

Effect of anti-thymocyte serum on acquisition of resistance to infestation by *Rhipicephalus appendiculatus* larvae in rabbits

M.G. BINTA*, E.Z. MUSHI and F.R. RURANGIRWA

International Centre for Insect Physiology and Ecology
P.O. Box 30772 Nairobi, Kenya

ABSTRACT

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Administration of specific goat anti-thymocyte serum (ATS) to rabbits, prior to a primary infestation by *Rhipicephalus appendiculatus* larvae, blocked the acquisition of resistance significantly only in the third infestation. The larvae which fed on these rabbits had higher engorgement masses than did those feeding on untreated control rabbits. Also, a higher percentage (92%) of larval ticks fed on these animals than on the controls (88%). ATS also induced a leucopenia due to a lymphopenia in the treated rabbits. It was concluded that a T-cell-dependent component might be involved in acquired resistance to infestation by *R. appendiculatus*.

Keywords: Anti-thymocyte serum, ATS, rabbits, resistance to infestation, *Rhipicephalus appendiculatus*

INTRODUCTION

The phenomenon of acquired immunity to ticks feeding on animal hosts has been investigated by Trager 1939; Rechav & Dauth 1987; Rechav, Heller-Haupt & Varma 1989; Walker & Fletcher 1990.

Passive transfer of serum from tick-resistant guinea pigs, conferred resistance to ticks in naive guinea pigs (Askenase, Bagnall & Worms 1982). A cellular immune component has also been established in the resistance against ixodid ticks (Wikel & Allen 1976a; 1976b). Brown, Galli, Gleich & Askenase (1982) reported that acquired resistance to *Amblyomma ame-*

ricanum could be abolished by administering a highly specific anti-basophil serum to sensitized guinea pigs, prior to tick infestation. On the other hand, only partial abrogation of resistance was achieved by these authors when they used anti-eosinophil serum.

Since the role of T-cells in the expression of tick resistance has not been fully elucidated, the ability of T-cell-deprived rabbits to mount resistance to *R. appendiculatus*, was investigated.

MATERIALS AND METHODS

Experimental animals

Outbred New Zealand White rabbits of 1–2 kg body mass were used.

* Present address: National Veterinary Laboratory, Private Bag 035, Gaborone, Botswana

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Preparations of thymocyte- and lymphocyte-cell suspensions

Thymocytes and lymphocytes were separated from the thymus, and from the lymph nodes and spleen, respectively, according to the method of Sabolovic, Sabolovic & Guilmin (1977).

Briefly, this entailed forcing the thymus through a stainless-steel sieve with the use of a sterile rubber "policeman". The thymocytes were separated on Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden). The interphase cells, mainly thymocytes, were collected and washed in Hank's balanced salt solution (HBSS) by centrifugation at 100 *g* for 5 min at 4 °C. The thymocytes were counted by means of a haemocytometer, and their concentration was adjusted to 2×10^7 cells ml^{-1} .

The mesenteric, cervical and popliteal lymph nodes were collected from rabbits. Lymphocyte-cell suspensions were prepared as described for the thymocytes. Lymphocytes were isolated from spleen cells by means of the same technique. The lymphocytes were used to determine the cytotoxicity of the ATS.

Preparation of goat anti-thymocyte serum

Two mature goats were used to produce goat anti-thymocyte serum (ATS). The goats were immunized with 2×10^7 thymocytes in incomplete Freund's adjuvant at four subcutaneous sites on each of three occasions at two-weekly intervals.

Blood was collected from the goats 2 weeks after the last injections. The serum was harvested and absorbed three times with washed, packed, rabbit red blood cells and bone-marrow cells.

The serum was heat-inactivated at 56°C for 30 min before it was stored at -20°C in 10-ml aliquots.

The globulin fraction was prepared by precipitation of the serum with 33% ammonium sulphate solution. The precipitate thus prepared was reconstituted to its original volume in sterile phosphate-buffered saline and stored under the same conditions as the ATS.

Testing specificity of ATS

The specificity of the ATS was determined by the use of both thymocyte- and lymphocyte-cell suspensions of 10^7 cells/ml in Eagle's Minimum Essential Medium (MEM); 0.5 ml of the cell suspensions were incubated with equal volumes of the ATS (diluted 1:8) at 37°C for 30 min. An equal volume of guinea-pig complement (diluted 1:4 in sterile phosphate-buffered saline) was then added and the cultures were incubated for another 1 h. Four sets of tubes were used for each lymphocyte sample:

- lymphocytes +ATS +medium
- lymphocytes +complement +medium
- lymphocytes +medium

The cells were centrifuged at 100 *g* for 5 min and re-suspended to the original volume in MEM. A drop of trypan blue was added and the number of viable lymphocytes per 200 lymphocytes was estimated with the use of a haemocytometer chamber.

