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Cutaneous basophil-associated resistance to ectoparasites (ticks)

I. TRANSFER WITH IMMUNE SERUM OR IMMUNE CELLS

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Summary. Immune resistance experiments were carried out in guinea-pigs employing two tick species that as adults are ectoparasites of cattle (*Ixodes holocyclus* and *Rhipicephalus appendiculatus*). These studies showed that susceptibility of non-immune guinea-pigs to infestation with tick larvae varies according to the species of tick and the strain of guinea-pig. With both tick species, greater than 90% acquired resistance was achieved in several guinea-pig strains. Immune resistance was evident within a week following primary infestation and lasted up to 9 months following a single sensitizing exposure to tick feeding. The strength and duration of resistance was influenced strongly by the size of the initial sensitizing dose. Immune resistance was readily transferred to naive recipients by intravenous administration of either peritoneal exudate cells or immune serum from donors sensitized by a single prior infestation with ticks. Doses of serum as small as 0·5 ml transferred resistance. These studies demonstrate that both sensitized cells and immune serum factors contribute significantly to acquired host resistance to ticks that as adults are ectoparasites of cattle.

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INTRODUCTION

The pioneering work of Trager in 1939 established that infestation of the skin of guinea-pigs with the American dog tick, *Dermacentor variabilis*, leads to an immune resistance mechanism that is able to reject subsequent challenge with these arthropod ectoparasites. Recently, Allen (1973), working with the Rocky Mountain wood tick, *Dermacentor andersoni*, showed that this acquired resistance is associated with delayed cutaneous accumulation of basophil leucocytes at the sites of tick feeding. These responses are an extreme example of cutaneous basophil hypersensitivity (CBH; Dvorak, Dvorak, Simpson, Richerson, Leskowitz & Karnovsky, 1970; Askenase, 1977; Galli & Dvorak, 1980), with basophils comprising up to 90% of the infiltrate at tick feeding sites (Brown & Knapp, 1981). Wikel & Allen (1976) have transferred the resistance to normal guinea-pigs with immune cells, but not with immune serum. The present report is the first in a series concerned with similar experiments employing two tick species that as adults are ectoparasites of cattle. We have found that the susceptibility of non-immune guinea-pigs to feeding by tick larvae varies according to the species of tick and the strain of guinea-pig; and that acquired resistance can be transferred to normal animals with *either* immune cells or with immune serum. Subsequent papers will deal with the kinetics of

basophil arrival and subsequent ultrastructural degranulation at cutaneous sites of tick rejection.

MATERIALS AND METHODS

Ticks

Specific pathogen-free *Rhipicephalus appendiculatus* (Kabete strain) were from a colony originating in Kenya and maintained in laboratory culture on rabbits (larvae and nymphs) and cattle (adults) at the Wellcome Research Laboratories, Berkhampstead, Hertfordshire. Fully fed females, eggs and hatched larvae were regularly supplied through the generosity of Mr Michael D. Matthewson.

Ixodes holocyclus engorged females were obtained from the Lismore Tick Serum Laboratory, North New South Wales, Australia.

Larvae and nymphs of *I. holocyclus* were fed in the laboratory on rats and guinea-pigs, and adults were successfully fed on the head of rats with an attached Elizabethan collar to prevent host grooming of the head area.

Ticks were maintained in gauze-topped tubes inside dessicator jars over a saturated solution of KNO_3 (92%–93% relative humidity at 25°) and a photoperiod of 12 hr light/dark was employed.

Hosts

For experiments with *R. appendiculatus*, albino guinea-pigs of the Hartley Strain (outbred) and Strain 2 and 13 (inbred) were obtained from breeding stocks at the National Institute for Medical Research, Mill Hill. The majority of experiments employed female guinea-pigs weighing approximately 250 gm at first tick exposure.

For experiments with *I. holocyclus*, outbred Hartleys were obtained from the University of Sydney Animal House, Castle Hill, and inbred Hestons were obtained from C.S.I.R.O. McMaster Laboratory, Sydney, Australia.

Enumeration of larvae

A tube containing several thousand *R. appendiculatus* larvae, the pooled progeny of up to six simultaneously fed female ticks, was cooled in ice for approximately 20 min. Quiescent larvae were removed and placed in a small (35 mm diameter × 10 mm deep) water filled plastic petri dish (Falcon Plastics, Oxnard, Calif.) resting on ice. On pipetting off most of the water, the wetted larvae clumped together and small groups estimated by eye to contain approximately the required number of ticks were transferred using fine forceps to individual small petri dishes standing on ice. A larger (90 mm diameter × 14 mm deep) petri dish (Sterilin Ltd, Middlesex) filled with ice placed on the stage of a dissecting microscope acted as a cold stage. Individual dishes were transferred to this stage and the number of larvae were counted accurately at fifteen times magnification. Dessicated larvae were removed and the number in each dish was adjusted to the required dose per guinea-pig. All dishes were held on ice until all doses required for an experiment were complete; usually within a 1–2 hr period.

