

## Significant changes in epidermal Langerhans cells of guinea-pigs infested with ticks (*Dermacentor andersoni*)

S. NITHIUTHAI & J. R. ALLEN *Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada*

*Accepted for publication 12 August 1983*

**Summary.** Resistance to tick feeding has previously been shown to be an acquired, immunologically mediated phenomenon in guinea-pigs, associated with cutaneous basophil hypersensitivity to tick antigens.

In this study, Langerhans cells (LC) in the epidermis of guinea-pigs were monitored during tick infestations of susceptible and resistant animals. A specific adenosine triphosphatase (ATPase) staining technique was used to identify epidermal LC. The numbers of LC decreased significantly around the sites of tick attachment during primary infestations. Early in the secondary infestations, increases in numbers of LC were observed in the epidermis surrounding tick mouthparts.

These changes in LC populations resemble those previously described in contact dermatitis reactions of guinea-pigs.

### INTRODUCTION

*Dermacentor andersoni*, the Rocky Mountain Wood tick, is a slow feeding ixodid tick attaching to the skin of its host for 5–12 days before becoming engorged (Balashov, 1972). In certain hosts, resistance is developed to subsequent tick infestations. Resistance to

ticks is acquired in guinea-pigs once the animals have been sensitized with a certain number of tick larvae (Wikel, 1982). The immune hosts express resistance by preventing ticks from obtaining a normal blood meal. Relatively few ticks reach engorgement and the dry weight of fed ticks is decreased. In guinea-pigs, acquired tick resistance can be transferred from sensitized donors to naive syngeneic recipients either by lymphoid cells or by serum (Wikel, 1982; Brown & Askenase, 1981).

Histological studies of tick attachment sites show different skin reactions in susceptible and resistant animals. Resistant animals exhibited a marked infiltration of basophils in infested skin; eosinophils were also increased in number but they were less numerous than basophils (Allen, 1973; Brown & Knapp, 1981). Brown *et al.* (1982) demonstrated that introducing antibasophil serum to *Amblyomma americanum*-sensitized guinea-pigs abolished their tick resistance. Mechanisms leading to the cutaneous basophil hypersensitivity reaction of the immune host still remain unclear. Allen, Khalil & Wikel (1979) using indirect immunofluorescence techniques, showed evidence of dendritic epidermal Langerhans cells (LC) trapping salivary gland antigens during a secondary tick infestation with *D. andersoni* larvae.

LC are dendritic cells found in the epidermis and other stratified squamous epithelia, and also in lymphoid tissues of mammals. They have recently been found to play important roles in immunological

Correspondence: Dr John R. Allen, Dept. of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0.

responses of the skin (Stingl, 1980). These cells possess immunological markers and receptors similar to those found on Ia-bearing macrophages. They bear Fc and C3 receptors and express Ia histocompatibility antigens on their surface membranes. These cells are able to mimic the function of Ia-bearing macrophages in elicitation of antigen specific and allogeneic T lymphocyte proliferation *in vitro*. Histological and ultrastructural studies have revealed the active participation of LC in allergic contact hypersensitivity reactions. In guinea-pigs, Silberberg, Baer & Rosenthal (1974), Silberberg-Sinakin, Baer & Rosenthal (1976) and Silberberg-Sinakin, Baer & Thorbecke (1978) by electron microscopy showed responses of epidermal LC to dinitrochlorobenzene (DNCB) allergic contact hypersensitivity. A slight reduction of epidermal LC numbers with diffuse cell damage occurred during contact sensitization. In contrast, after being challenged, sensitized animals showed increased numbers of LC in the epidermis as well as in the dermis and dermal vessels.

In experiments reported here, the LC populations in the epidermis of guinea-pigs were examined during primary and secondary tick infestations, making use of the established fact that LC are the only dendritic cells in the normal epidermis of guinea-pigs that exhibit formalin resistant, sulphhydryl-dependent adenosine triphosphatase (ATPase) activity (Juhlin & Shelley, 1977).

## MATERIALS AND METHODS

### *Animals*

Thirty-six male albino Hartley outbred guinea-pigs, 350–500 g in weight, were supplied by Canadian Breeding Labs Ltd., Montreal. They were fed on a pellet diet and water supplemented with ascorbic acid (10–15 mg/animal/day). The animals were housed individually in metal cages.

### *Ticks and infestations*

One-month-old *Derma-centor andersoni* larvae used in the experiments were cultured in the laboratory. Adults were originally collected from the field in Saskatchewan. The larvae for each experiment came from the same egg mass in order to reduce variation in their feeding behaviour. They were maintained at 90% relative humidity over saturated potassium chloride solution at 24°.

