Cutaneous hypersensitivity induced in rabbits by extracts of the tick *Amblyomma cajennense* (Acari: Ixodidae)

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ABSTRACT

The cutaneous hypersensitivity test was used to correlate host resistance to ticks and type of reactions elicited by *Amblyomma cajennense* (Fabricius, 1787) tick extract in rabbits. Rabbits were divided into 3 groups of 2 animals each: naive, pre-infested and control. Cutaneous hypersensitivity was induced by intradermal inoculation of 25 μg extract in 0.03 mℓ of phosphate buffered saline (PBS) in rabbit ears. Control rabbits were inoculated with PBS only. The ear thickness was measured with a Mitutoyo® device before and 10 min, 1, 2, 4, 18, 24, 48, 72 and 96 h post-inoculation (PI). Pre-infested rabbits showed an immediate type reaction within the 1st 10 min PI (60 % increase in ear thickness) and a delayed reaction (18 h) (85 % increase), whereas the naive rabbits showed only the immediate reaction within the 1st 4 h (60 % increase). PBS induced only mild reactions. These results point out the crucial role of the cellular immune response of rabbits in the expression of resistance to *A. cajennense.*

Key words: *Amblyomma cajennense,* cutaneous hypersensitivity, tick extract.

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INTRODUCTION

Blood-feeding ectoparasites are a major worldwide problem for humans and animals, both as debilitating agents and as vectors of diseases[22]. It is well established that various species of ixodid ticks induce a degree of resistance in the host and that resistance is immunologically mediated[20]. The nature of the immunological response following tick infestation varies with the tick–host model. This aspect has been comprehensively reviewed by Wikel[19] and Brown[6].

Hosts acquire resistance against ticks either after natural and repeated infestation or after immunisation with tick-derived antigens[10,11], its immune response being mediated by cells and antibodies[7].

The skin hypersensitivity test has long been used for the determination of antigens responsible for allergic states. A delayed-type, cell-mediated hypersensitivity reaction has been demonstrated after the intradermal injection of tick extracts into resistant animals[3]. If it could be shown that this reaction correlates well with the resistance level to ticks, the test could then replace the labour-intensive method of counting ticks on individual animals to assess their immune status. In addition, it would be helpful in the identification of tick-resistant animals in order to save acaricides and employ selective breeding.

In this study, the cutaneous hypersensitivity test was used to correlate host resistance to ticks and type of reactions elicited by *Amblyomma cajennense* (Fabricius, 1787) tick extract in rabbits.

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MATERIALS AND METHODS

Experimental animals and ticks

Six New Zealand white rabbits were used throughout the experiment. Rabbits were supplied by the São Paulo State University's bioterium (Botucatu-SP, Brazil). The animals were either tick-bite naive or previously infested with adult ticks. *Amblyomma cajennense* ticks were obtained from a colony maintained in the Department of Animal Pathology, State University of São Paulo.

Infestation procedure

Acrylic feeding chambers with a screw-top lid were glued to the animals' backs. For each infestation, 6 adult female and 6 adult male ticks were used per host animal. The interval between infestations and skin tests was 15 days from the 1st day of infestation.

Tick extract

Unfed *A. cajennense* adult ticks were killed by immersion in liquid nitrogen, homogenised with a ground glass homogeniser in phosphate buffered saline (PBS, pH 7.4) and then sonicated 3 times for 10 s each time and once for 60 s (20 MHz). The extract was centrifuged at 4 °C for 1 h at 12 000 *g* and the supernatant fluid was filtered through a 0.22 μm Millex-GX (Millipore Corporation, Billerica, MA, USA) filter and stored at –40 °C until use. The protein concentration was determined according to Lowry *et al.*[12] and was used for inoculations at 25 μg/mℓ.

Skin test procedure

Unfed adult tick extract (UAE) was injected into the dermis of the left ear of rabbits (25 μg in 0.03 mℓ of PBS) and the reaction evaluated through measurement of skin thickness at 10 min, 1, 2, 4, 18, 24, 48, 72 and 96 h post inoculation. PBS only was injected into the right ear to measure non-specific reactions. The ear thickness was measured with the aid of a Mitutoyo® device 3 times on each occasion. Results were expressed as the percentage of ear thickness increase in relation to initial values, obtained before the UAE inoculation. Increase in the ear thickness induced by UAE was obtained by subtracting values for the right ear from those of the left ear.

RESULTS

Overall, the inoculum of tick extract, but not PBS, elicited an immediate local inflammatory reaction in both naive and pre-infested animals. This was characterised by swelling, heat and redness in all animals. The skin test results for rabbits are summarised in Figs 1 and 2.