For *I. holocyclus* it was found that 10 mg of eggs equals approximately 200 eggs (205 ± 5). Eggs at this stage were stored in lots of 200 and hatched larvae were later counted as above to assemble doses of 200 larvae which were 2–8 weeks old at the time of infestation.

Infestation of hosts

For *R. appendiculatus* experiments, guinea-pigs were anaesthesized by injection of Pentobarbitone Sodium (Sagatal, May and Baker Ltd, Dagenham) at 30 mg/kg i.p. which produced a light anaesthesia of 2–3 hr duration. The flank area was clipped with animal shears leaving a hair stubble approximately 1 mm deep. The outer surface of the petri dish containing tick larvae was wiped dry and the lidless dish inverted onto the clipped area and securely taped into position by passing a length of $\frac{1}{2}$ inch wide zinc oxide plaster around the abdomen of the animal. Care was taken to ensure that the plaster did not adhere directly to the skin of the host near the dish, but only to the contralateral unclipped hair. Dish and plaster were left in place for approximately 18 hr (usually overnight) to ensure adequate attachment of ticks on the hosts.

For *I. holocyclus* experiments, larvae were applied directly to the unshaven backs of unanaesthetized animals that were kept moving for $\frac{1}{2}$ hr by frequent disturbance to stimulate penetration through the hair coat to the skin surface.

Housing of infested animals

Guinea-pigs were caged in groups of three in the *R. appendiculatus* experiments (and groups of five in the *I. holocyclus* experiments) in galvanized wire cages (39 cm × 25 cm × 23 cm) fitted with a wire mesh (10 mm opening) 4 cm from the base. Cages were placed in deep plastic boxes (46 cm × 32 cm × 17 cm) containing water 2 cm deep to which a small amount of detergent had been added. During the first 48 hr after infes-

tation, animals were fed on pelleted diet (E. Dixon and Sons, Ware, Hertfordshire) *ad libidum*. Thereafter, until all larvae had become detached, they were fed fresh cabbage only to eliminate large faecal drops. Water was available *ad libidum*. Animals were housed in an insectary at 28° and 68%–80% relative humidity.

Recovery of larvae, calculation of resistance and statistical analysis

Water in the outer plastic boxes was changed once or twice daily. It was decanted in portions into a shallow white tray which enabled fed larvae to be seen, collected with a pipette, and counted. The recorded total cumulative return of engorged larvae was expressed as the mean percentage return of the total number of larvae applied to the group (600 per three animals for *R. appendiculatus* and 1000 per five animals for *I. holocyclus*).

Resistance was calculated as the % rejection = $100 \times [1 - (\%$ return of engorged larvae from immune animals)/(% return or engorged larvae for non-immunes)].

For statistical analysis the mean ± SE percentage return from a number (n_1) of control groups was compared to the mean ± SE percentage return from the number (n_2) of experimental groups and analysed by Student's t test.

Harvesting serum and cells and systemic transfers

Groups of *R. appendiculatus* donors were primed by exposure to 200 tick larvae and were boosted by a similar infestation on the contralateral flank at three weeks. Seven days later they were bled by severing the vessels of the neck. The blood was allowed to clot at 25° for 1–2 hr, then at 37° for 45 min, then at 4° overnight. Serum was decanted from the clot, contaminating red blood cells (RBC) were removed by centrifugation, and storage was at −20°. Three days before serum harvest, some donors were injected i.p. with sterilized liquid paraffin and peritoneal exudate cells were harvested 3 days later by peritoneal lavage with Hanks's buffered salt solution. Cells were 70%–90% viable by eosin dye exclusion and were transferred by intravenous injection through the dorsal foot veins of unanaesthetized Hartley recipients using a 27 gauge needle. Various doses of freshly thawed serum were transferred similarly. When the dose transferred was less than 1 ml, the serum was appropriately diluted with saline to make 1 ml of the volume transferred. One to two hours later, the challenging dose of 200 larvae was applied.

For experiments with *I. holocyclus*, immune serum was obtained from donor guinea-pigs that had received two–five infestations of 200 larvae over a period of 1–3 months. The animals were killed 1 week after the final infestation and serum was collected and stored at −20°. Recipients were anesthetized with O_2 halothane and given 5 ml of freshly thawed serum by slow intravenous infusion into the anterior ear vein via a 27 gauge needle. For cell transfers in the *I. holocyclus* system, Heston animals were killed 1 week after the final infestation. Their axillary and prescapular lymph nodes were removed under sterile conditions, trimmed, diced, and then ground through a sieve, washed and counted. Recipients were anesthetized with O_2/halothane and given $2{\cdot}5 \times 10^8$ cells in 0·5–0·1 ml by slow intravenous injection into the anterior ear vein via a 27 gauge needle. Three hours later the challenging dose of 200 larvae was applied.