Each guinea-pig was subjected to tick infestations

with 100 *D. andersoni* larvae, receiving a primary 5-day infestation on the right ear followed by 7 days free of ticks and a secondary 5-day infestation on the left ear. Ticks were confined to the ears within plastic capsules (Wikel & Allen, 1976). Grooming was prevented by placing plastic collars around the guinea-pigs' necks. Non-attached larvae were removed 6 hr after introducing them to the ears.

### *Skin biopsies*

Animals were killed and 5 mm punch biopsies which encompassed tick attachment sites were taken from the ears on day 1, 3 and 5 of the infestations. Biopsies were kept on ice before being processed the same day through histological and enzyme histochemical techniques.

### *Histology*

Standard haematoxylin and eosin (H & E), Giemsa, and toluidine blue staining techniques were used. Skin biopsies were fixed in 10% neutral formalin buffer for at least 24 hr. After dehydration with alcohol, the samples were embedded in paraplast and were cut at 5  $\mu$ m thickness.

### *Enzyme histochemistry*

Epidermal sheets were separated from the underlying dermis after incubating the biopsies in EDTA mixture (Juhlin & Shelley, 1977) at 37° for 2 hr. The sheets were rinsed in 0.85% saline solution for 20 min.

Fresh cryostat sections of 20  $\mu$ m thickness were cut serially from the biopsied skin, placed on 22 mm<sup>2</sup> glass coverslips, and air dried for 20 min.

Both epidermal sheets and cryostat sections were then fixed and stained for adenosine triphosphatase (ATPase) activity using a slight modification of the technique of Juhlin & Shelley (1977). The specimens were fixed in cold cacodylate-formalin buffer at 4° for 6–24 hr and then rinsed in 0.85% saline solution at room temperature. After being incubated in freshly prepared ATP-lead nitrate solution at 37° for 2 hr, the specimens were rinsed and immersed in 0.5% V/V ammonium sulphide solution at room temperature for 20 min. After rinsing in saline and distilled water to remove excess precipitated lead sulphide, the stained specimens were then mounted on glass slides with Berlese mounting medium (Humason, 1979).

### *Assays of ATPase-positive epidermal LC*

The density of ATPase-positive dendritic cell populations within each epidermal sheet was determined

using a light microscope at a magnification of  $\times 1000$ . In each specimen, the ATPase-positive LC were counted in five to 10 fields 0.2 mm in diameter, with the site of tick attachment in the centre. The cell populations were then expressed as mean numbers of cells ( $\pm$  SEM) per  $\text{mm}^2$  of skin surface.

The stained sections were examined, and changes of distribution, location and morphology of ATPase-positive LC were recorded.

#### Statistical analysis

Significant differences in the mean density of ATPase-positive LC population of control and experimental animals were assessed using Student's *t*-test.

## RESULTS

### General observations

The ears of guinea-pigs infested with 100 *D. andersoni* larvae showed no obvious reactions macroscopically during the first infestation. Grossly visible inflammatory reactions were evident during the second infestation. A mild inflammatory reaction at the tick attachment sites was found on the first day after challenging the animals with 100 tick larvae. The reaction reached a maximum between day 3 and 5 of the secondary infestation. Once the larvae were removed the reactions subsided. The percentages of larvae engorged in primary and secondary tick infestations were  $82.17 \pm 4.87$  and  $11.92 \pm 3.26$  (mean  $\pm$  SEM) respectively.

### Histology

Sections stained with H & E showed no detectable changes in the epidermis of nonsensitized animals around the sites of tick attachment. In the lower dermis, slight infiltrations of mononuclear cells were visible on day 3 and both mononuclear and polymorphonuclear cell infiltrations were evident on day 5. In skin sections from the secondary infestation, the epidermis showed marked changes by day 3. Intraepidermal vesicles, primarily infiltrated by basophils, had developed beneath the attached larvae. Many clear cells were prominent in the epidermis.

### Enzyme histochemistry

By the ATPase staining technique, epidermal LC from normal control animals were seen clearly and regularly

**Table 1.** Mean number of ATPase-positive LC ( $\pm$  SEM) during primary tick infestation with *D. andersoni* larvae

Group of Animals	LC/ $\text{mm}^2$ ( $\pm$ SEM)	P value (Student's <i>t</i> -test)
Control	1488 $\pm$ 5.82	—
1° day 1	1172 $\pm$ 27.15	*
1° day 3	416 $\pm$ 8.32	***
1° day 5	424 $\pm$ 11.15	***

\*  $P < 0.05$ .

