

Effect of cyclophosphamide on the acquisition of resistance to infestation by *Rhipicephalus appendiculatus* in rabbits

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ABSTRACT

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Parenteral administration of cyclophosphamide in rabbits made resistant to infestation by the tick *Rhipicephalus appendiculatus*, resulted in abrogation of the resistance phenomenon. As a result, a high percentage (83%) of the larval ticks fed to repletion. This was in contrast to the control rabbits which were expressing the phenomenon of resistance to infestation by the ticks. In the latter, only 28% of the ticks fed to repletion. Cyclophosphamide administration in rabbits induced a leucopenia and depressed antibody response to the heterologous antigen, sheep red-blood cells.

Keywords: Cyclophosphamide, effect, infestation, rabbits, resistance, *Rhipicephalus appendiculatus*

INTRODUCTION

Acquired resistance to *Rhipicephalus appendiculatus* feeding on rabbit hosts has been documented (Branagan 1974; Rubaire, Sheikh & Ahmed 1988; Fivaz 1990; Fivaz, Tuckers & Petney 1991). Attempts to characterize the nature of these antigens have been reported (Mongi & Aganyo 1990). The use of cyclophosphamide to abrogate the expression of acquired resistance to *Dermacentor andersonii* in guinea-pig has been demonstrated. (Wikel & Allen 1976). Similarly, methotrexate—another cytostatic drug—was also used to block the expression of acquired resistance to ticks (Wikel & Allen 1976). The immunosuppressive effects of cyclophosphamide in mink infected with the Aleutian Disease virus were studied

by Cheema, Heuson & Gorham (1972). This drug, according to Mackie (1981), causes the destruction of immunocompetent cells, culminating in the suppression of antibody formation. It is therefore possible that administration of certain chemicals can disrupt the immune response and subsequent expression of resistance to a parasitic organism or its products.

This is a report on the effect of cyclophosphamide on acquired resistance to the larval instars of *Rhipicephalus appendiculatus* on rabbit hosts.

MATERIALS AND METHODS

Experimental animals

Outbred New Zealand White rabbits with a mass of 1–2 kg each, were used 1 week after weaning. These were obtained from a well established colony maintained at Muguga Research Station, Kenya. The rabbits in the colony were housed singly or in cages and maintained disease free. Frequent anticoccidial treatment was given by dispensing sulphaquinoxaline in the rabbits' drinking water.

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Tick colony

Parasite-free *Rhipicephalus appendiculatus* (Muguga strain) ticks were bred in a tick colony at Muguga Research Station. The instars were allowed to feed on the rabbits' ears. The ticks were placed in cotton bags which were secured on the rabbits' ears by means of Elizabethan collars. This technique, described by Branagan (1974), successfully restricted grooming.

Tick infestation

Larval tick infestation on experimental animals was achieved by releasing 1 000 larvae 2 weeks after hatching on each rabbit (500 ticks on each ear). It was previously estimated that one hatching tube produced 500 larvae. In this study two such tubes were used. Previous pilot experiments by the authors showed that 1 000 was the optimum number of larvae to optimally sensitize the rabbit host (Binta 1984). A tick infestation normally lasts 3–5 d, depending on the tick-resistance status of the rabbit. In this study, daily collections of replete ticks were made from the ear bag after the debris and dead ticks had been removed. They were weighed collectively in precooled tubes at 4°C to immobilize the larvae. The ticks were counted individually by placing them on the sticky side of masking tape.

Cyclophosphamide

Cyclophosphamide (Endoxan-Asta; Asta Werke Ag, Chemische Fabrik, D-480, Bielefeld, Germany) was dissolved in sterile deionized water containing penicillin and streptomycin calculated to give each rabbit 200 IU penicillin G and 20 mg streptomycin.

Preparation of sheep red-blood cells (SRBC)

A 20% suspension of SRBC was prepared in PBS 0.01 M, pH 7.2, as described by Gold & Fudenberg (1967).