Preparation of tick extracts for vaccination

Crude extracts of *I. holocyclus* were prepared by grinding 10,000 freshly frozen larvae (500 mg) in a glass mortar for 10 min with 1–2 ml saline. The mixture was brought to 5 ml and particulate material was removed by centrifugation. Extracts were stored at −20°. Protein content was 5–6 mg/ml. Thus 0·1 ml or about 0·55 mg were regarded as being the equivalent of 200 larvae.

RESULTS

Susceptibility to infestation in various strains of non-immune guinea-pigs exposed to various species of ticks

Groups of guinea-pigs were exposed on one flank to 200 larvae of either *I. holocyclus* or *R. appendiculatus*. The mean percentage return of engorged larvae that fed on these non-immune guinea-pigs varied between experiments with each tick species. This could be ascribed to the age of the larvae, as *I. holocyclus* larvae less than 8 weeks showed a higher rate of attachment and engorgement than older larvae, and a higher percentage of younger *R. appendiculatus* larvae also fed successfully. In addition to variation according to the age of the larvae within a given tick species, differences were noted between the two tick species examined. Overall (in more than twenty-five experiments) *I. holocyclus* showed nearly double the numbers of engorged larvae returned (36·5% ± 1·6%) from non-immune Hartley guinea-pigs compared with *R. appendiculatus* (17·1% ± 1·1%). Another factor in

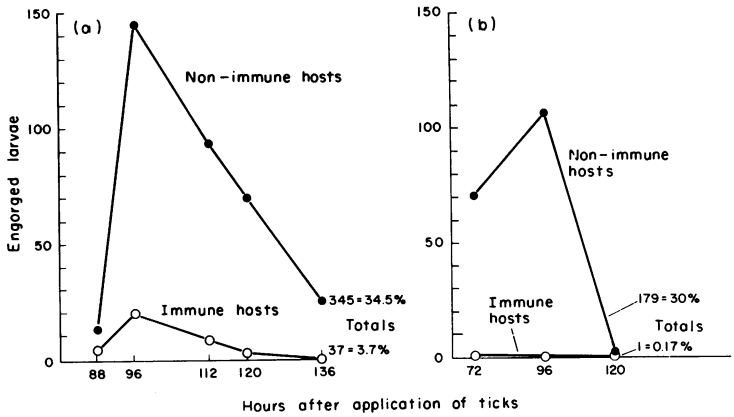

Figure 1. Kinetics of larval tick feeding on non-immune compared with immune guinea-pigs. (a) *I. holocyclus* larvae (200 per host) were applied to a group of five non-immune guinea-pigs (●) and to a group of five immune animals (○). (b) *R. appendiculatus* larvae (200 per host) were applied to three non-immune (●) *versus* three immune (○) animals. For (a) and (b), detached and engorged larvae returned from the animals were counted at the times indicated. The total cumulative return for the group, and the percentage engorged ticks of the total number applied, is shown to the right of each curve.

this susceptibility to infestation was the strain of guinea-pig. For *I. holocyclus*, nearly twice as many fed on inbred Heston guinea-pigs (69·9%±2·3%) compared with outbred Hartleys (36·5%±1·6%; $P<0.0001$) and for *R. appendiculatus* an equivalent percentage of applied larvae fed successfully on inbred Strain 2 (20·7%±1%) and Hartley guinea-pigs (22·5%±5·6%), but feeding was only half as good on inbred Strain 13 guinea-pigs (10·3%±1%; $P<0.025$). Thus, susceptibility to successful feeding of tick larvae on guinea-pigs varied according to the age of the larvae, the species of tick employed, and the strain of guinea-pig infested.

Acquired resistance of guinea-pigs to tick infestation

Exposure of guinea-pigs to cutaneous feeding of tick larvae induced acquired resistance to a subsequent challenge at a previously unexposed skin site. Figures 1a and 1b show the time course of detachment of engorged larvae of, respectively *I. holocyclus* and *R. appendiculatus*, from non-immune Hartley guinea-pigs versus animals that were previously exposed to a single cutaneous infestation with larvae. It can be seen that detachment of engorged larvae from non-immune hosts took place for each tick species largely on days 3, 4 and 5 after commencement of feeding, with a peak at day 4. Far fewer larvae of each species successfully fed to engorgement on previously exposed guinea-pigs. Those larvae that did complete engorgement on immune hosts did so within the same period as on non-immune hosts. Table 1 shows a summary of such immune resistance experiments that employed feeding of either *I. holocyclus* or *R. appendiculatus* larvae on different strains of guinea-pigs. Greater than 90% rejection was achieved in all cases. Thus, susceptibility varied according to the species of tick and strain of host, whereas acquired immune resistance was so complete that such a variation was not observable in challenge feedings. For immune Hartley guinea-pigs challenged with *R. appendiculatus* the mean group resistance was 97·9% with a range of 72% to 100%. However, of thirty-eight immune groups, only two had resistance below 90% and twenty-seven had a resistance of 99·9% or 100%.

