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Langerhans cells present tick antigens to lymph node cells from tick-sensitized guinea-pigs

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Summary. Resistance to the feeding activities of ixodid ticks has previously been shown to be an acquired, immunologically-mediated phenomenon in guinea-pigs, associated with cutaneous hypersensitivity to tick antigens. Also, there is circumstantial evidence to support the suggestion that epidermal Langerhans cells may play roles in the acquisition and expression of tick resistance.

In this *in vitro* study, lymphocyte blastogenesis assays were performed using column-purified lymph node cells from naive or tick-sensitized guinea-pigs as responder cells. Syngeneic macrophages or epidermal Langerhans cells, incubated with tick salivary antigens, were used as stimulator cells.

Epidermal cell populations, containing viable Langerhans cells, were prepared by two different protocols. Epidermal cell populations containing ATPase-positive, Fc receptor- and Ia-bearing cells (Langerhans cells), when incubated with tick antigen, produced detectable proliferative responses in responder cells from tick-sensitized guinea-pigs. Antigen-incubated, Ia-bearing macrophages produced similar responses. It is concluded that, as has been shown in man and the guinea-pig with other antigens, Langerhans cells, like Ia-bearing macrophages, can act as antigen-presenting accessory cells in the immunological responses of guinea-pigs to tick infestations.

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INTRODUCTION

Guinea-pigs acquire an immunologically-mediated resistance to ixodid ticks following a primary tick infestation. Resistant animals prevent most ticks from obtaining normal blood meals upon secondary and subsequent infestations (Trager, 1939; Allen, 1973; Wikel & Allen, 1976; Brown & Knapp, 1981; Askenase, Bagnall & Worms, 1982). Acquired resistance can be transferred from resistant donors to normal recipients with viable lymph node cells or serum suggesting that both humoral and cellular factors are involved in resistance, and resistance is associated with cutaneous basophil hypersensitivity reactions to tick antigens (Wikel & Allen, 1976; Brown & Askenase, 1981; Askenase *et al.*, 1982). In addition, the local release of histamine, or other mediators when basophils degranulate in the skin, appears to represent an important part of the tick resistance mechanism (Wikel, 1982; Brown *et al.*, 1982; Paine, Kemp & Allen, 1983).

Wikel, Graham & Allen (1978) examined the specific *in vitro* proliferative responses to tick salivary antigens in lymph node cells from guinea-pigs undergoing primary and secondary tick infestations. Maximal lymphocyte responsiveness was obtained early in the secondary infestations when tick resistance had been acquired. In those experiments, it was assumed that macrophages were acting as antigen-presenting cells. In contact hypersensitivity and other cutaneous immunological reactions, it has been shown that

epidermal Langerhans cells (LC) may also act as antigen-presenting cells (Stingl, 1980) and, recently, it has been proposed that LC may also act as antigen-presenting cells in the immunological reactions associated with tick resistance in guinea-pigs (Nithiuthai & Allen, 1984a). Evidence to support this proposal was, at best, circumstantial. Adenosine triphosphatase (ATPase)-positive epidermal LC populations were shown to be markedly reduced in primary tick infestations and increased early in infestations of resistant guinea-pigs (Nithiuthai & Allen, 1984a). These LC population changes resembled those previously shown to occur during sensitizing and challenge contact hypersensitivity reactions to dinitrochlorobenzene in guinea-pigs (Silberberg, Baer & Rosenthal, 1974, Silberberg-Sinakin, Baer & Rosenthal, 1976; Silberberg-Sinakin, Baer & Thorbecke, 1978).

In the work described here, we have investigated the abilities of LC-containing and LC-depleted epidermal cell populations to present tick salivary antigens to lymphocytes from syngeneic tick-resistant guinea-pigs. The LC-containing and LC-depleted epidermal cell populations were obtained by two different methods, those described by Stingl *et al.* (1978a) and by Braathen & Thorsby (1980). Such epidermal cell populations, or macrophages, were incubated with tick salivary antigens, allowed to react with lymphocytes from syngeneic tick-resistant or control animals, and specific proliferative responses of lymphocytes were assessed by increases in DNA synthesis as determined by [^{3}H]thymidine incorporation.