Pre-infested rabbits (Fig. 1) developed an immediate reaction during the 1st 10 min post-inoculation (60 % increase in ear thickness), that declined slowly until

2 h post-inoculation (36 % increase in ear thickness). This was followed by the secondary response (85 % increase in ear thickness at 18 h post-inoculation) and decreased up to 96 h post-inoculation, reaching 15 % increase in ear thickness.

Naive rabbits (Fig. 2) displayed a similar immediate reaction pattern which was a little more intense (62 %) than the pre-infested rabbits. PBS on the other hand induced minor and non-specific reactions.

DISCUSSION

The technique for skin testing used here was very practical, reliable, and possibly more suitable and precise that using - callipers since the ear skin is measured without folding. The skin hypersensitivity test or intradermal testing has long been used for the determination of antigens responsible for allergic reactions. In tick–host relationships, a cutaneous hypersensitivity test has been used for different purposes. Willadsen *et al.*[21] used purified antigens from whole larvae of *Boophilus microplus* (Canestrini, 1887) and found that skin reactions correlated well with the levels of host resistance measured by tick feeding. Binta and Cunningham[5] observed that an extract of *Rhipicephalus appendiculatus* (Neumann, 1901) larvae induced an immediate hypersensitivity reaction in sensitised cattle following intradermal inoculation. Generally, the extent of cutaneous reaction depends upon duration of exposure of the cattle to tick infestation. The skin reaction was absent in steers on which ticks had not previously fed and in field trials the reaction intensity increased with the age of the animal. Smith *et al.*[15], who used a hypersensitivity test for assessing tick-immune status of cattle in Zambia, found a negative correlation between the intensity of the reactions and the total number of ticks found on the animals.

Girardin and Brossard[8] observed the blocking of immediate cutaneous reaction (type I) by cyclosporin A after intradermal injection of *Ixodes ricinus* (Linnaeus, 1758) salivary antigens, as detected by measuring the skin-fold thickness in rabbits repeatedly infested with *I. ricinus* adults. The same method revealed a decrease in delayed (type IV) hypersensitivity to these antigens. These experiments showed the major role of cyclosporin A-sensitive cells, mostly T-lymphocytes, mast cells and basophils, involved in the complex phenomenon of resistance to ticks.

Walker and Fletcher[18] used a skin test involving intradermal injection of salivary gland extract of *R. appendiculatus* to measure the genetic resistance of cattle to this tick as a way of predicting the ability

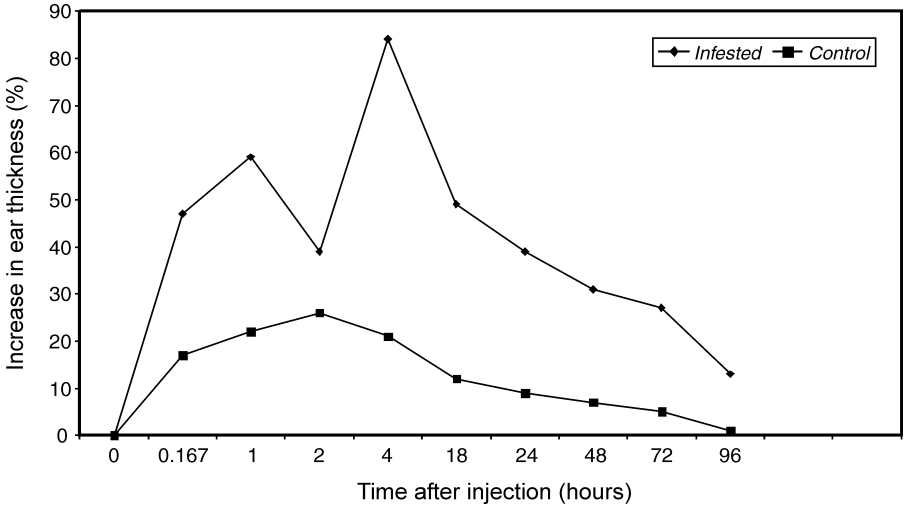

Fig. 1. **Cutaneous reaction to intradermal inoculation of *Amblyomma cajennense* unfed adult extract** (25 µg) **in pre-infested rabbit ears. The results are expressed as the percentage increase in ear thickness compared to the initial measurement. Values obtained from PBS-injected ears were subtracted from those inoculated with the adult tick extract.**