Experimental design

A total of eight rabbits were used. Four of these rabbits were injected with cyclophosphamide at a dosage rate of 20 mg/kg body mass, I.V. for 9 d. Subsequently, cyclophosphamide was administered intraperitoneally every other day. Two control rabbits (control 1) were injected with sterile saline and the antibiotic combination for the same period as for the test rabbits.

On day 0 of cyclophosphamide administration, the rabbits (M_1 – M_4) were also intravenously immunized with 2 ml of 20% sheep red-blood cells (SRBC). Simultaneously, 1 000 larvae were released onto the ears (500 ticks per ear) of rabbits M_1 , M_2 , M_5 and M_6 , according to the technique of Branagan (1974). The

secondary tick infestation was initiated 5 d after the primary infestation on rabbits M_1 – M_8 . A 2-d rest period between infestations was allowed. The rest of the rabbits, M_7 and M_8 , were used as normal saline-treated controls for the secondary infestation (control 2).

Collection of blood and serum

All the rabbits were bled: before cyclophosphamide administration; at the time of immunization with SRBC; during the tick infestation, primary and secondary; and on days 5, 7, 9 and 15, post-immunization with SRBC.

The serum thus collected from the rabbits was heat-inactivated at 56°C for 30 min and stored at –20°C.

Whole blood was collected in EDTA and used to determine total white-blood-cell counts and differential cell counts.

Serum antibody determination to sheep red-blood cells (SRBC)

Rabbit serum (heat inactivated at 56°C, 30 min prior to use) was titrated in doubling dilutions in microtitre plates according to the method of Gold and Fudenberg (1967). An equal volume (50 µl) of the 1+ % sensitized SRBC was added to the serum dilutions. The plates were thoroughly shaken and incubated at 37°C for 1 h. After 1 h they were removed and kept at 4°C for 1 h before the agglutination titre was read. The endpoint was that plate beyond which there was no red-blood-cell agglutination.

Statistical analysis

The data on mean engorgement mass of larvae as well as that on percentage larvae engorgement to repletion, were analysed as a two-way classification between treatment groups and infestations (Snedcor & Cochran 1967).

RESULTS

Haematological changes

When cyclophosphamide (Cy) was injected into four rabbits at an I.P. dose of 20 mg/kg body mass, a leucopenia was induced in the cyclophosphamide-treated rabbits. The decline in the mean leucocyte count was discernible as early as day 1 post Cy administration (Fig. 1). The differential leukogram indicated a decrease in the absolute lymphocyte counts. There was no alteration in the differential leukograms of the control rabbits.

Antibody response of rabbits to SRBC

Direct-haemagglutination antibody titres to a heterologous antigen (SRBC) were also depressed in the

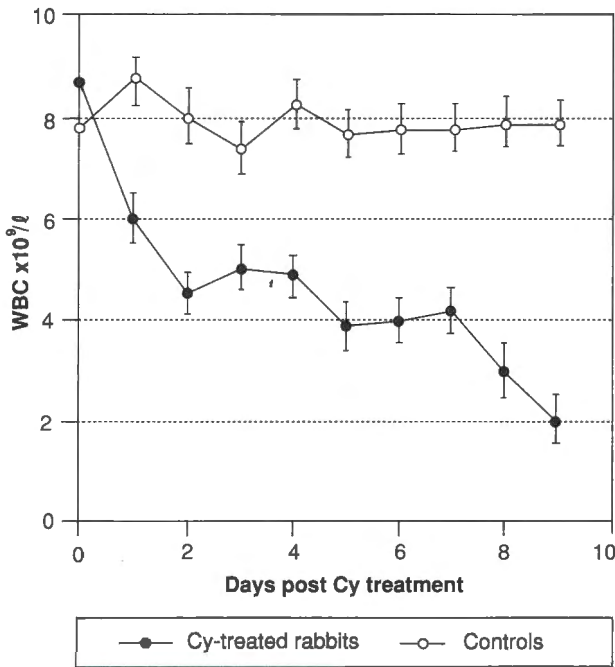


FIG. 1 Effect of Cy on rabbit leucocytes (WBC)

TABLE 1 Serum haemagglutination antibody titres to sheep red-blood cells (SRBC) in cyclophosphamide-treated (CY+) and control rabbits