MATERIALS AND METHODS

Guinea-pigs

Male, Strain 2 guinea-pigs were provided from Elm Hill Breeding Laboratories, Chelmsford, MA. They were at least 350 g weight and were maintained as described by Nithiuthai & Allen (1984a). Each test animal received a 5-day infestation with 100 *Dermacentor andersoni* larvae on each ear. Maintenance of tick larvae and infestations were performed as described by Nithiuthai & Allen (1984a) and single cell suspensions from parotid and cervical lymph nodes were prepared 7 days after infestations ended. Control guinea-pigs received no tick infestations before similar lymph node cell suspensions were prepared from them.

Preparation of lymph node cell suspensions

Parotid and cervical lymph nodes were removed from tick-sensitized or control guinea-pigs. Nodes were immersed in RPMI-1640 (Gibco, Grand Island, NY) culture medium supplemented with 20% heat-inactivated bovine serum and 50 μg/ml gentamycin, trimmed free of fat, teased apart with forceps and forced through a sterile stainless steel wire cloth (type 304, 400 mesh, Small Parts Inc, Miami, FL). Cell suspensions were washed three times with culture medium lacking serum. Contaminating red blood cells were lysed with cold 0·83% NH_4Cl in bicarbonate buffer, pH 7·2, on ice for 2–3 min. This was followed by three washes in culture medium. Such lymph node cell suspensions were used as lymph node cells (non-column purified) in preliminary experiments. In final experiments, column-purified lymph node cells were prepared by passing the cell suspension through EDTA-treated rayon wool columns (Leuko Pak, Fenwal Laboratories, Morton Grove, IL) to remove adherent cells. Cells which passed through the column were washed three times and used as column-purified lymph node cells. Viability of these and other cell preparations was assessed by dye exclusion (0·4% trypan blue in 0·01 M phosphate-buffered saline, pH 7·2). Samples with less than 80% cell viability were discarded.

Preparation of macrophages

Macrophages were prepared from tick-naive guinea-pigs by peritoneal lavage. Twenty-five millilitres of cold RPMI-1640 supplemented with 10 IU/ml preservative-free heparin (Sigma Chemical Co., St Louis, MO) were injected into the peritoneal cavity. The abdomen was massaged for a few minutes and cell suspensions were removed using a sterile 18-gauge needle attached to a 30-ml syringe. Cells were washed three times, resuspended in culture medium and incubated in glass petri-dishes at 37° for 2–5 hr. Non-adherent cells were removed and adherent cells were transferred to siliconized tubes. Contaminating red cells were lysed as before and the remaining cells were used as macrophages. Some aliquots of macrophage suspensions were treated with anti-Ia strain 2 guinea-pig antisera and complement to destroy Ia-bearing macrophages, as described below.

Preparation of epidermal cell suspensions, including Langerhans cells

Two different protocols were used to prepare epidermal cell populations which contained LC. The first

protocol was that described by Stingl *et al.* (1978a), in which LC-enriched populations were prepared from single epidermal cell suspensions by rosetting of the FcIgG receptor-bearing LC. Briefly, skin sections were obtained from tick-naive guinea-pigs using a Davol/Simon (Mississauga, Ont.) dermatome. Epidermal sheets were separated from the underlying dermis following treatment of skin sections with 0·5% trypsin in phosphate-buffered saline, pH 7·2 (PBS), for 12 hr. Single cell suspensions were produced after treatment of epidermal sheets in 0·025% deoxyribonuclease (Sigma Chemical Co.) in RPMI-1640. Cell suspensions were washed three times, resuspended, layered onto Ficoll–Paque (Pharmacia, Dorvall, PQ) and centrifuged at 400 ***g*** at 4° for 30 min. Epidermal cells in the interphase were collected and used as epidermal cells suspensions.

Equal volumes of epidermal cell suspensions (10^6 cells/ml) and 1–2% IgG-coated bovine red cells were mixed, centrifuged at 300 ***g*** for 5 mins, and incubated on ice for 3–5 hr. The concentrations of rosette-forming cells (approximately 2% of the epidermal cell population) were determined. The cells were then resuspended, layered onto Ficoll–Paque and centrifuged as before. Non-rosette-forming cells, the LC-depleted fraction of epidermal cells, were collected from the interphase and washed three times. Such LC-depleted fractions contained less than 1% ATPase-positive or rosetted cells. Cells in the sediments were collected, and the percentage of rosetted cells (14–20%) was determined. Bovine red cells were lysed by incubation with cold 0·83% NH_4Cl in bicarbonate buffer, pH 7·2, for 10 min on ice. The remaining cells were washed three times and used as LC-enriched epidermal cell populations.