\*\*\*  $P < 0.001$ .

in the suprabasal layer with extensions of the dendritic processes in all directions, reaching to basal and spinous layers of the epidermis (Fig. 1A). The epidermal sheets exhibited a regularly spaced distribution of ATPase-positive LC (Fig. 1B). Their dendritic processes radiated evenly from a central body. Mean numbers ( $\pm$  SEM) of the LC per  $\text{mm}^2$  were  $1448 \pm 5.82$  (Table 1) and  $1358 \pm 3.34$  (Table 2).

Changes in the numbers and morphology of ATPase-positive LC occurred at the sites of tick attachment during both primary and secondary infestations. In primary infestations, a significant decrease in the number of LC per  $\text{mm}^2$  was evident in areas surrounding attachment sites. The ATPase-positive LC populations, estimated from the density of cells in the epidermal sheets from control animals and those undergoing primary tick infestations are summarized in Table 1. One day after tick attachment (Fig. 2A), a slight but significant reduction ( $P < 0.02$ ) of LC population was detected. By the third day of tick feeding, the number of ATPase-positive LC had decreased to approximately 35% of the normal population. Areas

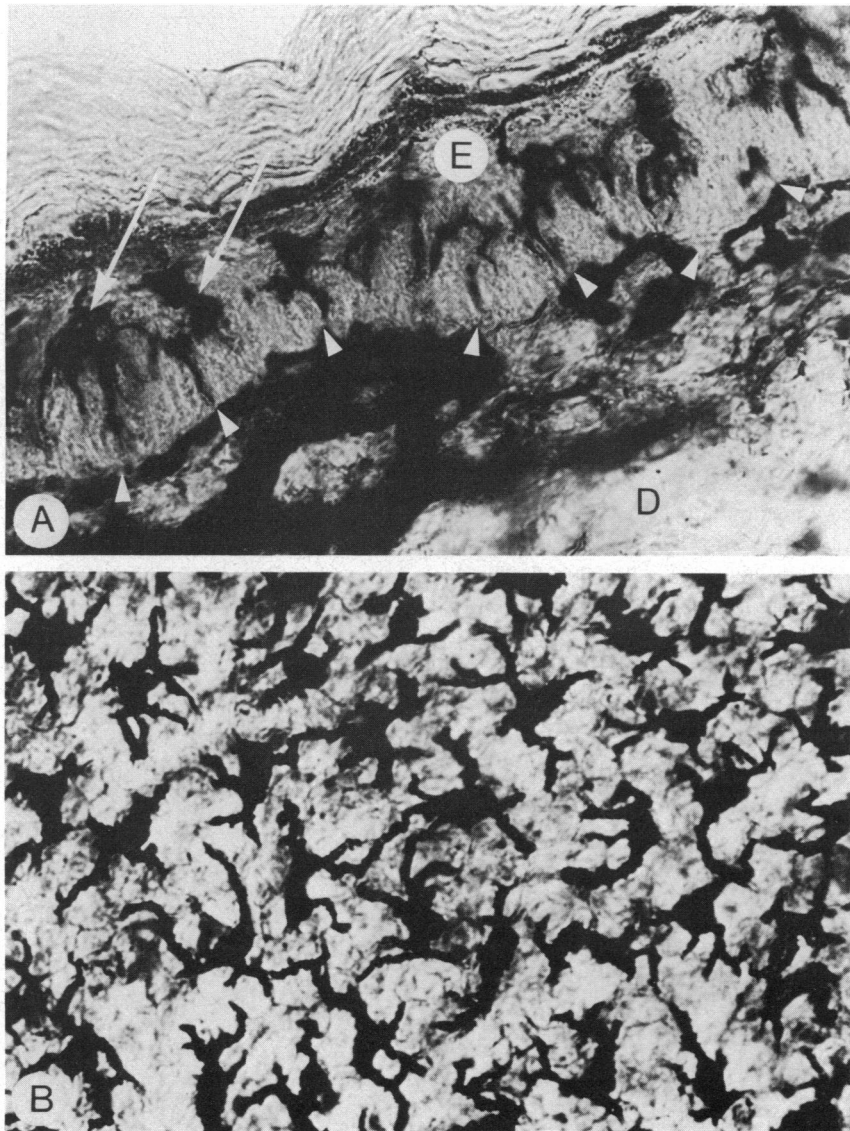
**Table 2.** Mean number of LC ( $\pm$  SEM) at tick attachment sites during secondary *D. andersoni* larval infestation

Group of Animal	LC/ $\text{m}^2$ ( $\pm$ SEM)	P value (Student's <i>t</i> -test)
Control	1358 $\pm$ 3.34	
2° day 1	1644 $\pm$ 25.22	$P < 0.01$
2° day 3	*	
2° day 5	*	