The degree of resistance was not improved by multiple infestations. Resistance seemed to be a property of the primary interaction between host and tick, rather than the number of prior infestations. With *R. appendiculatus* larvae there was a suggestion that a low priming dose and a high challenging dose could reveal a somewhat lower level of resistance (Table 2).

Kinetics of the development and duration of immune resistance to tick larvae

The onset of immunity occurred within 1 week following a primary cutaneous infestation with either tick. With *R. appendiculatus*, resistance was nearly

Table 1. Resistance to feeding of larval ticks on immune guinea-pigs*

Guinea-pig strain	Tick species†	Non-immune animals: Number of Experiments	Groups	Animals	Return (%) ±SE	Immune animals: Number of Groups	Animals	Return (%) ±SE	Rejection (%)‡
Hartley	*I. holocyclus*	11	16	80	34·07±3·9	16	80	2·58±0·8 ($P<0·0001$)	92·4
Heston	*I. holocyclus*	1	1	5	69·3§	1	5	1·2	97·3
Hartley	*R. appendiculatus*	17	37	111	18·3±1·5	38	114	0·37±0·08 ($P<0·0001$)	97·9
Strain 2	*R. appendiculatus*	1	1	3	21·0§	2	6	0	100
Strain 13	*R. appendiculatus*	1	1	3	11·2§	2	6	0·1	99·6

* Guinea-pigs were sensitized by exposure to 200 tick larvae on one flank skin and were challenged 2 to 3 weeks later by exposure to 200 tick larvae on the opposite flank skin.

† Animals exposed to *I. holocyclus* were five per group; those exposed to *R. appendiculatus* were three per group.

‡ The % rejection = 100 × (% return of engorged larvae from immune animals)/(% return of engorged larvae from non-immunes)].

§ No SE because yields were per group and only one group was used in these experiments.

complete after 1 week and thus no further increase in resistance could be discerned with longer periods between sensitization and challenge.

Figure 2 shows the duration of resistance in Hartley guinea-pigs sensitized with *I. holocyclus*. A single infestation led to significant resistance for up to 4 months. The level of resistance correlated with the priming dose. When the primary infestation resulted in 42% or greater return of engorged larvae (a mean sensitizing dose of eighty-four larvae per guinea-pig), then resistance of greater than 75% ensued for up to 17 weeks. In contrast, a priming yield consisting of 22% or less of engorged larvae (a mean sensitizing dose of forty-four or less larvae per animal), led to resistance

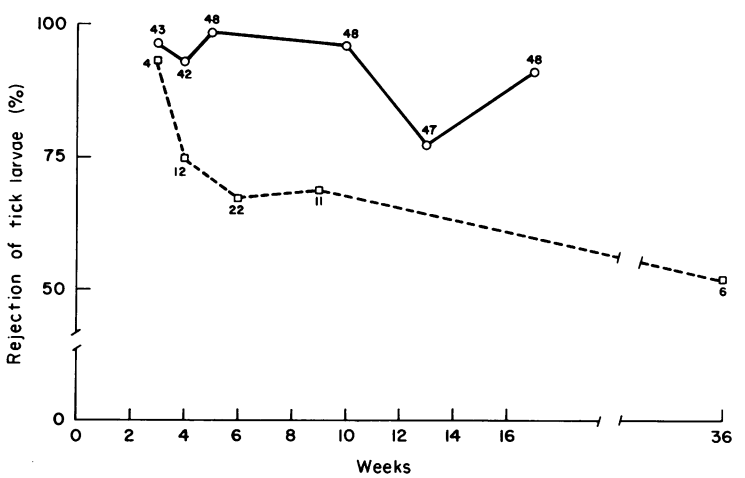

Figure 2. Relationship of strength and duration of immune resistance to the initial sensitizing dose of *I. holocyclus* larvae. In all experiments 200 larvae were applied to each guinea-pig at the sensitizing infestation, and at challenge 3–36 weeks later. The numbers appearing at each point on the curves are the percentage of the 200 applied larvae that fed successfully at the sensitizing infestation. High sensitizing dose (—); low sensitizing dose (- - -).

