

THE ROLE OF LANGERHANS CELLS IN CONTACT ALLERGY

I. An Ultrastructural Study in Actively Induced Contact Dermatitis in Guinea Pigs¹

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Abstract. Guinea pigs were sensitized to 2,4 dinitro-1-chlorobenzene (DNCB) and biopsies of skin-reaction sites to DNCB were studied by electron microscopy. Apposition of mononuclear cells to Langerhans cells in the epidermis was the striking change seen and was noted as early as 3 to 5 hours after application of 0.3% DNCB in olive oil. Apposition of mononuclear cells to Langerhans cells was not seen in the epidermis in primary irritant reactions or clinically negative skin tests to DNCB. These findings in guinea pigs are similar to those previously reported by us at sites of contact-allergic reactions in man, and represent an animal model for further study of the role of Langerhans cells in contact allergic reactions.

In our previous ultrastructural studies in man (12, 13, 14) we showed the features of early apposition of mononuclear cells to Langerhans cells² and subsequent damage to some Langerhans cells³ in contact allergic reactions. No such changes were seen in contact irritant reactions. To determine whether this apposition is unique for man or whether it is also found in other species, we studied contact-allergic and primary irritant reactions to 2, 4 dinitro-1-chlorobenzene (DNCB) in guinea pigs.

MATERIALS AND METHODS

Hartley strain, male guinea pigs, randomly bred and weighing between 450 and 600 g were used. Their diet consisted of Wayne guinea pig diet (Allied Mills, Inc., Chicago, Ill.), fresh greens twice weekly and water *ad lib*.

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² The term "apposition of mononuclear cells to Langerhans cells" is used whenever some areas of the membranes of these cells appear to touch.

³ The term Langerhans cell refers to any cell, irrespective of location, containing Langerhans cell granules and lacking features typical of keratinocytes or melanocytes.

Sensitization to DNCB, recrystallized from hot alcohol, was induced in the following ways: in 3 animals by the technique of Maguire & Chase (8); in 4 animals by topical application to the clipped nuchal skin of 2% DNCB in ethanol daily for 5 consecutive days and in 3 animals by injection of 180 µg of DNFB in complete Freund's adjuvant into the hind footpads.

Sensitization to 3-pentadecylcatechol (PDC) and citraconic anhydride (CA) was induced by using techniques similar to those of Maguire & Chase (8). Challenge skin-tests for active sensitization were done on day 34 with DNCB 0.3% in olive oil, PDC 1.0% in olive oil, or CA 20% in equal parts of olive oil and butyl cellosolve. Skin-test reactions were graded as previously reported (2). Only those guinea pigs having test reactions graded as 2+ to 3+ were used.

Experimental groups

Four groups of guinea pigs were studied. The first consisted of 10 guinea pigs which were sensitized to DNCB and challenged with 0.3% DNCB in olive oil. The second group consisted of 6 non-sensitized guinea pigs which were also challenged with 0.3% DNCB in olive oil. The third group consisted of 10 non-sensitized guinea pigs which were challenged with the primary irritant concentration of 5% DNCB in olive oil. Only those guinea pigs having primary irritant reactions graded as 2+ to 3+ were used. The fourth group consisted of 3 guinea pigs which were sensitized with structurally unrelated allergens (one to PDC and two to CA). These 3 animals were challenged with 0.3% DNCB in olive oil. All animals had biopsies taken prior to the challenge application of DNCB and at 0, 3, 5, 6, 19, 24 and 48 hours after application of DNCB. In addition, a few animals had biopsies taken 12 hours after application of DNCB. The biopsies were prepared for electron microscopy as previously described (12). In an attempt to obtain quantitative data, mononuclear cells and Langerhans cells were counted in six areas each approximately 0.1 mm², in the epidermis of each specimen. These areas were randomly selected from six different blocks of every skin biopsy obtained.

A. Normal guinea pig skin

No apposition of mononuclear cells to Langerhans cells was seen either in the epidermis or dermis.



Fig. 1. Contact-allergic reaction at 3 hours. A mononuclear cell (M) is apposed to a Langerhans cell (L) in the epidermis.

One Langerhans cell granule is seen at the arrow. Stained with uranyl acetate and lead citrate, $\times 17\,000$.

B. After sensitization to DNCB, PDC or CA but prior to challenge with DNCB

Apposition of mononuclear cells to Langerhans cells was not seen in the epidermis. However, in those
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animals which had been sensitized with the use of Freund's adjuvant, mononuclear cells apposed to Langerhans cells were rarely seen in the dermis.