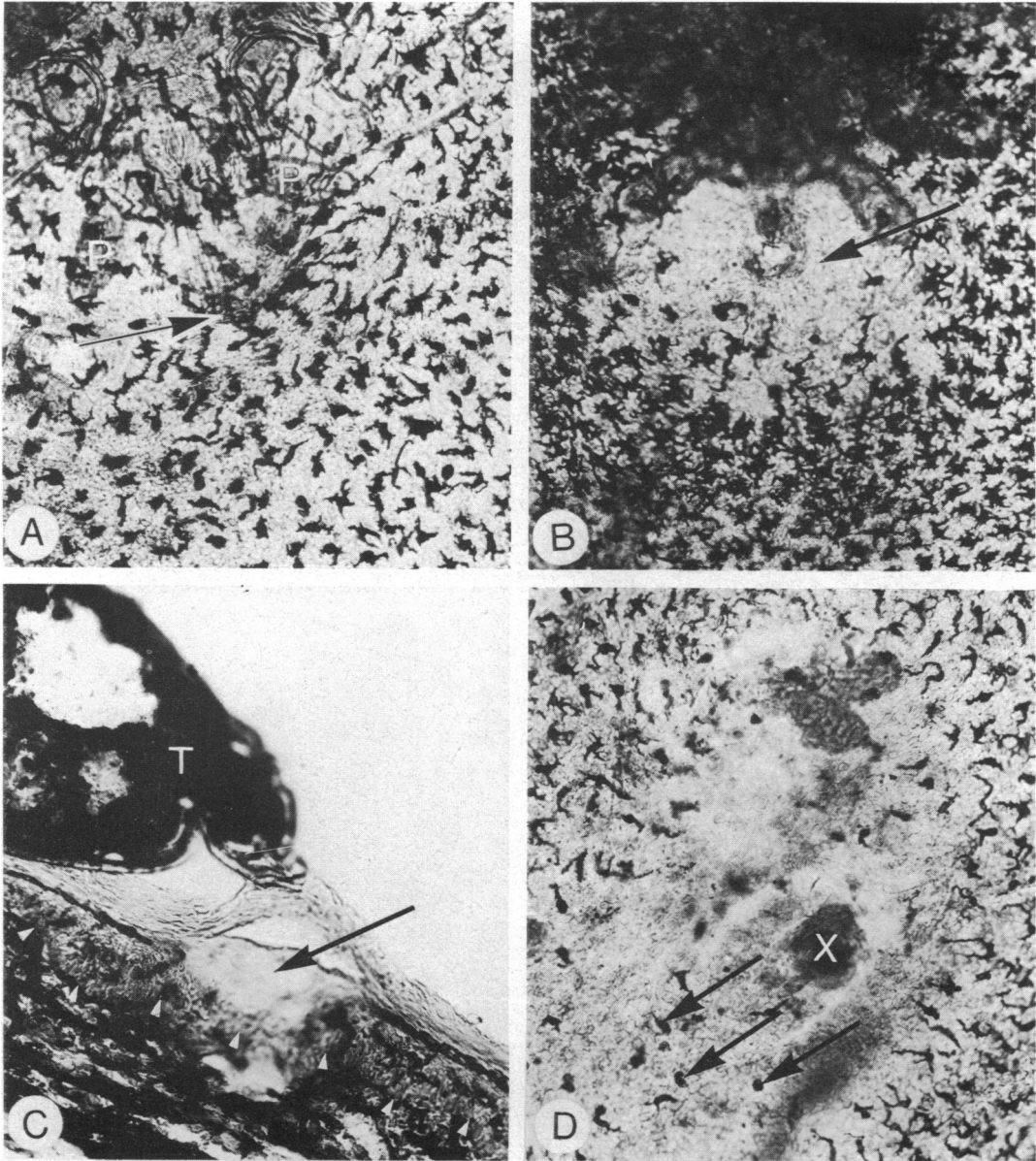
\* Due to the presence of other ATPase-positive cells, numbers of LC could not be reliably estimated.

lacking ATPase-positive cells were observed adjacent to the attachment site of ticks (Fig. 2B, C). A few of the ATPase-positive cells remaining in some of these sites showed abnormal morphology. The morphology and density of LC in the epidermis was normal in appearance in areas free of tick attachments. At the end of the 5-day primary tick infestation, the density of ATPase-

