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Progressive sensitization of circulating basophils against *Ixodes ricinus* L. antigens during repeated infestations of rabbits

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Summary The sensitivity of rabbit basophils to antigens from *Ixodes ricinus* females has been studied by a degranulation test. Observations of basophil numbers and degranulation were made on the 6th day of each of four sequential infestations. Maximal degranulation of cells was observed after challenge of cells with antigen at a concentration of 10^6 and 10^7 pg/ml. At these concentrations, during a 1st infestation, 21·8 and 23·6% of cells degranulated. During a 2nd infestation, these percentages increased (34·8 and 33·8%) and reached 59·8 and 63·8% by the 4th infestation. A plasma factor which partially blocks basophil degranulation, is described. This was already present during the 1st infestation, since in its presence the percentage of degranulation was reduced by 2·8 and 4·0% respectively on challenge with 10^6 and 10^7 pg antigen/ml. Inhibition was maximal at the 4th infestation (difference: 16·5 and 20·5%). Basophil sensitization and inhibition of the degranulation are thus both progressive phenomena. After 10–15 infestations on four other rabbits, 75·0 and 79·8% degranulation was obtained. The inhibition of degranulation by plasma was also greater (difference: 25·5 and 27·4%). IgG specific anti-*I. ricinus* antibodies were identified by indirect immunofluorescence. In two animals, they were detected at the 6th day of the 1st infestation. Subsequently, they were generally present for all the animals.

Keywords: *Ixodes ricinus*, rabbit, immunity, basophils, antigens

Introduction

Rabbits infested by female *Ixodes ricinus* acquire a resistance which affects the nutrition and egg laying of this ectoparasite (Bowessidjaou, Brossard & Aeschlimann 1977). Experiments on the effects of immune serum transfer have shown that humoral factors participate in the expression of immunity (Brossard 1977, Brossard & Girardin 1979). Cell infiltration of the skin at the point of tick attachment occurs progressively during the course of infestation and changes in character during sequential infestations (Brossard & Fivaz, in press). For example, degranulated mast cells are more numerous at the beginning of re-infestation. However, degranulated basophils appear in the cutaneous

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lesion mainly towards the end of reinfestation, probably as a result of hypersensitivity phenomena. The reaction may be similar to the cutaneous basophil anaphylaxis described in other systems (Askenase *et al.* 1978).

In this work, we will show, by using a degranulation test (Benveniste *et al.* 1977), that circulating basophils are sensitized against the antigens of tick salivary glands. Further we will show the existence of a plasma factor blocking the specific degranulation of the cells.

We have also established for each infestation, the rate of egg production and the duration of preoviposition and embryogenesis. Thus, this study extends our knowledge (see Bowessidjaou *et al.* 1977) of the biological effects on *I. ricinus* of the resistance acquired by rabbits.

Materials and methods

I. ricinus ticks were bred in our laboratory. The infestation conditions were as described previously (Bowessidjaou *et al.* 1977). Four infestations were made on the ears of five Himalayan male rabbits (aa c^Hc^H) of about 2 kg each. Four animals infested 10–15 times were also used. The rate of tick egg laying (mean weight of egg laying/ mean weight of fed females) was determined according to Graf (1978). The periods of preoviposition (time lapse from the end of feeding until the laying of the first eggs) and of embryogenesis (time separating the depositing of the first eggs and observation of the first larvae hatched) were also observed.

The IgG anti-*I. ricinus* antibodies were detected by an indirect immunofluorescence technique. Samples were taken on the 6th day of each infestation (Ambroise-Thomas 1969, Brossard 1976). Five micron histological sections of salivary glands taken from ticks fed for 3 days were used as antigen. Fluorescein-labelled, goat anti-rabbit IgG (Miles) was diluted (5%) in a counter colour (Evans Blue 1/10 000).

To carry out the basophil degranulation test according to Benveniste *et al.* (1977), the antigen was prepared with the salivary glands of female *I. ricinus* which had been fed 3–5 days. After dissection, these glands were washed three times at 500*g* in PBS pH 7·2, then homogenized and centrifuged ($13 \times 10^3 g$, 30 min at 4°C). The supernatant was dialysed against distilled water overnight. The dialysate was frozen at −20°C in aliquots sufficient for an experiment.