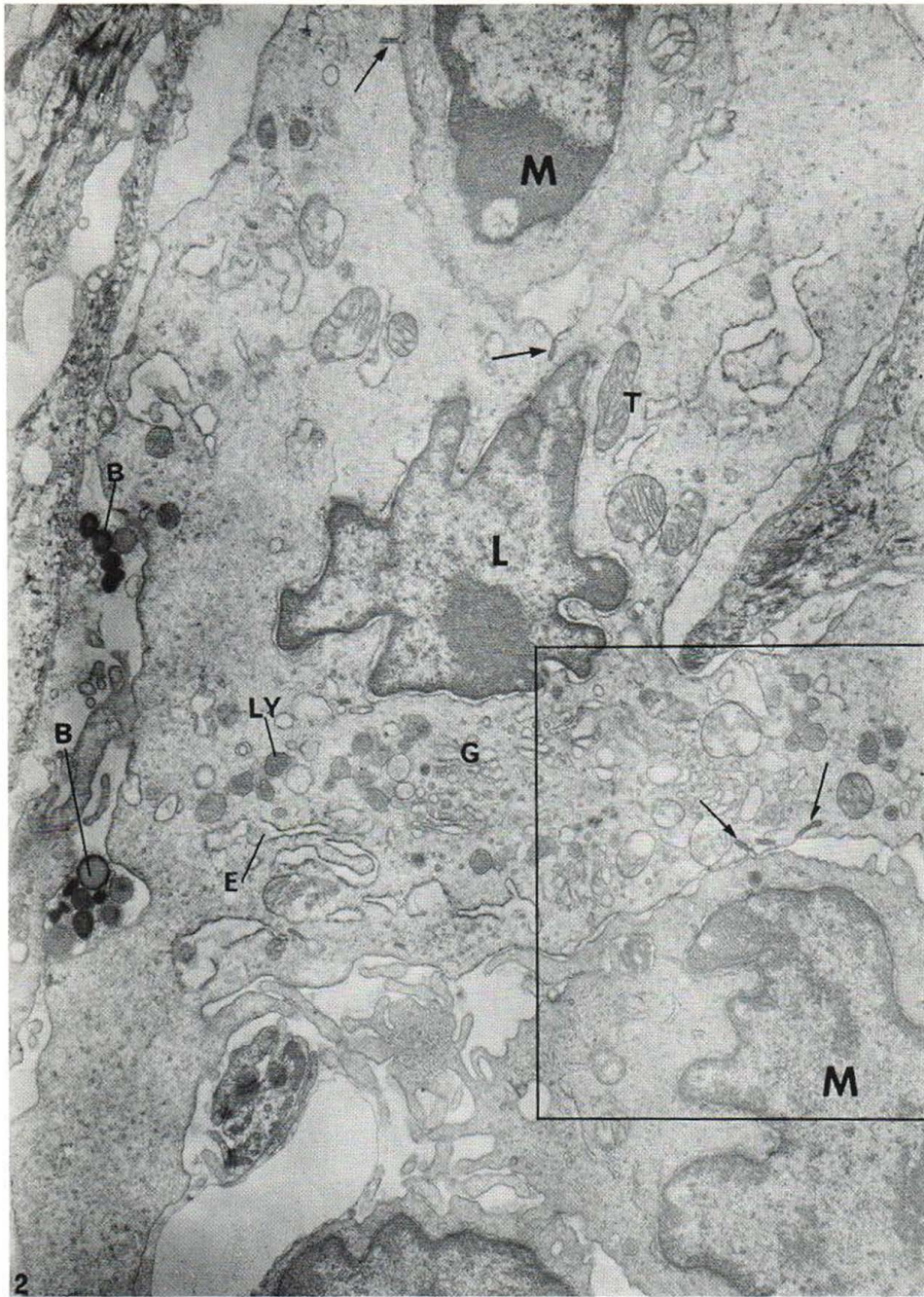


Fig. 2. Contact-allergic reaction at 6 hours. Two mononuclear cells (*M*) are in juxtaposition to one Langerhans cell (*L*) in the epidermis. The cytoplasm of the Langerhans cell contains prominent Golgi region (*G*), several lysosome-like bodies (*LY*), numerous mitochondria (*T*), and several channels of endoplasmic reticulum, some of which contain moderately

opaque material (*E*). The arrows denote Langerhans cell granules which communicate with the extracellular space at sites of apposition of mononuclear cells. Several leukocyte granules (*B*) are seen in the intercellular space. Stained with uranyl acetate and lead citrate. $\times 12\,000$. The area within the lines is shown at higher magnification in Fig. 3.



Fig. 3. Part of Fig. 2 at higher magnification. Part of mononuclear cell (M) is visible. Arrows denote Langerhans

cell granules communicating with the extracellular space. $\times 32\,000$.