Rabbit No.	Treatment	Days post immunization with SRBC			
		0	5	9	15
M ₁	Cy+	< 2*	4	4	4
M ₂	Cy+	< 2	4	4	4
M ₃	CY+	< 2	4	4	4
M ₄	CY+	< 2	< 2	< 2	< 2
M ₅	Control 1	< 2	64	32	16
M ₆	Control 1	< 2	64	32	16

* Titres expressed as reciprocal of end-points

TABLE 2 Mean engorgement masses (mg) of larvae

Treatment	Cy+			Control 1			Control 2	
Rabbit group	M ₁	M ₂		M ₅	M ₆		M ₇	M ₈
Infestation 1	0,56	(0,58 0,54)		0,54	(0,52 0,56)		—	—
Infestation 2	0,54	(0,56 0,52)		0,40	(0,38 0,42)		0,52	(0,56 0,52)

TABLE 3 Mean percentage larvae engorging to repletion

Treatment	CY+			Control 1			Control 2	
Rabbit group	M ₁	M ₂		M ₅	M ₆		M ₇	M ₈
Infestation 1	90	(97 87)		90	(88 92)		—	—
Infestation 2	83	(85 81)		28	(24 32)		90	(93 87)

cyclophosphamide-treated rabbits (Table 1). The reciprocal of anti-SRBC antibody titres on day 0 was less than 2 for both cyclophosphamide-treated and control rabbits. However, on day 5 post SRBC immunization, the titres were 64 in the control and only 4 in three out of four of the cyclophosphamide-treated rabbits. One rabbit in the latter group did not agglutinate sheep red-blood cells (Table 1).

Feeding performance of larvae on Cy-treated rabbits

During the primary tick infestation, the larvae engorged to repletion without any inhibition in both cyclophosphamide-treated and control rabbits (Table 2). Cyclophosphamide alone had no detrimental effects on the larvae. Similarly, the antibiotics with saline did not interfere with the immune system of the rabbits. Repeated tick infestation had little effect on the mean engorgement of larvae feeding off cyclophosphamide-treated rabbits, in contrast to the tick-sensitized, untreated and the tick-naive controls.

There was a statistical significance between groups as well as between the first and second infestations ($P < 0,05$) for the mean engorgement masses (Table 2).

For the percentage of larvae engorging, a statistical significance ($P < 0,01$) was found between the cyclophosphamide-treated, Cy-untreated groups, as well as between infestations (Table 3).

A progressive drop in the number of replete ticks was recorded for the untreated, sensitized control on the secondary infestation (Table 3).

DISCUSSION

Repeated infestation with the larval ticks of *R. appendiculatus* on rabbits induced resistance in the rabbits. There was a reduction in the mean engorgement masses of the larvae feeding on the tick-bite-sensitized rabbits shown in Table 2. The percentage of larvae feeding to repletion was also reduced (Table 3). Similar results were reported by Branagan (1974); Wikel & Allen (1976); Rubaire-Akiiki & Mutuku (1980); Sheikh & Ahmed (1988); Fivaz (1990); Fivaz *et al.* (1991). Administration of cyclophosphamide at the time of tick infestation blocked the ability to mount an immune response to the larval ticks.

Cyclophosphamide is known to exert a mitostatic effect on lymphoid tissues, more marked against the short-lived B-lymphocytes than against T-lymphocytes (Stockman, South, Heim & Trentin 1973; Turk & Poulter 1972). The

selective destruction of lymphocytes with B-cell characteristics by cyclophosphamide has been documented (Sabolovic, Sabolovic & Guilmin 1977). In our study, the lymphopenic leucopenia and the depressed haemagglutination antibody titres to SRBC antigen were indicative of depressed B-cell function.

In this study, failure of Cy-treated rabbits to mount acquired resistance to the larvae of *R. appendiculatus* was in accordance with the results of Wikel & Allen (1976), who used guinea-pig hosts infested with *Dermacentor andersoni*. This failure was attributed to blockage of induction of immune memory for the tick antigens.

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