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Leishmania Immunoassay: IgE Antibody to Culture Medium Components

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The study of leishmaniasis has been aided by the in vitro culture of the protozoan (Rowe and Hirumi 1980) and by highly sensitive immunoassays (Voller and De Savigny 1981). However, such immunoassays can cause interpretational complications due to cross-reactivity between distinct infectious agents (Yarzabal et al. 1979; Voller and De Savigny 1981). We describe here the possible occurrence of additional spurious results caused by the protozoal culture medium employed. A total of 194 sera from individuals (56% under 15 years of age) in zones of American cutaneous leishmaniasis were tested for their content of IgE antibody by the radio-allergosorbent test (RAST; Lynch et al. 1975) against extracts (Lynch et al. 1982) of the AVZ strain (Perez et al. 1979) of *L. mexicana*. The parasites were cultured in two different types of medium: Liver infusion tryptose (LIT) and a defined medium (REN) containing no extraneous protein (Steiger and Steiger 1977). Although the RAST employed was developed in our laboratory, it was standardized against the commercially available "Phadebas" (Pharmacia, Sweden) kit, the IgE antibody levels being expressed, therefore, as RAST units (RU)/ml.

We found 43 sera to contain low, but detectable, levels ($\geqq 0.1$ RU/ml) of specific IgE antibody against LIT-cultivated *Leishmania* (mean $\pm$ SEM level of 1.02 ± 0.11 RU/ml). However, 5 of these positive sera exhibited < 0.1 RU/ml when tested against REN-cultivated organisms, while containing 0.37 to 0.78 RU/ml against LIT-cultured *Leishmania* extracts. As the most evident difference between LIT and REN media is the presence of animal protein in the former, these 5 sera were examined for their content of IgE antibody against bovine milk proteins, and were found to contain relatively high levels (4.9 to 8.4 RU/ml). As some of the 194 test sera contained up to 6.8 RU/ml against milk proteins, this probably explains the somewhat higher RAST levels detected with LIT-*Leishmania* over REN-cultured organisms.

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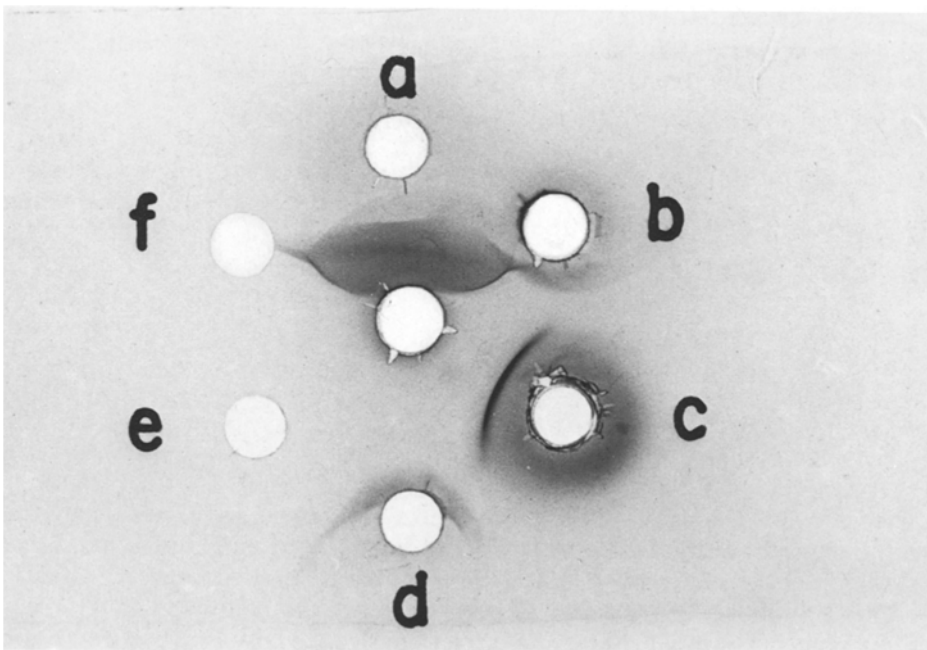

Fig. 1. Centre well: Hyperimmune rabbit antibovine plasma. Well *a*: LIT medium, *b*: REN medium, *c*: Extract of LIT-cultivated *Leishmania* (3 washes, saline) at 15.6 mg protein/ml, *d*: LIT-cultivated *Leishmania* (6 washes in saline with 0.005% Tween 20, 0.1 mM EDTA) 18.6 mg/ml, *e*: REN-cultivated *Leishmania* (3 washes, saline) 27.8 mg/ml, *f*: REN-cultivated *Leishmania* (6 washes, 0.005% Tween 20, 0.1 mM EDTA) 21.2 mg/ml. Immunodiffusion allowed to proceed for a supra-optimal period (32 h) to allow adequate visualization of precipitation band *d*

Using immunochemical techniques that have been previously described (Lynch and Turner 1974; Lynch et al. 1975), the *Leishmania* extracts were tested against a hyperimmune rabbit antibovine plasma antiserum. The LIT-cultured *Leishmania* were contaminated by significant quantities of bovine proteins, even after extensive washing of the organisms (Fig. 1). In contrast, no reaction was detected between REN-cultured *Leishmania* and anti-bovine plasma antibody. This latter observation, and the fact that a hyperimmune rabbit antiserum that was prepared against REN-cultured organisms did not react with bovine plasma, demonstrate that no significant cross-reactivity existed between this protozoan and bovine proteins.

LIT-cultured *Leishmania* extracts were also immunoadsorbed by passage through a Sepharose-antibovine plasma column and then used in the immunoassay. The sera that were previously LIT positive-REN negative then became LIT negative-REN negative. When one of these test sera was passed through a Sepharose-bovine plasma column, the reactivity towards LIT-*Leishmania* was removed, the IgE antibody concentration being <0.1 RU/ml, compared to a level of 0.67 RU/ml after passage through a control column (Sepharose-antibovine plasma).

These results demonstrate the occurrence of false-positive reactions in sensitive immunoassays for anti-*Leishmania* antibody caused by the use of complex culture media. Protozoa can incorporate medium constituents (Bretaña and O'Daly 1976), and extracts can be contaminated by animal proteins (LeRay et al. 1974). In addition, the occurrence of antibodies, including IgE (Savilahti 1981), against bovine proteins in human sera is not rare (Foucard et al. 1975). A number of semi-defined protozoal culture media have, however, been described (Merino and Gabaldon 1978; O'Daly 1980; Rowe and Hirumi 1980), and we tested a small batch of *Leishmania* grown in a medium (Steiger and Steiger 1977) that is free of extraneous protein. While the yield and reliability of the latter type of medium is still irregular (JL Avila, unpublished observations), the use of single protein supplements (Avila et al. 1979; O'Daly 1980) is a promising direction for further investigation. Such defined media not only reduce the possibility of spurious results in sensitive immunoassays, but also allow entire cultures to be used in the preparation of antigenic extracts, including the soluble protozoal products (Hernandez et al. 1981) that are found in the supernatants.

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