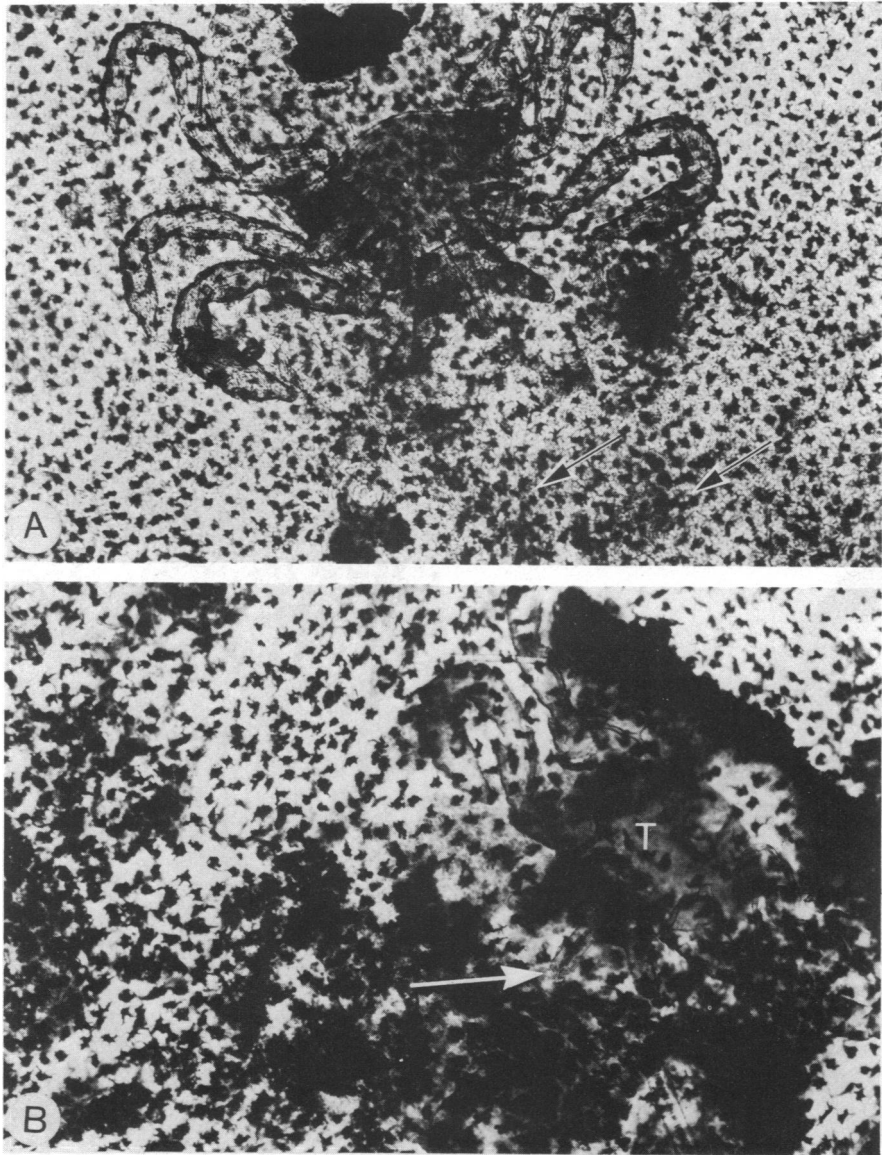
positive LC around attachment sites was significantly decreased ( $P < 0.001$ ). Morphological changes of LC were obvious, the dendritic processes were shortened and fewer in number (Fig. 2D). Many of the LC became round or oval. The cell size appeared to be smaller than normal, and the ATPase-staining became somewhat uneven. The spacing of the LC population



**Figure 1.** Distribution and location of epidermal ATPase-positive Langerhans cells (LC) from normal guinea-pigs (magnification  $\times 708$ ). (A) Transverse section: E = epidermis; D = dermis; *triangles* show dermoepidermal junction; *arrows* show ATPase-positive LC. (B) Epidermal sheet.



**Figure 2.** Epidermal ATPase-positive Langerhans cells (LC) at tick attachment sites during primary infestations. (A) Epidermal sheet (day 1): tip of mouth part (*arrow*); sensory palp (P). (B) Epidermal sheet (day 3): area lacking ATPase-positive LC (*arrow*). (C) Transverse section (day 3): area lacking ATPase-positive LC (*arrow*); dermo-epidermal junction delineated by *triangles*; section of attached tick (T). (D) Epidermal sheet (day 5): site where tick was attached (X); ATPase-positive LC showing abnormal morphology (*arrows*). (All magnifications  $\times 148$ .)