In the second protocol, epidermal cell suspensions were prepared as previously described. These suspensions contained small percentages of LC (2–5% on the basis of ATPase activity and 2–3% Ia-positive cells as assayed by indirect immunofluorescence) and were used as LC-containing epidermal cell populations. Langerhans cell-depleted epidermal cell populations were prepared by a method similar to that described by Braathen & Thorsby (1980). Anti-Ia, Strain 2 guinea-pig antiserum was prepared from strain 13 guinea-pigs as described by Geczy *et al.* (1975). Epidermal cell suspensions were incubated with a 1 : 10 dilution of this antiserum and complement for 30 min at 37°. Treated cells were washed three times in RPMI-1640 before use as LC-depleted cell populations.

Preparation of tick salivary gland extract

Salivary gland antigens (SGA) were prepared as described by Wikel, Graham & Allen (1978). Briefly, salivary glands were dissected from female *D. andersoni* ticks which had fed on rabbits for 5 days. They were washed in PBS and ground for 30 min at 4° in a hand tissue grinder. The suspension was centrifuged at 10,000 ***g*** at 4° for 30 min and the supernatant was passed through a 0·45 μm millipore filter. The protein concentration of SGA was determined by the method of Lowry *et al.* (1951).

Procedures used in lymphocyte blastogenesis assays

In all experiments, triplicate cell cultures were set up in flat-bottomed 96-well microculture plates (Flow Laboratories Inc., Mississauga, Ont.) and cultures were maintained in a humidified incubator at 37° under 5% CO_2.

In preliminary experiments, optimal concentrations of responder and stimulator cells, and optimal concentrations and incubation times with SGA and pokeweed mitogen (PWM) were determined. In final experiments, the following volumes of components were used: 100 μl of each cell type ($0{\cdot}5–1 \times 10^6$ cells/ml), 25 μl of SGA (10 μg/μl), PWM (1:10 dilution), culture medium (RPMI-1640 supplemented with 20% fetal bovine serum and 50 μg/ml gentamycin). Cultures were incubated for 5 days, and cells were harvested onto fibre glass filters (Whatman) using an automatic multiple harvester (Bellco, Vineland, NJ). Twenty-four hours prior to harvesting, cells were pulsed with 1 μCi of methyl [^{3}H]thymidine (specific activity 5 mCi/mM, New England Nuclear, Dorval, PQ). The uptake of methyl[^{3}H]thymidine was determined with a Beckman liquid scintillation counter, using a toluene, PPO, POPOP scintillation cocktail.

Two experiments were conducted with LC populations prepared by the first protocol, and two experiments with LC populations prepared by the second protocol.

Proliferative responses were recorded as mean counts per min (c.p.m.) from triplicate samples ± standard deviation. Ratios of responses with lymphocytes from tick-resistant, as opposed to control, guinea-pigs were recorded as follows:

$$\text{E/C} = \frac{\text{mean proliferative response of lymphocytes from tick-resistant animals}}{\text{mean proliferative response of lymphocytes from control animals}}.$$

RESULTS

Proliferative responses of column-purified lymph node cells from control and tick-sensitized guinea-pigs are shown in Tables 1 and 2.

Table 1 includes results with LC obtained by Protocol 1. The LC-enriched cell populations contained 14–20% rosetted cells (LC), and LC-depleted populations contained < 1% rosetted cells. Detectable stimulation of lymphocytes from tick-resistant guinea-pigs (producing E/C values of 3·9 and 4·9 in the two different experiments) was obtained using SGA-treated LC-enriched populations as stimulators. E/C values of 4·5 and 7·8 were obtained with SGA-treated macrophages. No detectable stimulation of lymphocytes was produced using SGA-treated LC-depleted cell populations as stimulators.

