasites cultured for 1 h (data not shown). This lends support to parasite metabolites being responsible for the observed effect on DC rather than changes in pH or nutrient availability.

PCM-induced inhibition of DC motility is reversible. Transwells containing DC were incubated for 1 h in either medium alone or PCM and then were transferred to new wells contain-
ing fresh medium. Following a second 1-h incubation, the Transwells were again moved to new wells containing fresh medium. After all incubations were complete, medium from the bottom chambers was collected and the number of migrated cells was determined. PCM-mediated inhibition of DC motility (66% after 1 h) was reversible when DC had been removed from the PCM for 1 and 2 h (Fig. 2C).

DC are characteristically motile in vitro (2, 7, 10, 18, 19, 20), and this work demonstrates that products of L. major PCM for 1 and 2 h (Fig. 2C). After all incubations were complete, medium from the bottom chamber was collected, and the number of migrated cells was determined. PCM-mediated inhibition of DC motility (66% after 1 h) was reversible when DC had been removed from the PCM for 1 and 2 h (Fig. 2C).

Whether the reduced DC motility observed was due to parasite-induced changes in DC adhesion molecules has still to be investigated; however, previous work (8) has shown that coculture of DC with promastigotes does not affect the general aspects of DC activation.

Migration, from the skin to the regional lymph nodes, of DC bearing parasite antigens is likely to be a pivotal step in the development and immunotherapy. In particular, the reversible nature of the block in DC movement may mean that therapeutic agents which can overcome the effect of the parasite and stimulate movement of antigen-bearing DC into the lymph nodes may be of value in infected individuals.

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**REFERENCES**


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**TABLE 1. DC motility is inhibited by PCM**

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<th>Expt no.</th>
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<tr>
<td>Average ± SEM</td>
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<td>54 ± 13</td>
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*a Inhibition of DC motility by PCM is shown after DC were incubated in PCM for 1 h.*
integrins are required for Langerhans cell migration from the epidermis. J. Exp. Med. 186:1725–1735.