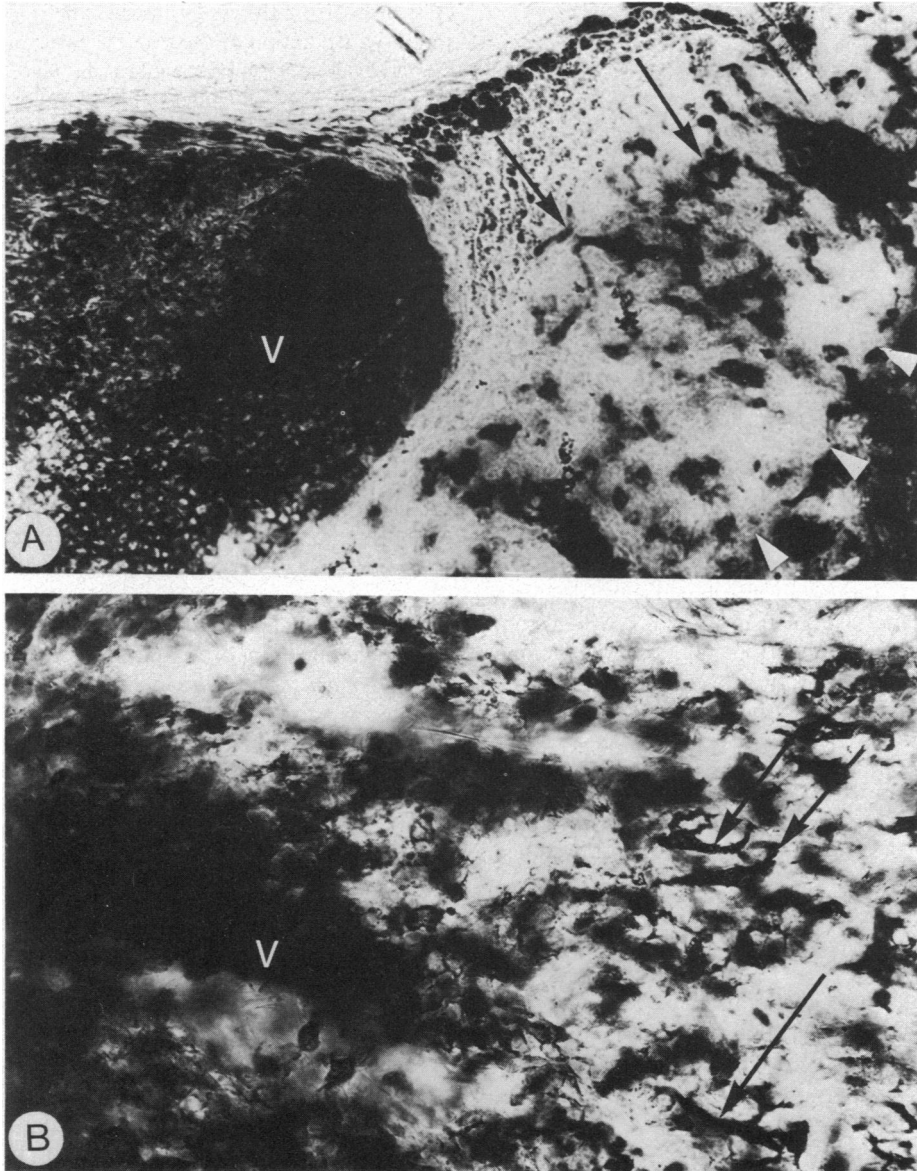
**Figure 3.** ATPase-positive Langerhans cells (LC) at tick attachment sites during secondary infestations (epidermal sheet). (A) Day 1 (magnification  $\times 108$ ): area showing increased populations of ATPase-positive LC (arrows). (B) Day 3 (magnification  $\times 125$ ): accumulation of ATPase-positive cells (LC and other infiltrating cells); tick (T); tip of mouth part (arrow).



was not uniform. The numbers of cells recorded in Table 1 include all ATPase-positive LC, including those with abnormal morphology.

During the second infestation, epidermal hyperplasia was evident at the sites of tick attachment. One day after challenge with ticks, the dendritic ATPase-positive LC population had significantly increased

close to the tick attachment sites (Fig. 3A, Table 2). In epidermal sheets, a heavy stain of ATPase activity was evident by day 3 and 5 of the infestation. Because of the presence of epidermal vesicles and the infiltration of other ATPase-positive cells from the dermis into the epidermis, the ATPase-positive cells present could no longer be identified positively as epidermal LC and



**Figure 4.** Guinea-pig skin at the sites of tick attachment at day 5 of secondary infestations showing ATPase-positive LC (magnification  $\times 500$ ). (A) Transverse section: LC (arrows); intraepidermal vesicle (V); dermo-epidermal junction (triangles). (B) Epidermal sheet: LC (arrows); vesicle (V).

enumeration of LC became impossible in the epidermal sheets (Fig. 3B). Transverse sections of skin, however, showed the continued presence of epidermal dendritic ATPase-positive LC in the suprabasal layer adjacent to the intraepidermal vesicles which developed later in the secondary infestation (Fig. 4). In the vesicles, some ATPase-positive dendritic cells were also evident.

## DISCUSSION

Tick resistance has previously been shown to be acquired by guinea-pigs (Allen, 1973). The acquired resistance was associated with a cutaneous hypersensitivity reaction with marked accumulation of basophils at tick attachment sites of the resistant animals. Our results confirmed these observations.