Treatment of rabbits with ATS

Three rabbits were given the globulin fraction of ATS i/v at a dosage rate of 3 ml kg^{-1} body mass daily for 4 d, together with 2 ml of Combiotic (Pfizer) containing 400 000 units of procaine penicillin G and dihydrostreptomycin sulphate equivalent to 0.5 g dihydrostreptomycin per 2 ml.

Two control rabbits were injected daily with 3 ml kg^{-1} of phosphate-buffered saline (PBS) pH 7.2 and the same dosage of Combiotic for a similar period. Blood samples were collected daily in EDTA from all rabbits, to determine the leukogram.

Tick infestations

Four days after commencement of the ATS treatment, the test and control rabbits were each infested with 1 000 disease-free larval ticks of *R. appendiculatus* (Muguga strain).

These ticks were from a colony maintained at the Veterinary Research Centre, Muguga, Kenya. Two tubes, each containing approximately 500 larvae, were applied to the ears of each rabbit with the use of cotton ear bags as described by Branagan (1974). The number of larvae used in this study was thought to be optimal in eliciting the cutaneous hypersensitivity response to larval ticks in this species (Binta 1984).

Larvae which had fed to repletion, were collected manually after the primary infestation. They were weighed collectively, but counted individually after having been attached to the sticky side of cello tape.

Secondary infestations were initiated 24 h after completion of the primary infestation. Two control rabbits were used for each infestation.

Statistical analysis

The data in Table 3 were first transformed (arcsine transformation) and a two-way analysis of variance was performed.

The primary tick infestation was compared with the secondary and the tertiary infestations, respectively.

RESULTS

Cytotoxicity of ATS for lymphocytes

The effect of ATS on lymphocytes from the various lymphoid organs, is shown in Table 1. In the presence

TABLE 1 Percentage of dead cells resulting from the effect of goat anti-thymocyte serum and complement on lymphocyte viability

Treatment	Source of cells		
	Thymus	Lymph node	Spleen
+ATS +Complement	100,0	73,0	79,8
+ATS +Media	9,6	6,8	6,9
+Complement +Media	1,4	7,1	3,9
+Media	3,9	6,9	19,0

TABLE 2 Mean white-blood cell count ($\times 10^9/l$) in ATS-treated and untreated rabbits

Treatment	Days post treatment				
	0	5	7	9	15
ATS+	9,5	6,5	6,3	5,8	4,4
ATS-	9,7	9,7	9,6	9,6	9,6

TABLE 3 Mean engorgement masses (mg) of larvae feeding on ATS-treated and untreated rabbits

Treatment groups	ATS+ (mg) M ₁ -M ₂	ATS- (mg) M ₅ -M ₆	C ₁ (mg) M ₇ -M ₈	C ₂ (mg) M ₉ -M ₁₀
Infestation 1	0,68-0,58	0,61-0,51	-	-
Infestation 2	0,65-0,55	0,36-0,26	0,66-0,55	-
Infestation 3	0,65-0,58	0,18-0,18	-	0,66-0,66

ATS+ = Treatment with globulin of anti-thymocyte serum
 ATS- = Untreated controls
 C₁ and C₂ = Additional tick-naive controls, not treated with ATS, for secondary and tertiary infestations
 M₁-M₁₀ = Rabbits

TABLE 4 Percentage of ticks engorging on ATS-treated and untreated rabbits

Treatment groups	ATS+		ATS-		C ₁		C ₂	
	M ₁	M ₂	M ₅	M ₆	M ₇	M ₈	M ₉	M ₁₀
Infestation 1	87	83	92	88	-	-	-	-
Infestation 2	92	88	37	33	93	87	-	-
Infestation 3	92	88	25	24	-	-	95	90

ATS+ = Treatment with globulin of anti-thymocyte serum
 ATS- = Untreated controls
 C₁ and C₂ = Additional tick-naive controls, not treated with ATS for secondary and tertiary infestations
 M₁-M₁₀ = Rabbits

of complement, the serum killed all the thymocytes from normal rabbits. The proportion of dead cells from the spleen and pooled lymph nodes was 79,8% and 73,0%, respectively.

Haematological changes

Administration of goat anti-thymocyte serum (ATS+) induced a leucopenia as a result of a lymphopenia (Table 2).

