Effect of host age on the expression of acquired resistance to ticks[1]

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Summary. Young guinea-pigs (350 g) expressed significantly greater levels of acquired resistance to challenge by larval *Amblyomma americanum* ticks than older guinea-pigs (520–800 g). This finding suggests that younger guinea-pigs are immunologically more responsive to tick infestation than older mature individuals. Therefore, host age is an important variable in evaluating acquired resistance to ticks.

Studies directed at elucidating the immune response of hosts to feeding by *Amblyomma americanum* ticks have demonstrated mediation by antibody and T-cell components[2-4] and dependency upon intact basophils[5]. Current knowledge concerning immune resistance to *A. americanum, Dermacentor andersoni, Boophilus microplus* and *Ixodes holocyclus* has been reviewed[6-9]. However, one variable not yet critically evaluated and only eluded to in 2 reports[10,11], is the effect of host age on the expression of resistance to ticks. Studies in our laboratory aimed at defining the parameters of acquired immune resistence to ticks have always utilized young guinea-pigs (1–2-week-old; 150–200 g). The rational behind the selection of younger animals was that they would be immunologically more responsive than older animals because of their relative limited exposure to environmental antigens. The object of this study was to examine this hypothesis.

Materials and methods. Female albino Hartley guinea-pigs (CrL: (HA) BR) weighing 200–800 g were used as hosts. Larval *A. americanum* ticks were enumerated into groups of 100 and confined to the shaved flank of hosts using the capsule (35 × 10 mm petri dish) technique[2]. Initial (sensitization) tick infestation was on the right flank and challenge infestation on the left flank 26 days later. Hosts were housed in groups of 3 in plastic cages (43 × 20 × 18 cm) and given food (guinea-pig chow) and water ad libitum. Animals were weighed at the time of initial and challenge tick infestation.

At the end of the tick feeding period, fed ticks were collected from hosts, counted and weighed. Tick yield was calculated (number fed/100) and mean percent yield and weight ± SE determined for each group. In addition, the percent tick rejection and weight reduction for each experimental group was calculated: 100 × (1-yield or weight reduction from experimentals/yield or weight reduction from controls).

Results. Primary tick feeding success from all hosts was similar ($p > 0.05$), ranging from 72 to 83% yield and all exhibited a mean weight of 0.9 mg (table). During the challenge infestation 26 days later, however, all animals expressed significant resistance (47.1–63.1% tick rejection), but younger animals (group A) expressed significantly greater ($p < 0.01$) resistance than older animals (group C). Intermediate aged animals (group B) expressed a level of immunity that was only qualitatively different ($p > 0.05$) from the youngest (group A) and oldest animals (group C). This finding suggests an age-dependent quantitative gradation in immune responsiveness to tick feeding. Mean engorgement weight of ticks from each group of challenged hosts exhibited significant decreases compared to controls. Individuals from group A and C animals weighed significantly less ($p < 0.05$) than individuals from group B animals which were not significantly different from controls. These results suggests that the mechanism of acquired resistance by guinea-pigs to larval *Amblyomma americanum* ticks is mediated by an immune process that is agedependent.

Discussion. Acquired resistance to ticks was first described in cattle[12]. The guinea-pig, however, also expresses acquired resistance to tick feeding[2-7] and is a preferred laboratory model host. Criteria for assessing host resistance to ticks are a decrease in the number of fed ticks followed by a decrease in mean tick engorgement weight. It is well known that certain breeds of cattle are more resistant *(Bos indicus)* to tick infestation than others *(B. taurus)*[8], but the importance of host age on the magnitude of the immune response has not yet been evaluated critically. It has been reported that older dogs are more susceptible than younger dogs (no ages given) to feeding by *Rhipicephalus sanguineus* ticks, and that fewer *R. rossicus* engorge on young rabbits compared to older rabbits[10]. Contrarily, juvenile cottontail rabbits were shown (P-K test) to have lower circulating anti-*Haemaphysalis leporispalustris* antibody and carried a significantly greater tick burden than older rabbits[11]. The apparent lack of circulating antibody in juveniles does not preclude the existance of an immune response because the P-K test is dependent upon homocytotropic antibody. In addition, assays for cell-mediated immunity (CMI) were not reported. Passive transfer studies using immune guinea-pig serum and lymphocytes demon-

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Group[a]	Approximate age (weight) Primary	Challenge (Control)[b]	Tick yield (%)	Tick rejection (%)	Tick weight (mg)	Weight reduction (%)
A	1.5 weeks (200g)	–	83 ± 4[c]	–	0.9 ± 0.1	–
	–	5 weeks (350 g)	31 ± 4	63.1	0.6 ± 0.1[d]	37.5
		(350 g)	84 ± 3	–	0.9 ± 0.1	–
B	4 weeks (370 g)	–	78 ± 2	–	0.9 ± 0.02	–
	–	8 weeks (520 g)	39 ± 6[e]	55.7	0.8 ± 0.1	12.8
		(550 g)	88 ± 2	–	0.9 ± 0.02	–
C	9 weeks (600 g)	–	72 ± 4[f]	–	0.9 ± 0.03	–
	–	13 weeks (800 g)	46 ± 5[gh]	47.1	0.7 ± 0.03[d]	20.9
		(750 g)	87 ± 4	–	0.9 ± 0.1	–