LC have been shown to be the only cells in the normal epidermis of guinea-pigs possessing ATPase-positive cell membrane reactivity (Juhlin & Shelley, 1977). Our observations showed that the population of LC of normal guinea-pigs' ears occurred in a uniformly distributed pattern with mean numbers of 1448 and 1358 cells/mm<sup>2</sup> (Tables 1, 2). Other investigators (Woolff & Winkelmann, 1967; Bergstresser, Fletcher & Streilein, 1980; Billingham & Medawar, 1953) demonstrated that in guinea-pigs' ears the epidermal LC were evenly distributed with a density of approximately 1000 cells/mm<sup>2</sup>. Wolff & Winkelmann (1967) showed a uniform population of epidermal ATPase-positive LC with an average of  $974 \pm 68$  and  $941 \pm 85$  cells/mm<sup>2</sup> from red and albino guinea-pigs' ears respectively. Recently, Bergstresser *et al.* (1980) used gold chloride and ATPase staining techniques to enumerate epidermal LC of one guinea-pig's ear. The densities of LC were 970 and 990 cells/mm<sup>2</sup> respectively. It is possible that the higher LC density found by us may be due to the use of a modified technique providing better resolution, or to differences in strain, age, sex, number and skin site of the animals used by other authors.

In our study of tick infestations of guinea-pigs' ear skin, the distribution and morphological changes of ATPase-positive epidermal LC population were remarkable. A significant reduction of LC number/mm<sup>2</sup> at tick attachment sites was evident during primary tick infestation. It should be noted that the numbers of cells counted in primary infestations included all ATPase-positive cells, whether or not they exhibited abnormal LC morphology. Thus the reduc-

tion in numbers of normal LC may have been much greater than indicated in Table 1. It is not known whether the reduction of LC population during primary tick infestations was due to loss of the cells from the epidermis or to changes in cell membrane properties reflected by the loss of their ATPase staining reactions. Aberer *et al.* (1981), proposed that failure to detect ATPase-positive LC was not necessarily due to loss of LC from the epidermis. In experiments involving u.v. irradiation of the skin of man and mice they found evidence of ultrastructural damage and loss of ATPase activity in epidermal LC remaining in the epidermis after u.v. irradiation of the skin. In mice, Bergstresser *et al.* (1980) showed that a significant reduction in the numbers of ATPase positive LC occurred within 12 hr after primary topical application of dinitrofluorobenzene (DNFB), whereas the number of LC increased after sensitized animals were challenged with DNFB.

In sensitized guinea-pigs, during the secondary tick infestations, the numbers of epidermal LC at tick infested sites significantly increased (Table 2). These results are similar to those found in DNFB and DNCB contact hypersensitivity reactions in mice and guinea-pigs respectively (Bergstresser *et al.* 1980; Silberberg-Sinakin *et al.* 1978).

Other studies have shown that epidermal LC may be affected by tick infestations. Schleger & Bean (1973) described a marked reduction in the number of epidermal alkaline phosphatase-positive dendritic cells in the epidermis of tick-infested cattle 24 hr after a larval *Boophilus microplus* infestation. Alkaline phosphatase-positive cells in bovine epidermis have recently been proved to be LC (Khalil, Nithiuthai & Allen, 1982). In an experiment which was designed to locate tick salivary gland antigens by indirect immunofluorescence in the skin of guinea-pigs infested with *D. andersoni* larvae, Allen *et al.* (1979) demonstrated the retention of such antigens in cells resembling LC in secondary but not primary tick infestations. Possibly the inability to show this in primary tick infestations was a reflection of the decreased ATPase-positive LC population which we have shown to occur at that time.

In conclusion, we have shown, in this study of the epidermis of tick infested guinea-pigs, significant changes in the ATPase-positive LC populations of naive and tick-resistant animals. These changes are similar to those shown to occur in response to sensitizing and challenge applications of DNCB or DNFB in contact hypersensitivity reactions. It



appears possible that in tick resistance, epidermal LC may play roles similar to those proposed in contact dermatitis. In tick-resistant guinea-pigs, basophil leucocytes appear to play a key effector role, infiltrating the skin close to attached ticks and degranulating there. One might speculate that LC trap tick salivary antigens and present them to appropriate lymphocytes during primary, sensitizing infestations. In sensitized animals they could act as antigen-laden target cells in the epidermis, providing an early focus for humoral or cellular immune reactions as they are suggested to do in contact hypersensitivity (Silberberg-Sinakin *et al.*, 1978; Stingl, 1980). Following these reactions, factors chemotactic for basophils such as those described by Ward *et al.* (1975) could cause the accumulation of these effector cells at the tick attachment sites.