Briefly, the test procedure is the following: 10 ml of rabbit blood are collected in a test tube containing heparin and centrifuged (500*g*, 10 min at 4°C). Plasma is collected and stored at 4°C. Cells are washed twice in Tyrode's solution without Ca^{2+} and Mg^{2+}, containing 1×10^{-4} M EDTA. Half of the sample is resuspended with plasma to the original blood volume (5 ml) and the other half with complete Tyrode's solution. Then 0·5 ml of those solutions (with or without plasma) are mixed with increasing quantities of antigen (0–10^7 pg/ml). The mixtures are incubated at 37°C for 15 min. The reaction is stopped with 25 µl 0·2 M EDTA, pH 7·4. To an aliquot of 10 µl, 90 µl of toluidine blue were added. Basophils are counted in a Malassez haemocytometer. The results are expressed as average percentage of degranulated cells after comparison of mixtures incubated with or without antigen:

$$\frac{\text{No. of basophils in mixtures without antigen} - \text{No. of basophils in mixtures with antigen}}{\text{No. of basophils in mixtures without antigen}} \times 100$$

Results

TICK BIOLOGY

We have calculated the rate of egg laying for the four infestations considered (Table 1). This decreases progressively from 0·52 to 0·29 going from the 1st to the 4th infestation. The duration of preoviposition and embryogenesis were also measured. Both increase by 3 days from the 1st to the 4th infestation. The evolution of the weight of fed females and the period of nutrition is comparable to that observed by Bowessidjaou *et al.* (1977).

Table 1. Biology of *I. ricinus* females

Infestations	Mean weight of fed females (mg)	Rate of egg laying	Mean duration of blood meal (h)	Mean duration of preoviposition (d)	Mean duration of embryogenesis (d)
1	196·2 ± 97·9 (n = 36)	0·52	163 ± 27 (n = 36)	12·6 ± 3·0 (n = 32)	48·4 ± 3·3 (n = 28)
2	98·7 ± 65·6 (n = 32)	0·35	195 ± 63 (n = 32)	13·0 ± 3·1 (n = 20)	46·6 ± 5·8 (n = 20)
3	76·5 ± 58·8 (n = 27)	0·22	200 ± 47 (n = 27)	17·7 ± 5·3 (n = 13)	50·1 ± 6·9 (n = 9)
4	101·9 ± 80·0 (n = 32)	0·29	221 ± 60 (n = 32)	15·4 ± 3·9 (n = 15)	51·1 ± 4·4 (n = 12)

h = hours; d = days; n = number of ticks.

EVOLUTION OF IgG ANTIBODIES AGAINST *I. RICINUS* SALIVARY GLANDS

All the serological tests were made on the 6th day of each infestation. Antibodies appeared from the 1st infestation in two cases out of five only (titre 1/20). From the 2nd infestation on they were always detected. Average reciprocal titres increased from then on progressively, from 47·6 to 269·1 (Table 2).

EVOLUTION OF THE NUMBER OF CIRCULATING BASOPHILS AND OF THEIR SENSITIZATION AGAINST THE ANTIGENS OF *I. RICINUS* SALIVARY GLANDS

According to Spector (1956), the normal range for the concentration of rabbit basophils lies between 120 and 310 per mm^3. With the exception of the counts for the 1st infestation,

Table 2. Evolution of IgG antibodies to *I. ricinus* salivary glands

	Negative	1/20	1/40	1/80	1/160	1/320	1/640	n	GMRT
1st infestation	3	2	0	0	0	0	0	5	13·2
2nd infestation	0	0	3	1	0	0	0	4	47·6
3rd infestation	0	0	0	2	2	0	1	5	160·0
4th infestation	0	0	0	1	0	2	1	4	269·1

n = number of tests; GMRT = geometrical mean of reciprocal titres.

Table 3. Evolution of the number of basophils and results of degranulation tests for four successive infestations (a: without plasma; b: with plasma). The results are calculated as average percentage degranulation seen with five rabbits (see Materials and methods)

	Mean number of basophils (mm^3)	Concentration of antigen (pg/ml)			
		a		b	
		10^6	10^7	10^6	10^7
Controls	129 ± 27	7·0 ± 3·0	5·0 ± 1·4	not done	not done
1st infestation	103 ± 29	21·8 ± 7·4	23·6 ± 4·0	19·0 ± 5·2	19·6 ± 4·2
2nd infestation	260 ± 110	34·8 ± 7·4	33·8 ± 7·9	23·3 ± 6·2	22·8 ± 5·4
3rd infestation	248 ± 73	56·8 ± 5·2	57·7 ± 6·9	46·7 ± 5·4	47·5 ± 4·8
4th infestation	166 ± 31	59·8 ± 7·8	63·8 ± 4·9	43·3 ± 7·1	43·3 ± 5·7

our results were always within these limits (Table 3a). From the 1st to the 2nd infestation, there is a considerable increase (103 to 260). Subsequently, at the moment of observation considered, counts were always decidedly higher than at the 1st infestation.