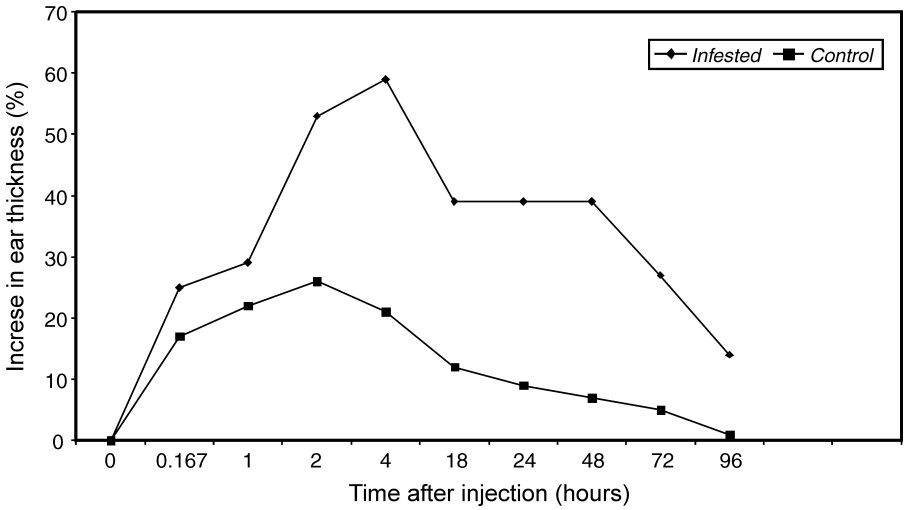

Fig. 2. **Cutaneous reaction to intradermal inoculation of *Amblyomma cajennense* unfed adult extract** (25 µg) **in naive rabbit ears. The results are expressed as the percentage increase in ear thickness compared to the initial measurement. Values obtained from PBS-injected ears were subtracted from those inoculated with the adult tick extract.**

of host individuals to acquire resistance.

In this study, the cutaneous hypersensitivity test was used to compare local skin reactions in rabbits with whole *A. cajennense* extract. The scientific rationale for the approach is the possibility of full development of the host's tissue reaction to tick antigens due to the absence of the tick's saliva modulating effect. Tick saliva is known to affect host's inflammatory reactions[13] and to have immunosuppressive properties[23].

Variations in the intensity of reactions to tick extract in the same experimental group, might be attributed to (1) individual sensitivity to the tick and its extract, (2) cross-reactivity reactions induced by other blood-feeding arthropods such as fleas or mites, and (3) varying reactions of hosts to parasite extracts according to number, frequency and intensity of exposures, as well as age at which exposure first occurs. This was observed with flea extracts in guinea pigs[4] and dogs[9], and also with tick extracts in dogs[17].

Our results showed that rabbits react to tick extracts with a strong immediate hypersensitivity reaction (type I), with a delayed type reaction in rabbits that were pre-infested 15 days earlier. The immediate hypersensitivity reaction is mediated by IgE and/or IgG and is associated with an increase in vascular permeability and corresponding oedema due to local mast cell degranulation. Mast cells were seen in significant numbers at the *Rhipicephalus sanguineus* (Latreille, 1806) feeding sites on dogs by Szabó and Bechara[16]. These mast cells are probably coated with IgE or IgG following the 1st exposure to ticks and might degranulate upon challenge with tick antigens. The immediate reac-

tion was less intense than the delayed type reaction. This suggests that the delayed reaction might be associated with the resistance expressed by rabbits to ticks. Most probably this delayed type reaction occurs due to a cellular infiltration and triggering of cellular immunity. The onset of this delayed type hypersensitivity within 18 hours of UAE injection and the possible role for basophils in the resistance of rabbits to the tick *A. cajennense* suggests a Jones-Mote reaction also known as cutaneous basophil hypersensitivity[2]. A possible explanation for the lack of resistance and the development of an immediate-type hypersensitivity reaction in naive rabbits is the deviation of immunity to a Th2 type response. Murine $CD4^+$ cells have been classified into 2 distinct subsets, Th1 and Th2[1]. Th1 cells produce interleukin-2 (IL-2), tumour necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) and are involved in delayed type hypersensitivity responses[14]. Th2 cells produce IL-4, IL-5 and IL-10 and help B cells to develop into antibody producing cells[1]. The essential role of IL-4 in both IgE production and Th2 cell commitment and of IL-5 in terminal differentiation of eosinophils, led to the hypothesis that the Th2 panel of cytokines in the pathogenesis of asthma and allergy is pivotal[1]. It seems that the Th2 response might be involved with the immediate-type hypersensitivity reaction but not the delayed one. It is tempting to speculate that tick saliva might induce, in rabbits, a predominantly Th2 response displacing local cellular immunity.

In conclusion, the results showed that *A. cajennense* induced an immediate type hypersensitivity reaction in naive rabbits, with a delayed type reaction in previously infested rabbits. The reactions induced by the tick extract can be different from that observed during natural tick infestation due to saliva interference.

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