[a] 9 animals/group; [b] first tick feeding; [c] mean ± SE; [d] $p < 0.001$ (vs control); [e] $p > 0.05$ (vs challenge yield from group A); [f] $p > 0.05$ (vs primary yield from groups A and B); [g] $p < 0.01$ (vs challenge yield from group A); [h] $p > 0.05$ (vs challenge yield from group B).

strate the existence of both antibody and CMI responses to *A. americanum*[2], *R. sanguineus*[2], *R. appendiculatus*[13], *Ixodes holocyclus*[13] and *D. variabilis*[14], but only CMI to *D. andersoni*[15] and antibody mediated immunity to *B. microplus*[16].

The observation that young guinea-pigs are better responders to tick infestation than older mature animals suggests age-dependent depression of immune responsiveness. A similar phenomenon has been described in other vertebrates associated with the depression of specific subpopulations of T-lymphocytes (CMI)[17]. Resistance to *A. americanum* involves both cell and antibody-mediated reactions[2-4,6], therefore, the importance of antibody in this observed age-dependent decrease in immune responsiveness should not be overlooked. The finding of an age-dependent immunity in guinea-pigs to ticks is critical to our studies aimed at elucidating the hosts immune response to ectoparasites; where high titered antibodies are needed as probes for antigens, and where large populations of highly specific lymphocytes are used for in vitro assays. These findings support our initial prejudice that young guinea-pigs are immunologically more reactive to tick feeding than older mature individuals, and presents another variable that must be considered in evaluating host resistance to ticks.

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Monoclonal antibodies to L-asparaginase

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Summary. Five hybridomas secreting monoclonal antibody to *E. coli* L-asparaginase were isolated. These monoclonal antibodies were classified into 3 different subclasses; Ig G_1 (1 clone), Ig G_2 (2 clones) and Ig G_3 (2 clones). One of them possessed anti-L-asparaginase neutralizing activity. Four antibodies examined demonstrated a linear Langmuir binding plot and binding affinities, with equilibrium dissociation constant (K_d) ranging between 2.5×10^{-9} M and 6.3×10^{-10} M. The monoclonal antibodies should be useful probes for investigation of the enzyme activity.

There are several reports of the preparation and exploitation of documented monospecific antibodies against enzymes such as phenylalanine hydroxylase[1], glucose-6-phosphate dehydrogenase[2], acetylcholinesterase[3], urokinase[4] and phosphofructokinase[5].

This paper describes the isolation and characterization of monoclonal antibodies against *E. coli* L-asparaginase. This is the first report describing the preparation of monoclonal antibody to L-asparaginase.

Materials and methods. Enzyme assay: Enzyme solution (0.1 ml) is added to sodium borate buffer (0.1 M, pH 8.5) to give a volume of 1.5 ml. The reaction is started by the addition of 0.5 ml of L-asparagine monohydrate (0.04 M) and allowed to proceed for 30 min at 37 °C. The incubation is stopped by adding 0.5 ml of 15% trichloroacetic acid, and the mixture if centrifuged. The supernatant is transferred quantitatively to 10-ml graduated test tubes, and diluted to 9 ml with water. 1 ml of Nessler's reagent is then added, and the mixture is allowed to stand for 15 min at room temperature. Ammonia is estimated by determining the optical density at 500 nm. Enzyme and substrate blanks are incubated in all assays[6].

Immunization protocol: Five male BALB/c mice, 2 months of age, received i.p. injections of L-asparaginase (500 units, Kyowa Hakko Kogyo Co.) with complete Freund's adjuvant on days 0, 10, 20, and 30 and i.v. injections of 300 units of enzyme on day 40. The mice were killed 3 days later for somatic cell hybridization.

Cell fusion protocol: Lymphocyte suspensions from spleens of immunized mice were prepared by gentle teasing, and the red blood cells were removed by NH_4Cl (0.83%). In each fusion experiment, spleen cells (1×10^8 cells) were mixed with mouse myeloma cells (P3 × 63Ag8, 1×10^7 cells) and co-pelleted with 30% of polyethyleneglycol (PEG 1000) for a total of 8 min at room temperature. Pellets were washed with Dulbecco's modified Eagle's medium (DMEM) to remove PEG. The pellets containing fused cells were gently resuspended in a small volume of DMEM-HAT medium (10% fetal calf serum, penicillin, streptomycin, nonessential amino acids, glucose, glutamine,

Antibody	Subclass	Binding activity (K_d)	Neutralizing activity
84	Ig G_1 (k)	1.8×10^{-9} M	–
116	Ig G_{2a} (k)	8.3×10^{-10} M	–
119	Ig G_3 (k)	2.5×10^{-9} M	–
280	Ig G_{2a} (k)	6.3×10^{-10} M	–
105	Ig G_3 (k)	+	+