The specificity threshold of the basophil degranulation test for the tick antigen has been fixed at 10%. Indeed for four rabbits observed, the degranulation average varied between 1·3 and 7·0 (means: 7·0 and 5·0 respectively) for antigen concentrations of 10^6 and 10^7 pg/ml (Table 3a).

One observes a degranulation maximum for antigen concentrations of 10^6 and 10^7 pg/ml (Figure 1). Thus one can compare the results obtained at these rates of antigen during sucessive infestations (Table 3a). At the 1st infestation, 21·8 and 23·6 of the cells degranulated. From the 2nd infestation, these percentages increased (34·8 to 33·8%) to reach 59·8 and 63·8% at the 4th infestation. Clearly during the series of infestations, basophils became sensitized progressively towards the antigens of tick salivary glands.

The presence of a plasma factor partly blocking the degranulation of basophils was also demonstrated. Replacement of Tyrode's buffer by plasma in the test, with other factors kept constant, resulted in less degranulation of the cells at the same concentrations of antigen (Table 3, Figure 1). This factor was probably present during the primary infestation, since the percentage of degranulation was reduced by 2·8 and 4·0% (see concentrations 10^6 and 10^7 pg/ml, Table 3a & b). The blockage of the reaction then increased progressively during reinfestations. It was maximal at the 4th reinfestation (difference: 16·5 and 20·5).

After 10–15 infestations carried out on four other animals, the sensitization of basophils was very strongly marked. We obtained 75·0 and 79·8% degranulation for 10^6 and 10^7 pg/ml antigen (Table 4a). Application of the test before the last of the reinfestations showed that the values were already high (69·0 and 74·8%) and greater than those obtained at the 6th day of the 4th infestation (Table 3a). Furthermore, blockage of the reaction was also more marked. A difference of 25·5 to 27·4% between the tests carried out with and without plasma was observed (Table 4b, observations carried out after the last infestation).

