Specific Delayed-type Hypersensitivity Footpad Response in Mice Artificially Immunized with Two *Trypanosoma* (*Schizotrypanum*) Species

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INTRODUCTION

There is much evidence that T-cell (thymocyte) mediated immunological responses occur in patients or laboratory animals infected or artificially immunized with *Trypanosoma* (*Schizotrypanum*) *cruzi* (see review by Hanson, 1976). As part of a study of species of this subgenus which do not infect man, we investigated the specificity of a cell-mediated immune response in mice artificially immunized with *T. (S.) dionisii* or *T. (S.) vespertilionis* (of Chiroptera), using as an indicator the delayed hypersensitivity footpad skin test (Crowle, 1975). Some preliminary work has been reported (Liston, 1974).

METHODS

Origin and maintenance of strains. *Trypanosoma (S.) dionisii* strain P3 and *T. (S.) vespertilionis* strain N6, isolated from *Pipistrellus pipistrellus* and *Nyctalus noctula* respectively (Baker & Thompson, 1971) were maintained as frozen (-196°C) stabulates and *in vitro* at 28°C in medium L4NHS (medium L4N of Baker *et al.*, 1972, with halved concentrations of rabbit serum and rabbit erythrocyte lysate).

Immunization of mice. Adult male Parke's mice (Animal Virus Research Institute, Pirbright, Surrey) were immunized with two intradermal injections, 2 weeks apart, of 0.05 ml of one of the following three preparations: 5-day-old cultures of (i) *T. dionisii* or (ii) *T. vespertilionis*, washed three times and resuspended in phosphate-buffered saline, pH 7.3 (PBS; Dulbecco's solution A, Oxoid) at $4 \times 10^8 \text{ ml}^{-1}$ and emulsified with an equal volume of Freund's complete adjuvant (FCA; Difco), so that each mouse received 1×10^7 flagellates; or (iii) culture medium L4NHS emulsified with an equal volume of FCA.

Preparation of antigen. Purified soluble antigen (PSA) for skin testing was prepared from cultures of *T. dionisii* and *T. vespertilionis* as described by Bryceson *et al.* (1970), freeze-dried and stored at -20°C ; it was reconstituted at 1.0 mg ml^{-1} in PBS.

Skin testing. Groups of immunized or normal mice (see above) were inoculated intradermally in the plantar surface of the hind feet with 0.01 ml PBS or PSA, with the aid of an Agla micrometer syringe (Wellcome), 4 weeks after the first immunizing injection. The thickness of the foot was measured immediately before, and at intervals after, this procedure, with a Quicktest model A02T gauge (Carobronze Ltd, School Road, Belmont Road, London W4). All experiments were conducted 'blind', the person measuring the response not knowing which immunogen, if any, had been given to a mouse, nor which foot had received antigen or control PBS.

Fig. 1. Skin test reactions of mice challenged intradermally in the footpad with 0.01 mg antigen or 0.01 ml culture medium L4NHS 4 weeks after the first of two immunizing injections (2 weeks apart) of 10^7 trypanosomes or 0.025 ml medium in Freund's complete adjuvant given intradermally. The experiments were performed 'blind' (see Methods). Each point represents the mean difference in thickness between the foot challenged with antigen and that of the same mouse receiving PBS only, measured in millimetres at the time shown. Vertical bars show 95 % confidence limits (twice the standard error of the mean); the difference in thickness is significant at this level when the bars do not meet the line indicating zero difference.

Immunogen	Challenge antigen	No. of mice	Immunogen	Challenge antigen	No. of mice
(a) None	<i>T. dionisii</i> P3	21	(g) <i>T. vespertilionis</i> N6	<i>T. vespertilionis</i> N6	16
(b) None	<i>T. vespertilionis</i> N6	12	(h) <i>T. vespertilionis</i> N6	<i>T. dionisii</i> P3	12
(c) None	Medium L4NHS	11	(i) <i>T. vespertilionis</i> N6	Medium L4NHS	12
(d) <i>T. dionisii</i> P3	<i>T. dionisii</i> P3	29	(j) Medium L4NHS	Medium L4NHS	4
(e) <i>T. dionisii</i> P3	<i>T. vespertilionis</i> N6	18	(k) Medium L4NHS	<i>T. dionisii</i> P3	13
(f) <i>T. dionisii</i> P3	Medium L4NHS	22	(l) Medium L4NHS	<i>T. vespertilionis</i> N6	6

RESULTS AND DISCUSSION

No delayed response significantly greater than that occurring in the footpad challenged with saline resulted from challenging non-immune mice with either trypanosomal or medium antigens (Fig. 1 *a, b, c*), nor did any delayed response occur in mice immunized with FCA and challenged with either trypanosomal antigen or medium.

Strong, highly significant ($P < 0.05$) delayed responses occurred in mice immunized with trypanosomal antigen or medium and challenged with homologous antigen (Fig. 1 *d, g, j*).

Slight but significant delayed cross-reactions occurred in mice immunized with *T. dionisii* and challenged with *T. vespertilionis* (Fig. 1 *e*) and in the reciprocal test (Fig. 1 *h*), indicating that the two species possessed some shared antigens. Other workers have shown the existence

of common antigens within the subgenus *Schizotrypanum*: Bice & Zeledón (1971) showed electrophoretically that *T. cruzi* and a Costa Rican strain identified as *T. vespertilionis* shared five out of seven antigenic fractions and Soria & Dusanic (1975) demonstrated by immunodiffusion the existence of shared and specific antigens in the strains of *T. dionisii* and *T. vespertilionis* used by us; such antigens were not, however, necessarily those responsible for delayed hypersensitivity skin reactions.

Similar cross-reactions occurred in mice immunized with *T. vespertilionis* and challenged with medium (Fig. 1*i*), in the reciprocal test (Fig. 1*l*) and in mice immunized with medium and challenged with *T. dionisii* (Fig. 1*k*); these were probably caused by contamination of the trypanosomal antigen by endocytosed medium. Mice immunized with *T. dionisii* and challenged with medium did not give a delayed response (Fig. 1*f*), possibly because *T. dionisii*, a smaller organism *in vitro* than *T. vespertilionis*, contained after washing only traces of medium which were insufficient to provoke an immune response after challenge with medium; mice immunized with medium in the reverse reaction would be highly sensitized to the very immunogenic medium components so that traces of medium in the *T. dionisii* challenge antigen could produce a response.

These results, which showed a considerable degree of specificity of the delayed response, and those of Soria & Dusanic (1975), indicated some, though incomplete, antigenic difference between *T. dionisii* and *T. vespertilionis*. Immediate responses were less specific but the homologous reactions were more pronounced.

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