Table 2. Immunity to larval and nymphal ticks following infestation with ticks at various stages in the life cycle

Species of tick*	Tick life cycle stage used for: Sensitization (no. *feeding* per guinea-pig)	Challenge (no. *applied* per guinea-pig)	Rejection (%)†
I. holocyclus	Larvae (4)	Larvae (200)	93·7
I. holocyclus	Larvae (70)	Nymphs (10)	100
I. holocyclus	Nymphs (2–3)	Larvae (200)	92.8
R. appendiculatus	Larvae (29)	Larvae (1000)	80·4
R. appendiculatus	Larvae (19)	Larvae (200)	99·2
R. appendiculatus	Larvae (27)	Larvae (200)	100
R. appendiculatus	Larvae (27)	Larvae (400)	100
R. appendiculatus	Larvae (33)	Larvae (200)	100
R. appendiculatus	Nymphs (3–4)	Larvae (200)	92·8

* Animals exposed to *I. holocyclus* were five per group; those exposed to *R. appendiculatus* were three per group.

† The % rejection = 100 × 1 − [(% return from immune animals)/(% return from non-immunes)].

that was similarly long lived, but was less complete. Thus, a high or low sensitizing dose led to long lasting immune resistance, but a high dose led to more complete resistance. With guinea-pigs exposed to 200 *R. appendiculatus* larvae, resistance at 10 weeks, 18 weeks and 6 months was, respectively, 99·9%, 100%, and 100%, while animals challenged 9 months after priming showed 72% resistance. Thus, a single infestation with tick larvae sensitized guinea-pigs for nearly complete immune resistance for several months.

Sensitization with minimal numbers of larvae or with nymph or adult ticks; and resistance to nymphs

Table 2 shows that a primary sensitization with as few as four *I. holocyclus* larvae were sufficient to sensitize guinea pigs for greater than 90% resistance to a subsequent challenge with larvae. Small numbers of *R. appendiculatus* larvae infesting guinea-pigs at the time of sensitization also were sufficient to immunize for potent resistance to challenge with larvae (Table 2).

The hard tick life cycle consists of three successive stages—larva, nymph and adult in which cutaneous feeding and engorgement occurs at each stage. Table 2 shows that *I. holocyclus* larvae sensitized guinea-pigs for complete rejection of nymphs, and that feeding of only two–three nymphs sensitized guinea-pigs for rejection of a subsequent challenging dose of 200 larvae. Similarly, feeding of three or four nymphs of *R. appendiculatus* sensitized guinea-pigs for rejection of a subsequent challenge dose of 200 larvae.

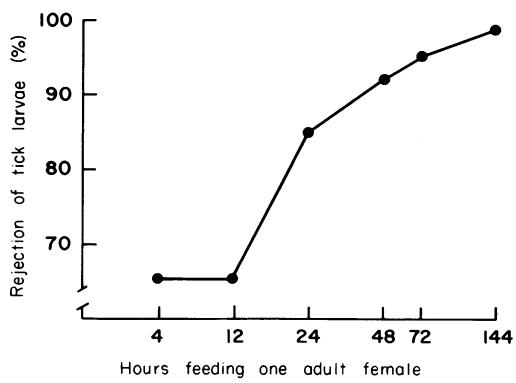

Figure 3. Rejection of tick larvae from guinea-pigs sensitized by prior feeding of one adult female for the interval shown. Each time point represents the feeding period for a single adult female feeding on each guinea-pig of a separate group of five animals. Data were pooled from two separate experiments.

Figure 3 shows that feeding one adult female *I. holocyclus* tick on a guinea-pig for as little as 4 hr conferred substantial but incomplete resistance to subsequent challenge with 200 larvae 3 weeks later, and that feeding of one adult female for greater than

Table 3. Transfer of resistance to *R. appendiculatus* larvae with immune serum

Volume transferred per recipient*	Non-immune animals				Immune serum recipients†				
	Number of			Return (%) ±SE	Number of		Return (%) ±SE	Rejection‡ (%)	Significance§
	Experiments†	Groups	Animals		Groups	Animals			
5 ml	2	4	12	8·1±1·7	3	9	1·44±0·5	82	$P<0·05$
2 ml	3	5	15	12·7±3·5	3	9	0·45±0·15	97	$P<0·025$
1 ml	3	5	15	18·6±1·2	3	9	1·55±0·24	92	$P<0·005$
0·5 ml	9	15	45	17·5±1·7	9	27	4·14±0·95	76	$P<0·0005$
0·25 ml	4	7	22	17·5±2·1	4	12	13·8±4·8	8	NS
2 ml¶ (trichinella immune serum)	1	2	6	23·2±4·6	1	3	20·5	12	NS

* Pooled serum was obtained from donors that were sensitized by exposure to 200 larvae, boosted 3 weeks later by a similar challenge, and bled out 1 week later.