Further studies on the effects of u.v. irradiation on the epidermal LC population in guinea-pigs, and on the acquisition and expression of tick resistance by the animals, will be reported subsequently.

#### ACKNOWLEDGMENTS

This work was funded in part by grant MA5315 from the Medical Research Council of Canada.

#### REFERENCES

- ABERER W., SCHULER G., STINGL G., HONIGSMANN H. & WOLFF K. (1981) Ultraviolet light depletes surface markers of Langerhans cells. *J. invest. Dermatol.* **76**, 202.
- ALLEN J.R. (1973) Tick resistance: Basophils in skin reactions of resistant guinea pigs. *Int. J. Parasitol.* **3**, 195.
- ALLEN J.R., KHALIL H.M. & WIKEL S.K. (1979) Langerhans cells trap tick salivary gland antigen in tick-resistant guinea pigs. *J. Immunol.* **122**, 563.
- BALASHOV Y.S. (1972) Blood-sucking ticks (Ixodoidea)—vectors of disease of man and animals. *Misc. Publ. Entomol. Soc. Am.* **8**, 161.
- BERGSTRESSER P.R., FLETCHER C.R. & STREILEIN J.W. (1980) Surface densities of Langerhans cells in relation to rodent epidermal sites with special immunologic properties. *J. invest. Dermatol.* **74**, 77.
- BILLINGHAM R.E. & MEDAWAR P.B. (1953) A study of the branched cells of the mammalian epidermis with special reference to the fate of their division products. *Trans. Roy. Soc. Lond. Series B*, **237**, 151.
- BROWN S.J. & ASKENASE P.W. (1981) Cutaneous basophil responses and immune resistance of guinea pigs to ticks: Passive transfer with peritoneal exudate or serum. *J. Immunol.* **127**, 2163.
- BROWN S.J., GALLI S.J., GLEICH G.J. & ASKENASE P.W. (1982) Ablation of immunity to *Amblyomma americanum* by antibasophil serum: cooperation between basophils and eosinophils in expression of immunity to ectoparasites (ticks) in guinea pigs. *J. Immunol.* **129**, 790.
- BROWN S.J. & KNAPP F.W. (1981) Response of hypersensitized guinea pigs to the feeding of *Amblyomma americanum* tick. *Parasitol.* **83**, 213.
- HUMASON G.L. (1979) *Animal tissue techniques*, 4th edn, p. 106. W. H. Freeman and Company.
- JUHLIN L. & SHELLEY W.B. (1977) New staining techniques for the Langerhans cells. *Acta Dermatol. Venerol.* **57**, 289.
- KHALIL H.M., NITHIUTHAI S. & ALLEN J.R. (1982) Alkaline phosphatase-positive Langerhans cells in the epidermis of cattle. *J. invest. Dermatol.* **79**, 47.
- SCHLEGER A.V. & BEAN K.G. (1973) The melanocyte system of cattle skin. II. Melanotic melanocytes of epidermis and dermis. *Aust. J. Biol. Sci.* **26**, 985.
- SILBERBERG I., BAER R.L. & ROSENTHAL S.A. (1974) The role of Langerhans cells in contact allergy. I. An ultrastructural study in actively induced contact dermatitis in guinea pigs. *Acta Dermatovener. (Stockholm)*, **54**, 321.
- SILBERBERG-SINAKIN I., BAER R.L. & ROSENTHAL S.A. (1976) The role of Langerhans cells in allergic contact hypersensitivity: a review of findings in man and in guinea pigs. *J. invest. Dermatol.* **66**, 210.
- SILBERBERG-SINAKIN I., BAER R.L. & THORBECKE G.J. (1978) Langerhans cells: a review of their nature with emphasis on their immunologic functions. *Prog. Allergy*, **24**, 268.
- STINGL G. (1980) New aspects of Langerhans' cell function: review. *Int. J. Dermatol.* **19**, 189.
- WARD P.A., DVORAK H.F., COHEN S., YOSHIDA T., DATA R. & SELVAGGIO S.S. (1975) Chemotaxis of basophils by lymphocyte-dependent and lymphocyte-independent mechanisms. *J. Immunol.* **144**, 1523.
- WIKEL S.K. (1982). Immune responses to arthropods and their products. *Ann. Rev. Entomol.* **27**, 21.
- WIKEL S.K. & ALLEN J.R. (1976) Acquired tick resistance to ticks. I. Passive transfer of resistance. *Immunology*, **30**, 311.
- WOLFF K. & WINKELMANN R.K. (1967) The quantitative studies on the Langerhans cell population of guinea pig epidermis. *J. invest. Dermatol.* **48**, 504.