C. Positive contact allergic reactions to DNCB

In all 10 guinea pigs apposition of mononuclear cells to Langerhans cells was seen in the epidermis, in the dermis, or both, as early as 3 hours after application of 0.3% DNCB. Some mononuclear cells resemble

lymphocytes in their morphology (Fig. 1). Two or more mononuclear cells may occasionally be seen in juxtaposition to a single Langerhans cell (Fig. 2 and 3). There are many signs of activity in the cytoplasm of the Langerhans cell: the Golgi region

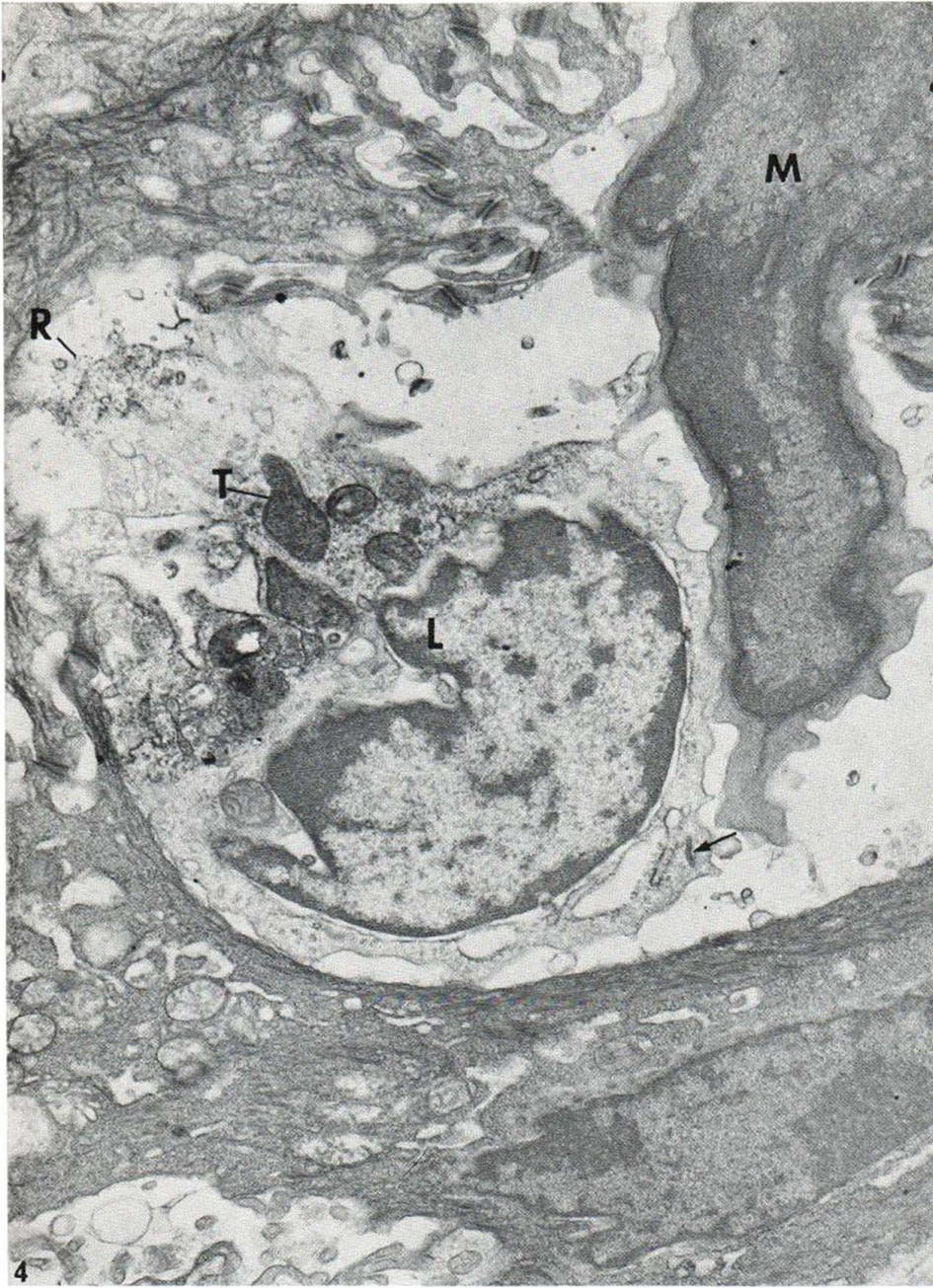


Fig. 4. Contact-allergic reaction at 12 hours. Part of a mononuclear cell (M) is apposed to a Langerhans cell (L). A Langerhans cell granule is shown at the arrow. The lower portion of the Langerhans cell has distinct cell borders, but the upper

half is disrupted by spilling over of ribosome-like material (R). Some of the mitochondria are electron-dense with partially obscured cristae (T). Stained with uranyl acetate and lead citrate. $\times 17\,000$.

is prominent; several lysosome-like bodies, numerous mitochondria and several channels of endoplasmic reticulum are seen; Langerhans cell granules which communicate with the extracellular space at

sites of apposition to the mononuclear cells are present at the arrows.

