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Short Report

Monoclonal antibodies for the identification of New World *Leishmania*

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Although some species-specific and complex-specific monoclonal antibodies (Mabs) are available for the identification of *Leishmania*, they recognize only approximately 50% of isolates from Amazonian Brazil (SHAW *et al.*, 1986). The aim of this work was therefore to produce new Mabs which could be used for identification of these *Leishmania* spp. by an indirect fluorescent antibody test (IFAT).

BALB/c mice were used to produce Mabs as described by HANHAM *et al.* (1990). For the production of Mabs against *L. (Viannia) naiffi*, mice were immunized and boosted with membrane preparations of a mixture of 3 isolates of this species (MDAS/BR/79/M5648, MDAS/BR/78/M5196 and MDAS/BR/79/M5533). For Mabs against *L. (L.) deanei* and *L. (L.) hertigi*, membrane mixtures of 2 isolates of *L. (L.) deanei* and a single isolate of *L. (L.) hertigi* (MCOE/BR/78/M5068, MCOE/BR/78/M5088 and MCOE/PA/65/C8) were used; for *L. (V.) lainsoni*, membrane preparations of 4 isolates of that species (MHOM/BR/81/M6426, MHOM/BR/84/M8040, MHOM/BR/84/M8157 and MHOM/BR/84/M11073) were used; for *L. (L.) mexicana*, the single isolate MNYC/BZ/62/M379 was used to immunize and boost mice before fusion. Hybridomas were screened by enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA), and the specificity of Mabs was determined by extensive cross-testing against a wide range of *Leishmania* isolates using both ELISA and IFAT. ELISA was performed on whole parasites cross-linked to antigen plates.

Five Mabs were obtained which could be used to identify *Leishmania* by IFAT. Mabs LA4 and N3 recognized *L. (V.) lainsoni* and *L. (V.) naiffi*, respectively. These parasites were recently characterized and described as new species of *Leishmania* (LAINSON & SHAW, 1989; SILVEIRA *et al.*, 1987). Mab N2 recognized both *L. (V.) naiffi* and *L. (V.) braziliensis*, and could therefore be used in conjunction with N3 to identify the latter species. Mab H1 recognized parasites belonging exclusively to the *L. hertigi* group (*L. (L.) hertigi* and *L. (L.) deanei*). Mab WA2 reacted with all isolates of *L. (L.) mexicana* and *L. (L.) amazonensis* tested to date, and will now be tested against other isolates of the *L. mexicana* complex. Mabs N2, N3, H1 and WA2 all gave very strong signals by IFAT, and did not cross-react non-specifically with any other strains of *Leishmania*. The intensity of fluorescent signal obtained by reacting LA4 with isolates of *L. (V.) lainsoni* was highly variable, but always positive. This variation may indicate heterogeneity in surface antigen expression between different members of this species. ELISA results were in close agreement with those of IFAT for all Mabs, although WA2 awaits full characterization.

Mab WA2 was produced after immunization with a single isolate of *L. (L.) mexicana*. We have subsequently, however, had greater success in producing both group- and species-specific Mabs by using mixtures of membrane antigens from several isolates of the same species. Immunization with a single isolate frequently resulted in Mabs which recognized only some members of the species and often cross-reacted with unrelated parasite strains.

We have, therefore, produced Mabs which may be used for the identification of *L. (V.) naiffi*, *L. (V.) braziliensis*, *L. (V.) lainsoni*, parasites of the *L. hertigi* group, and *Leishmania* species within the *L. mexicana* complex, by either ELISA or IFAT.

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