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Application of passive haemagglutination test for detection of antibodies to *Trichophyton verrucosum* in immunized rabbits and naturally infected cattle

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Summary

Sera of rabbits immunized with living and killed *T. verrucosum* and of naturally infected 50 cattle were examined by the passive haemagglutination test. The living antigen was superior to the killed one. The immunogenicity of the latter was improved when it was coupled with complete Freund's adjuvant. The best sensitization of RBC's was achieved by the crude and lipopolysaccharide extracts of the fungus; the culture filtrate and sonicated antigens were not suitable. A titre of 1 : 512 was obtained in rabbits immunized by the living *T. verrucosum*. In naturally infected cattle a titre above 1 : 32 was obtained in 41 animals; the other animals and the control healthy animals had titres between 1 : 8 and 1 : 32.

The production of antibodies by immunization of animals, especially rabbits, with killed dermatophytes has been demonstrated by several authors (KURODA, 1953; BEZOS, 1966; COX and MOORE, 1968). Also naturally infected cattle with *T. verrucosum* have been subjected to serological examination (KIELSTEIN, 1968; STANKUSHEV et al., 1971).

In the present work the passive haemagglutination has been used for evaluation of the immunization of rabbits by living and killed *T. verrucosum* antigens. Moreover, the test has been applied

for the study of antibodies in naturally infected cattle.

Material and methods

Antigens were prepared by growing *T. verrucosum*, freshly isolated from infected cattle, in fluid medium containing 4 % maltose, 1 % neopeptone, 0.005 % inositol, 0.001 % thiamine and 0.05 % yeast extract. After 4—5 weeks incubation at 37 °C the mycelial mat was separated.

For immunization of rabbits whole mycelial living antigen and autoclaved

dried powdered whole mycelial antigen were used. The killed antigen was applied with and without complete Freund's adjuvant. Each of two rabbits received 4 i. v. injections of 0.5 % suspension of the living antigen in increasing doses at a week interval for four weeks (0.1, 0.2, 0.5 and 1.0 ml). The killed antigen was injected 3 times weekly (1 ml of 0.2 % saline suspension) for 4 weeks. Injections were i. v. on one day and i. p. on the other two days.

Tanned sheep RBC's were sensitized with various antigen preparations of *T. verrucosum* (crude extract, lipopolysaccharide extract, sonicated antigen and culture filtrate). All sera of the 6 immunized rabbits and of 50 naturally infected cattle were inactivated by heat at 56 °C for 30 minutes and absorbed by untreated sheep RBC's; serial two-fold dilutions (0.5 ml) were prepared in buffered saline (pH 7.2) and 0.1 ml of the sensitized cells were added to each dilution. The plates were shaken gently and left at room temperature for 2 hours and at 4 °C for 16—18 hours and examined for haemagglutination.

Results

The passive haemagglutination test was positive in case of all 6 rabbits immunized by *T. verrucosum*. The highest titre (1 : 512) was obtained in sera prepared by using living or killed antigen coupled with complete Freund's adjuvant. Killed antigen without the adjuvant was less immunogenic and only a titre of 1 : 64 or 1 : 128 was obtained (Table 1).

Table 1: Titre of antibodies in sera of rabbits immunized by *T. verrucosum* using haemagglutination test

Rabbit antisera	Titre of antibodies								
	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	1 : 1024
1	++++	++++	++++	++++	++++	++++	++++	++++	—
2	++++	++++	++++	++++	++++	++++	+++	++	—
3	++++	++++	++++	++++	++++	++++	++++	+++	—
4	++++	++++	++++	++++	++++	++++	+++	+	—
5	++++	++++	++++	++++	+++	!	—	—	—
6	++++	++++	++++	++++	+++	—	—	—	—

1 and 2: antisera prepared against living *T. verrucosum*

3 and 4: antisera prepared against killed *T. verrucosum* + adjuvant

5 and 6: antisera prepared against killed *T. verrucosum* without adjuvant