† Pooled data from eleven experiments in which various doses of immune serum were transferred i.v. Groups are presented according to the dose received. In each experiment one or two control groups of three non-immune guinea-pigs and experimental groups of three guinea-pigs per group that received various doses of immune serum were challenged by exposure to 200 larvae 1–2 hr after transfer.

‡ The % rejection = $100 \times [1 - (\%$ return from immune or control serum recipients)/(% return from non-immunes)].

§ Comparison was made between the percentage return in recipients of a given dose and paired non-immune controls from the same experiment according to a paired *t* test.

¶ In one experiment each animal in a group received 2 ml of control immune serum from donors that were exposed to 2000 *T. spiralis* larvae by oral feeding three times over 2 months, and were bled out 1 week after the last challenge.

48 hr conferred greater than 90% resistance to such a challenge.

It was concluded that feeding of all the various stages in the life cycle of the tick *I. holocyclus* conferred strong resistance against subsequent challenge with larvae, and that larvae induced resistance to nymphs. These results suggest that antigens crucial to induction of immune resistance are present in each stage of the life cycle.

Transfer to tick resistance with immune serum or immune cells

Table 3 shows that immune serum harvested from Hartley guinea-pigs that were previously exposed to *R. appendiculatus* larvae, transferred significant and reproducible resistance to normal Hartleys that were challenged by feeding with *R. appendiculatus* larvae. In fact, doses as small as 0·5 ml immune serum, that were harvested 1 week after the second of two sensitizing feedings 3 weeks apart, provided significant resistance. The data in Table 3 was compiled from experiments in which sera from six different batches were active for transfer. Control immune serum from donors multiply sensitized by infestation with the intestinal nematode *Trichinella spiralis* was not able to transfer resistance to tick larvae (Table 3). In the *I. holocyclus* system immune serum did not transfer resistance to normal Hartley guinea-pigs.

Table 4 shows that rejection of *R. appendiculatus* tick larvae could also be accomplished by transfer of peritoneal exudate cells from immune donors. This was also true for infestation with *I. holocyclus* in Heston guinea-pigs where transfer of resistance was accomplished with lymph node cells (Table 4). It is interesting to note that in one experiment cells alone transferred 37% rejection, immune serum alone (5 ml) transferred 33% rejection, and the two together had an additive effect; transferring nearly 65% resistance. In the experimental system employing infestations with *R. appendiculatus* tick larvae in Hartley guinea-pigs, peritoneal exudate cells conferred nearly complete resistance. Thus, immune resistance to *I. holocyclus* seemed to require an additive effect of immune serum

Table 4. Transfer of resistance to larval ticks with immune cells

No. of exp.	Strain of guinea-pig recipient	Tick species*	Material transferred† (dose per recipient)	Non-immune animals		Immune recipients		Rejection‡ (%)	Significance (*P*)
				No.	Return (%) ±SE	No.	Return (%) ±SE		
3	Heston	*I. holocyclus*	LNC ($2{\cdot}5\times10^8$)	20	67·6±4	15	34·9±4	48·0	<0·025
1	Heston	*I. holocyclus*	LNC ($2{\cdot}5\times10^8$)	5	56·0**	5	35·0	37·0	—
	Heston	*I. holocyclus*	Serum (5 ml)§	5	56·0	5	33·0	33·0	—
	Heston	*I. holocyclus*	LNC+5 ml serum	5	56·0	5	19·7	64·8	—
4	Hartley	*R. appendiculatus*	PEC ($1–4\times10^8$)	12	8·8±1·2	12	0·8±4	91·0	<0·01
1¶	Hartley	*R. appendiculatus*	PEC (2×10^8)	6	10·3±0·4	3	1·3**	87·4	—
	Hartley	*R. appendiculatus*	PEC (10×10^8) (trichinella immune cells)	6	10·3±0·4	3	10·2	1·0	—

* Animals exposed to *I. holocyclus* were five per group; those exposed to *R. appendiculatus* were three per group.

† Donors were sensitized by exposure to 200 larvae, and were boosted 2 weeks later by a similar challenge. One week later lymph node cells (LNC) were harvested from sites draining the dorsal flank skin used for challenge, or peritoneal exudate cells (PEC) were harvested 3 days after intraperitoneal injection liquid paraffin oil.

‡ In each experiment one or two control groups of non-immune guinea-pigs, and experimental groups of guinea-pigs that received various transfers were challenged by exposure to 200 larvae 1 to 2 hours after transfer. The % rejection = $100\times[1-(\%\text{ return from transfer recipients})/(\%\text{ return from non-immunes})]$.