Table 2 includes results with LC obtained by Protocol 2. Epidermal cell (EC) populations contained 2–3% LC as identified by indirect immunofluorescence using anti-Ia antibodies, or 2–5% LC as revealed by ATPase activity. Epidermal cells incubated with SGA caused detectable stimulation of lymphocytes from tick-resistant animals (E/C values of 2·2 and 2·3 in the two experiments). Macrophages incubated with SGA also caused detectable lymphocyte stimulation (E/C values of 5·9 and 2·9). Macrophages or epidermal cell populations which had been treated with anti-Ia antiserum and complement prior to antigen treatment caused no detectable lymphocyte proliferation.

Table 1. Proliferative responses (mean c.p.m. ± SD) of column-purified lymph node cells from control and tick-resistant guinea-pigs: two experiments conducted with LC populations prepared by Protocol 1

Added cells and treatments	Lymphocytes from: Control guinea-pigs	Tick-resistant guinea-pigs	E/C*
1. LCE†	864 ± 546	905 ± 148	1·0
LCD‡	989 ± 16	838 ± 243	0·8
MØ§	595 ± 78	615 ± 148	1·0
LCE + SGA¶	634 ± 73	2454 ± 986	3·9
LCD + SGA**	868 ± 224	925 ± 533	1·0
MØ + SGA††	410 ± 111	1865 ± 502	4·5
PWM‡‡	12,891 ± 766	11,695 ± 581	0·9
–	356 ± 117	523 ± 333	1·5
2. LCE	655 ± 63	485 ± 168	0·7
LCD	545 ± 235	504 ± 187	0·9
MØ	524 ± 146	695 ± 62	1·3
LCE + SGA	631 ± 196	3096 ± 865	4·9
LCD + SGA	556 ± 94	758 ± 627	1·4
MØ + SGA	550 ± 134	4282 ± 649	7·8
PWM	15,598 ± 123	12,051 ± 426	0·8
–	311 ± 155	460 ± 233	1·5

$$* \text{E/C} = \frac{\text{mean proliferative response of lymphocytes from tick-resistant guinea-pigs}}{\text{mean proliferative response of lymphocytes from control guinea-pigs}}.$$

† LCE, Langerhans cell-enriched population.
‡ LCD, Langerhans cell-depleted population.
§ MØ, macrophages.
¶ LCE + SGA, LCE incubated with salivary gland antigen.
** LCD + SGA, LCD incubated with salivary gland antigen.
†† MØ + SGA, macrophages incubated with salivary gland antigen.
‡‡ PWM, pokeweed mitogen.

Table 2. Proliferative responses (mean c.p.m. ± SD) of column-purified lymph node cells from control and tick-resistant guinea-pigs: two experiments conducted with LC populations prepared by Protocol 2

Added cells and treatments	Lymphocytes from: Control guinea-pigs	Tick-resistant guinea-pigs	E/C*
1. EC†	986 ± 217	898 ± 330	0·9
EC + SGA‡	955 ± 204	2078 ± 572	2·2
αIa EC + SGA§	947 ± 269	816 ± 347	0·9
MØ¶	642 ± 125	716 ± 262	1·1
MØ + SGA**	693 ± 203	4112 ± 912	5·9
αIa MØ + SGA††	570 ± 311	726 ± 315	1·3
PWM‡‡	19,942 ± 1624	13,563 ± 3113	0·7
–	360 ± 117	417 ± 131	1·2
2. EC	529 ± 104	587 ± 152	1·1
EC + SGA	898 ± 203	2078 ± 380	2·3
αIa EC + SGA	473 ± 76	360 ± 104	0·8
MØ	489 ± 121	379 ± 89	0·8
MØ + SGA	728 ± 219	2116 ± 513	2·9
αIa MØ + SGA	437 ± 210	442 ± 116	1·0
PWM	42,037 ± 1909	37,023 ± 3071	0·9
–	412 ± 133	439 ± 97	1·1

$$* \text{E/C} = \frac{\text{mean proliferative response of lymphocytes from tick-resistant guinea-pigs}}{\text{mean proliferative response of lymphocytes from control guinea-pigs}}.$$

† EC, epidermal cells (including LC).
‡ EC + SGA, epidermal cells incubated with salivary gland antigen.
§ αIa EC + SGA, anti-Ia treated epidermal cells incubated with salivary gland antigen.
¶ MØ, macrophages.
** MØ + SGA, macrophages incubated with salivary gland antigen.
†† αIa MØ + SGA, anti-Ia treated macrophages incubated with salivary gland antigen.
‡‡ PWM, Pokeweed mitogen.