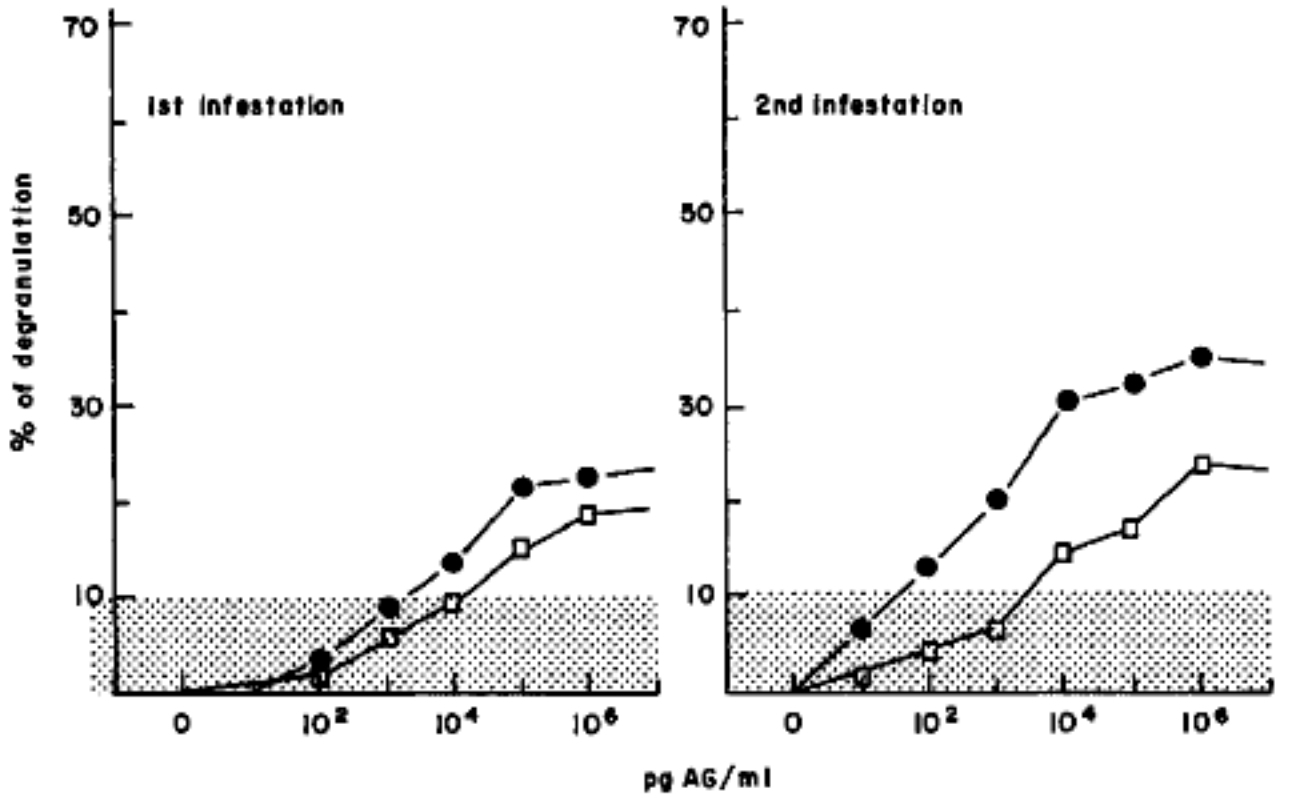

Figure 1. Progressive sensitization of circulating basophils (presence of a blocking factor). Stippled area: non-specific reactions (●——● without plasma; □——□ with plasma).

Discussion

The results of the present study on the effects of immunity on the weights of engorged *I. ricinus* ticks, on the duration of feeding, and on egg laying confirm and enlarge our earlier observations (Bowessidjaou *et al.* 1977). It is interesting to note the clear decrease of the

Table 4. Results of degranulation tests (10–15 successive infestations (a: without plasma; b: with plasma). The means are related to four rabbits

Concentration of antigen (pg/ml)	a		b	
	10^6	10^7	10^6	10^7
Before the infestation	69·0 ± 9·8	74·8 ± 7·9	50·0 ± 8·2	56·0 ± 4·6
After the infestation	75·0 ± 5·7	79·8 ± 7·0	49·5 ± 3·3	52·4 ± 4·3

rate of egg laying (0·52 to 0·29) and the prolongation of the periods of preoviposition and embryogenesis (Table 1).

Brossard & Fivaz (in press) have detected the presence of basophils and their quantitative evolution in the cutaneous lesion provoked by *I. ricinus* females attached to rabbits. In the course of a primary infestation, few of these cells are present and degranulated, but they infiltrate into the tissue to a larger extent at the end of a 2nd infestation. The infiltration of basophils in the feeding lesion of ticks is not unique for the rabbit *I. ricinus* system. It is even more intense in guinea-pigs infested by *Dermacentor andersoni* (Allen 1973) or *Ixodes holocyclus* larvae (Bagnall 1975). In the latter case, degranulated cells have been observed. These leucocytes could participate in cutaneous basophil hypersensitivity phenomena (Askenase *et al.* 1978), with cells sensitized by IgE degranulating specifically on contact with allergen. From this point of view, it is interesting that specific sensitization of rabbit basophils against *I. ricinus* antigens occurs.

The results of this study seem to account for the histological observations. The number of circulating basophils increase considerably from the 1st to the 2nd infestation (103–260/mm^3, Table 3a), as did the percentage degranulation of the same cells (21·8, 23·6–34·8, 33·8 for the concentrations of antigens of 10^6 and 10^7 pg/ml respectively: Table 3a). For the subsequent infestations, we have no histological information. However the progress of basophil sensitization has been noted. At the 3rd infestation, for the same antigen concentrations, the degranulated cells represented 56·8 and 57·7% of the total number of the circulating basophils. At the 4th infestation, the maximum degranulation was equal to 59·8 and 63·8%, respectively. Thus, the basophils are sensitized progressively in the course of subsequent infestations. In order to demonstrate a further evolution of this phenomenon, four rabbits have been submitted to 10–15 infestations. At the end of the last re-infestation, the percentage of degranulated basophils was very high, reaching 75·0 and 79·8% (Table 4a).

A blocking factor, probably an IgG antibody (Feingold 1973), reduced in our *in vitro* system the basophil degranulation induced by each antigen dilution, irrespectively of the number of previous infestations. Further, the blockade reaction increased from one infestation to the next (Table 3a & b). The anti-*I. ricinus* IgG antibodies appeared in two of five cases during the 1st infestation. The titres rose gradually during the course of re-infestations (Table 2). The *in vivo* efficiency of the blocking factor seems to be good in acute anaphylaxis phenomena where antigen is scattered with the blood (Vervloet & Charpin 1980). It could be less regular in immediate allergy where antigen interacts

directly with tissue mast cells. In infested rabbits, the allergen injected by the ticks reacts undoubtedly first with sensitized mast cells and basophils. Pharmacologically active substances released (e.g. histamine) could be harmful to the ticks (Brossard 1982). But these substances, in increasing vascular permeability could also facilitate access of protective elements: cells, complement and specific antibodies. The antibodies to tick salivary glands, notably the IgG demonstrated in this study, could then neutralize salivary components (e.g. enzymes) and thus impair normal food intake. Furthermore they could perhaps also react with the intestinal epithelium of the tick, thus impeding normal feeding and digestion.

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