Rabbit hosts

A very severe cutaneous hypersensitivity reaction characterized by hyperaemia and bullae formation and oedema, was observed, but only on the ears of the control rabbits during the tertiary infestation. The inflammatory exudate drowned many larvae.

This allergic skin reaction was not elicited in the ATS-treated rabbits.

Engorgement masses

The mean engorgement masses of larvae which fed on ATS-treated rabbits in the primary and secondary infestations, were comparable to those of the control rabbits. However, in the tertiary infestation, larvae feeding on the untreated control rabbits had significantly lower mean engorgement masses than those feeding on ATS-treated rabbits (Table 3). It was observed that the mean engorgement mass of larvae fed on the control rabbits (C₁ and C₂) was higher than that obtained from larvae fed on the controls that had not been treated with ATS and had undergone a secondary and tertiary tick infestation.

The mean engorgement masses of larvae from the C₁ and C₂ control rabbits were comparable to those from ATS-treated rabbits, both in the primary, secondary and tertiary infestations. Similarly, the percentage of larvae feeding to repletion on the ATS-treated rabbit groups, was comparable to that of larvae feeding on the controls, C₁ and C₂, which were tick naive (Table 4). Larvae feeding on the controls that had not been treated with ATS and had undergone three successive infestations, did not only have low engorgement masses, but were also pale and shrivelled. Many larvae feeding on these rabbits drowned in the exudate, subsequent to the cutaneous hypersensitivity reaction. The rabbits treated with ATS did not have this characteristic reaction.

DISCUSSION

Acquired resistance in rabbits to *R. appendiculatus* larvae, has been reported by Branagan (1974) and Rubaire-Akiiki & Mutinga (1980). After repeated larval infestations, both the engorgement masses of the larvae and the percentage of larvae feeding to repletion, were reduced (Rubaire-Akiiki and Mutinga 1980; Walker & Fletcher 1990). Resistance to ticks is generally expressed as an increase in the number of ticks which fail to complete their blood meal, and a decrease in the mean mass of the replete ticks (Rechav & Dauth 1987).

In the present study, the mean larval engorgement masses and the percentage of ticks feeding to repletion, were markedly reduced in the tertiary tick infestation in the control rabbits not treated with ATS. These rabbits elicited the classical cutaneous hypersensitivity reaction mentioned previously (Trager 1939; Binta & Cunningham 1984). This cutaneous hypersensitivity is thought to interfere with the feeding of the ticks.

The administration of goat ATS to the rabbits before and during the primary tick infestation, greatly reduced the hosts' ability to mount an acquired resistance to the larvae. The larvae which fed on ATS-treated and successively tick-infested rabbits, were a healthy dark grey. In contrast, the ticks feeding in the tertiary infestation, on the rabbits not treated with ATS, looked shrivelled and had a pale yellow colour. Similar changes in the appearance of larvae feeding on hosts with varying degrees of tick-resistant status, have been reported by several workers (Trager 1939; Branagan 1974; Rubaire-Akiiki & Mutinga 1980; Binta & Cunningham 1984). These authors were of the opinion that the pale appearance of the larvae was due to the ingestion of the hosts' leucocytes instead of red-blood cells. This observation has since been regarded as one of the criteria characterizing a tick-resistant host.

The larvae which fed on rabbits treated with ATS, were significantly heavier than those which fed on the controls in the tertiary infestation. This would suggest that ticks feeding on the ATS-treated rabbits imbibed more blood than did those from untreated rabbits. It is possible that the ATS treatment blocked the rabbits' acquisition of resistance to the larval ticks.

In the presence of complement, ATS killed all the rabbit thymocytes and, to a considerable degree, lymphocytes from the lymph nodes. These findings concur with those of other workers (Ratajczak, Richards & Richerson 1979). However, the 80% mortality found in the lymphocytes separated from the spleen, was contrary to the findings of other workers (Redelman, Scott, Shepherd & Sell 1976; Ratajczak *et al.* 1979). The reason for this is unclear, although it may be a function of the way the rabbits were handled before collection of the spleens.

For instance, an increased blood flow to the spleen may lead to an increase in the proportion of T-cells in the spleen, since peripheral blood contains a higher proportion of ATS-sensitive cells (Ratajczak, *et al.* 1979).

It may be assumed that treatment with ATS removed all cells possessing T-cell characteristics from the circulation of the rabbit thymus. It is possible that this depletion resulted in dysfunction of T-cell-dependent antibody formation. Whether the antibodies responsible for the phenomenon of tick resistance are partly or totally T-cell-dependent, is not yet known (Aske-

nase *et al.* 1982). However, such a mechanism could explain the results of this study.

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