§ Serum was obtained and transferred as in Table 4.

¶ In this experiment each animal in a group received control PEC from donors that were exposed to 2000 *Trichinella spiralis* larvae by oral feeding three times over 2 months. PEC were harvested 1 week after the last challenge.

** No SE because yield was per group and only one group was used in these experiments.

Table 5. Rejection of *I. holocyclus* larval ticks by guinea-pigs immunized with a crude extract of larvae

Exp.	Number of Guinea-pigs	Immunizing procedure* Vehicle	Antigen dose	Return (%) from Non-immune animals	Immunized animals	Rejection (%)
1†	5	Saline	0·7 mg×1	45·0	32·0	28·8
2	5	Saline	1·4 mg×1	62·3	38·6	38·0
2‡	5	Saline	1·4 mg×3	62·3	19·7	68·4
3	5	Saline	1·4 mg×1	43·9	16·5	62·4
3	5	IFA	1·4 mg×1	43·9	21·4	51·3
3	5	CFA	1·4 mg×1	43·9	18·0	58·5
4	5	CFA	1·4 mg×1	31·2	13·1	58·0

* Hartley guinea pigs (500 gm) were immunized by multiple subcutaneous injections in the foot pads and the neck with a total of 0·25 ml larval extract (approximately 1·4 mg protein) mixed with an equal volume of either saline or Freund's complete adjuvant or Freund's incomplete adjuvant. Animals were challenged 3 weeks later with 200 larvae.

† In this experiment one half dose was injected intradermally.

‡ In this group animals received two extra doses at weekly intervals.

and immune cells, whereas nearly complete immune resistance to *R. appendiculatus* was mediated to *either* immune serum (Table 3) or immune cells (Table 4). Controls that received peritoneal exudate cells from animals that were multiply exposed to oral challenges with *T. spiralis* had insignificant resistance to challenge with *R. appendiculatus* larvae (Table 4).

Vaccination for acquired resistance with tick extracts

Table 5 shows that extracts of *I. holocyclus* larvae injected into Hartley guinea-pigs were able to vaccinate these animals for significant immune rejection of larvae. In the first experiment, multiple injections of crude tick extract in saline seemed to improve its ability to function as a vaccine, but in a second experiment, one injection of this extract conferred substantial resistance that was not increased by incorporation in Freund's complete or incomplete adjuvant.

DISCUSSION

These experiments demonstrate that guinea-pigs can acquire a potent immune resistance to cutaneous infestation with ectoparasitic Ixodid ticks. This confirms the work of others who employed *D. variabilis* (Trager, 1939), *D. andersoni* (Allen, 1973; Wikel & Allen, 1976), and *Amblyomma americanum* (Brown & Knapp, 1981). We studied experimental immune resistance employing ticks that commonly infest cattle. These ticks, in themselves, are important causes of economic loss of the cattle industry (toxicosis, blood loss, anorexia) and are also vectors of infectious agents (*Babesia, Theileria*) that cause chronic diseases in cattle (Hoogstraal, 1956). Thus, exploitation of the acquired immune resistance response of cattle to ticks will contribute to the increased livelihood of important animal stocks.

Immune resistance to the tick vector may also affect immune resistance to the transmitted parasite. Recent studies in cattle have shown that the babesial infection causes an immunosuppression of the host that interferes with the immune resistance mechanism to the tick vector, leading to a greatly susceptibility to disease (Callow & Stewart, 1978). It had previously been shown that tick transmission of babesia to cattle that are resistant to infestation is greatly reduced in comparison to transmission of these protozoa to non-resistant hosts (Francis & Little, 1964). If acquired tick resistance is to contribute significantly to resistance to the transmitted infectious agents then complete resistance would have to be achieved, since a few ticks feeding successfully could transmit disease due to organisms able to multiply within the host.

In the model system described in this study nearly complete acquired resistance was achieved. Importantly, this strong resistance was found to be transferrable by either immune serum or cells. Immune resistance was induced by a single feeding with small numbers of organisms from various stages in the life cycle of the ticks, was long lasting, was evident within a week of the sensitizing feeding, and could be partially achieved with injection of crude extracts of the ticks. Strong resistance was shown to be present for several months after a sensitizing feeding with either of the ticks employed, but finally diminished with time. The key element in achieving strong and prolonged resistance after sensitization was the size of the original sensitizing dose.

