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IMMUNOLOGY

Protective Effect of Killed Trypanosome Vaccines with Incorporated Adjuvants

ATTEMPTS to control protozoal diseases by active immunization were unsuccessful until Mulligan *et al.*[1] showed that chickens could be partially protected against sporozoite-induced *Plasmodium gallinaceum* infection by means of an inactivated sporozoite preparation. Freund *et al.*[2,3] later immunized ducks against *P. lophurae* by incorporating the antigen in the aqueous phase of a water-in-oil emulsion containing killed mycobacteria.

We have been examining the adjuvant effect of a large number of miscellaneous substances on diphtheria toxoid and of some of these on *Trypanosoma congolense* antigen. A selected group of the adjuvants most effective with *T. congolense* antigen have also been tested with *T. cruzi* antigen.

The antigen was prepared by separating trypanosomes from the blood of heavily infected mice. Mouse red blood cells were agglutinated by the addition of mouse erythrocyte antiserum or phytohæmagglutinin. The trypanosomes trapped by the agglutinated red blood cells were collected by washing with saline. The separated trypanosomes were then washed twice with cold glucose saline and concentrated into a suitable volume. The trypanosomes were killed by rapid freeze-thawing. The killed trypanosome suspension (which constitutes the antigen) was pale pink to red in colour, depending on the numbers of red blood cells which it contained. It was stored at $-20°$ until used.

Many substances were examined for adjuvant action, the results of which will be described in a later publication. In this communication, results with three substances are described: (1) *Quillaia* saponin; (2) a quaternary ammonium surface active agent, 'Arquad 2HT'; (3) an alkylamine, *n*-hexadecylamine. Solutions or suspensions of these substances in water were prepared containing 1 mg/ml., 5 mg/ml. and 5 mg/ml., respectively. Mice were immunized against *T. congolense* by injecting subcutaneously a mixture of 0·1 ml. trypanosome antigen (equivalent to $1·0 \times 10^6$ trypanosomes) and 0·1 ml. of adjuvant solution or suspension. Two doses of vaccine were given with 14 days between doses. The mice were challenged by inoculating $1·0 \times 10^5$ trypanosomes subcutaneously, 14 days after the last immunizing dose. The *T. cruzi* vaccine consisted of a mixture of 0·1 ml. antigen (equivalent to $6·0 \times 10^5$ trypanosomes) and 0·1 ml. adjuvant. Groups of 9 or 10 mice were immunized with *T. cruzi* vaccine in the same way as with *T. congolense* vaccine and were challenged with $2·0 \times 10^5$ *T. cruzi* trypanosomes. The strains used were *T. congolense* strain *NIMR* and *T. cruzi* strain *Y*. After challenge, the tail blood of each mouse was examined microscopically for trypanosomes. Microscopical examinations were started 3–5 days after challenge and repeated thereafter 3 times per week.

The results with *T. congolense* (Table 1) and *T. cruzi* (Table 2) show that whereas the antigen or adjuvants given separately had no protective effect, the mixture of antigen and adjuvant provided a considerable degree of protection in mice. With both *T. congolense* and *T. cruzi* antigen, saponin proved to be the best adjuvant. The *T. congolense* vaccine gave a sterile immunity in 5 out of 6 mice, and the *T. cruzi* vaccine increased the survival time and decreased the parasitæmia. A sterile immunity was not achieved with *T. cruzi* vaccine.

Table 1. PROTECTION OF MICE AGAINST *Trypanosoma congolense* CHALLENGE

Treatment	Mean pre-patent period, days (max. = 24)	Proportion of negative survivors at 10 days
None	7·1	1/7
'Arquad 2HT' alone	4·4	0/7
n-Hexadecylamine alone	4·8	0/6
Saponin alone	6·2	1/6
Antigen alone	5·0	0/7
Antigen + 'Arquad 2HT'	6·6	1/7
Antigen + *n*-hexadecylamine	16·1	4/7
Antigen + saponin	21·2	5/6

Table 2. PROTECTION OF MICE AGAINST *Trypanosoma cruzi* CHALLENGE

Treatment	Mean survival time, days (max. = 33)	Mean parasitæmia*
Exp. 1		
None	12·4	186
Antigen alone	11·5	183
Antigen + 'Arquad 2HT'	18·4	107
Antigen + *n*-hexadecylamine	12·8	70
Antigen + saponin	19·3	72
Exp. 2		
None	15·0	128
Saponin alone	10·3	203

* Mean parasitæmia = number of trypanosomes seen in 20 microscopical fields (× 10 ocular, 4-mm objective) at height of infection per mouse.

Freund's adjuvant, with or without killed mycobacteria, was not effective with *T. congolense* antigen. No adjuvant activity was observed with potash alum.

The duration of protection conferred by *T. congolense* vaccine was tested by rechallenging the surviving mice at suitable intervals. Twelve weeks after the last immunizing dose of vaccine, 11 out of 22 mice were immune to infection with the same strain of *T. congolense*. At 19 weeks, 4 out of 6 mice were immune. The residual protection was further tested by injecting $1·0 \times 10^6$ trypanosomes of a different strain of *T. congolense* (*Buswale*). In this group of mice, 19 weeks after vaccination, 2 out of 5 animals remained immune to challenge.

Antigens, other than those derived from *T. congolense* or *T. cruzi* trypanosome cells, were examined for protective effect when mixed with adjuvant. Plasma or serum collected from mice heavily infected with trypanosomes but itself free from trypanosomes, injected with adjuvant in the same way as trypanosome antigen, protected against challenge. With *T. cruzi*, the plasma antigen (equivalent to Weitz's exo-antigen[4]) was more effective than trypanosome antigen. With the plasma antigen the average parasitæmia was 5 per 20 fields, compared with 272 in the untreated controls: 2/8 mice remained free from trypanosomes. The crithidial forms of *T. cruzi* grown *in vitro*, and the culture medium therefrom, were found to have considerable protective effect.

Other flagellates zoologically related to *Trypanosoma* were examined for protective properties when prepared in the same way as the trypanosomes and mixed with adjuvant. No cross-protection was seen against *T. congolense* challenge, but some degree of cross-protection was observed against *T. cruzi* challenge. Protection against *T. cruzi* challenge was observed with *Crithidia fasciculata* and *Leptomonas collosoma* and, to a much smaller degree, with *T. melophagium* and *T. mega*. The culture medium in which *C. fasciculata* and *L. collosoma* had grown also showed a protective effect against *T. cruzi*.

The experiments have shown that the addition of adjuvants to weak antigens from trypanosomes and related protozoa can increase resistance to challenge in mice. Further details of these experiments will be published shortly elsewhere.

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