DISCUSSION

Langerhans cells are the only epidermal cells of guinea-pigs known to possess both Fc-receptors and Ia antigens on their surface membranes (Stingl *et al.*, 1977, 1978b). In our experiments, LC-containing and LC-depleted epidermal cell populations were produced by two different protocols, one making use of Fc-receptors, the other making use of Ia antigens. Cell populations containing LC, when incubated *in vitro* with tick salivary antigens, caused detectable proliferative responses in column-purified lymphocytes from syngeneic tick-resistant guinea-pigs. Antigen-treated macrophages induced similar responses.

In the first experiment, LC-enriched and LC-depleted epidermal cell fractions were produced by the method of Stingl *et al.* (1978a), separating Fc-rosetting epidermal cells on density gradients. The LC-enriched fractions contained 14–20% rosetted cells, and, when incubated with antigen and lymphocytes, induced E/C values of 3·9 and 4·9 in replicate trials. The LC-depleted fractions, which contained < 1% rosetted cells, produced no such detectable proliferative responses in lymphocytes from resistant animals. However, antigen-treated macrophages induced E/C values of 4·5 and 7·8 (Table 1). These responses were similar to those reported by Wikel *et al.* (1978) who used similar concentrations of salivary gland antigen, together with non-column purified lymph node cell populations from resistant guinea-pigs. It is presumed that, in the

experiments of Wikel *et al.* (1978), the lymph node cell populations contained both lymphocytes and macrophages.

In the second experiment, detectable proliferative responses were obtained with antigen-treated macrophages or LC-containing epidermal cell populations; the E/C values obtained were 2·9 and 5·9 with macrophages and 2·2 and 2·3 with epidermal cells. No such responses were obtained when macrophages or epidermal cells had received prior treatment with anti-Ia antiserum and complement (Table 2). The lower E/C values associated with LC-containing populations in this experiment might be explained in terms of the lower percentages of LC present in these cultures (2–3%, as assayed by indirect immunofluorescence with anti-Ia antiserum, or 2–5% as assayed by ATPase activity). Results from this experiment were similar to those reported in human cell cultures by Braathen (1980), Braathen & Thorsby (1980) and Braathen *et al.* (1980) who demonstrated that Ia-positive epidermal cells (LC) could present antigens or allergens of PPD, herpes simplex virus and nickel sulphate to sensitized purified autologous lymphocytes, inducing specific proliferative responses.

On the basis of changes demonstrated to occur in epidermal LC populations during primary and secondary tick infestations of guinea-pigs, Nithiuthai & Allen (1984a) speculated that epidermal LC might act as accessory cells, presenting tick salivary gland antigens to appropriate lymphocytes in tick-resistance responses. Direct evidence of tick salivary antigen presentation by LC to lymphocytes from tick-sensitized guinea pigs is provided here.

In previous work, it has been shown that salivary gland antigens of *D. andersoni* ticks were present within the epidermis of infested guinea-pigs, and that these antigens were trapped by dendritic cells resembling LC (Allen, Khalil & Wikel, 1979). Also, Nithiuthai & Allen (1984b), in experiments with ultraviolet (u.v.C) irradiation of guinea-pig skin, found that irradiated skin showed very marked reductions in ATPase-positive LC and, when ticks infested irradiated skin, both the acquisition and expression of tick resistance were also significantly reduced. Since LC have now been shown to be capable of presenting tick salivary antigens to lymphocytes from tick-sensitized guinea-pigs, it is possible that such antigen by LC may be of importance in both the afferent and efferent arms of the tick resistance response. As previously suggested (Nithiuthai & Allen, 1984b), in the efferent arm of the response, it is possible that epidermal LC in resistant animals may act as antigen-laden target cells which represent early foci of cell-mediated immune reactions in the epidermis, which lead to the development of the basophil-packed epidermal vesicles which are the typical reactions of challenged tick-resistant guinea-pigs (Allen, 1973).

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