A remarkable finding of this study was that either immune serum or immune cells could transfer acquired resistance to ticks in guinea-pigs. With *I. holocyclus* infestations, immune serum conferred only modest resistance, that in addition to the modest resistance conferred by sensitized cells, seemed to approach reconstituting the recipient with the full acquired immune resistance of the donor. Thus, in this system it seems that both immune serum factors *and* immune cells may be necessary to achieve complete resistance. In the experiments of Wikel & Allen (1976), who employed *D. andersoni*, immune serum transfers of resistance were unsuccessful, and in the experiments of Trager (1939), who employed *D. variabilis*, immune serum conferred marginal resistance. Variations in the strain of guinea-pig, dose of serum, or route of administration might explain these different results.

In our study of immunity to *R. appendiculatus* in Hartley guinea-pigs, immune serum had a most potent effect. These serum transfers were repeatedly successful, employing several different batches of serum, in experiments conducted over a 2 year period. Remarkably, serum alone conferred almost complete resistance, and quite small doses of serum were effective. The site of tick rejection in these animals contains numerous degranulating basophils (see subsequent papers in this series). Similarly, cutaneous basophil reactions to haptens and proteins can be transferred systemically with low doses of immune serum (Askenase, Haynes & Hayden, 1976; Haynes, Rosenstein & Askenase, 1978), or microgram quantities of purified IgG1 antibodies (Haynes *et al.*, 1978). Three

crucial questions are: (1) how the responsible factors in such a small amount of serum are able to recruit sufficient numbers of host cells (such as basophils) to the site of rejection; (ii) which cells actually participate in the rejection mechanism; and (iii) how do these cells achieve this rejection? Recently, Brown, Galli, Gleich, Dvorak & Askenase (1981) reported that immune resistance to *A. americanum* can be abolished with the administration of highly specific anti-basophil serum to sensitized guinea-pigs before tick challenge. In addition, the administration of anti-eosinophil serum resulted in partial abrogation of tick resistance. It appears, therefore, that basophils and eosinophils co-operate in the resistance response; but how they affect tick feeding is not understood.

Successful transfer of acquired resistance to tick infestation was also achieved with immune peritoneal exudate cells. These cells are known to consist of 50%–80% phagocytic cells (macrophages and neutrophils), and the remainder are small lymphocytes, most of which are T cells. Thus, it is probable that the cell transfer of acquired resistance to ticks was mediated by T cells. However, because such small doses of immune serum also sufficed for successful transfer, it cannot be ruled out that the transferring T cells cause secretion of antibody by the few B cells present in the transferred cells, or even by B cells recruited in the recipient, and that these T-dependent antibodies ultimately lead to the resistance mechanism. However, for the moment, it seems most reasonable to conclude that in this system, as in various other CBH systems, that T cells and/or antibody products of B cells can lead to *both* the recruitment of basophils to cutaneous hypersensitivity sites, and perhaps to the activation of these cells once they have arrived at these sites.

This study contains two findings that are potentially encouraging for attempts to develop strategies that might induce immune resistance to ticks. First, it was shown that feeding of larvae, nymphs or adults sensitized for subsequent rejection of larvae, and that feeding with larvae could lead to immune rejection of nymphs. Similar results have recently been found in experiments on acquired immune resistance of guinea pigs to *Ixodes dammini*, the established vector of babesiosis and the purported vector of Lyme arthritis in New England (Krinsky, Brown & Askenase, 1981). These results suggest that crucial antigens that can lead to immune responses that confer resistance are found in the various life cycle stages of the ticks and that during feeding these antigens are presented to the immune apparatus of the host, perhaps via Langerhans cells of the skin (Allen, Khalil & Wikel, 1979). Attempts at tick control employing larvae, nymphs or adults that are able to feed, but not mate, may be an effective biological mode of tick control, in contradistinction to sprays and dips that can be environmentally toxic and short lived with respect to control because of the development of resistance by the ticks (Drummond, 1970). Infestation with a single adult female conferred immune resistance. This demonstrates that a unisexual infection will confer immunity without the possibility of probagation of the ticks, since females must mate with males during feeding in order to complete feeding and later oviposit.

The second result encouraging for vaccination attempts is our preliminary finding that crude extracts of *I. holocyclus* larvae were able to confer significant resistance even when administered without adjuvants, in a single injection in saline. Similar results have been obtained by others in guinea-pigs (Trager, 1939; Allen, 1973; Wikel & Allen, 1976; Wikel, Graham & Allen, 1978); rats (Ackerman, Floyd & Sonenshine, 1980); rabbits (Garin & Grabarev, 1972; McGowan, Homer, O'Dell, McNew & Barker, 1980), and importantly, in cattle (Allen & Humphreys, 1979). Clearly, attempts to isolate the crucial antigens in the ticks could lead to manufacture of highly effective vaccines. The potent and reproducible resistance we have achieved with immune serum may mean that specific antibody could serve as a useful probe to detect and isolate these crucial antigens.

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