Basophils are occasionally seen near mononuclear cells and Langerhans cells. Basophils, either granu-

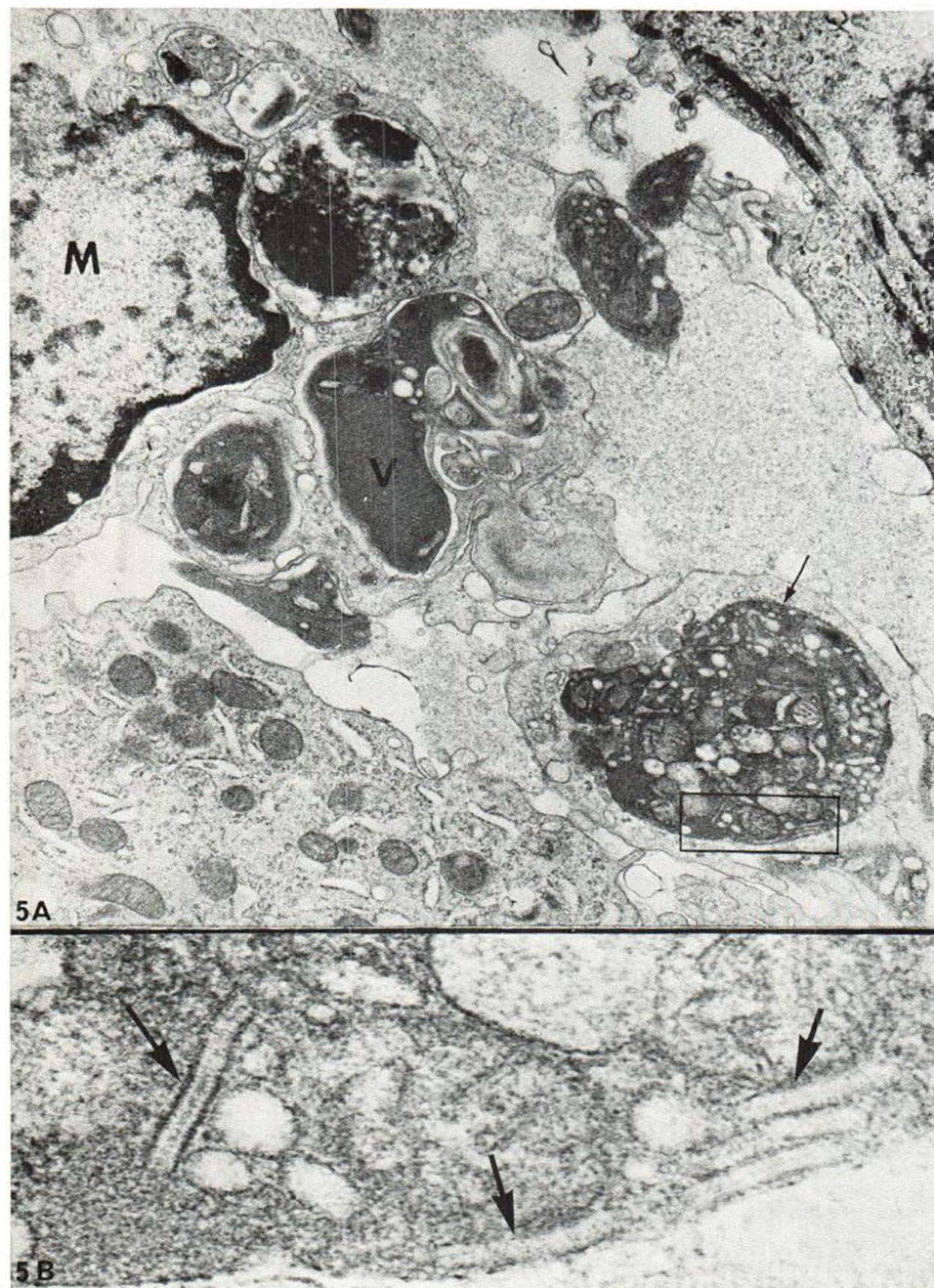


Fig. 5. Contact-allergic reaction at 48 hours. A macrophage (M) is seen with several phagocytic vacuoles (V). Some of these (see arrow) contain Langerhans cell granules. The inset shows a higher magnification of the phagocytic vacuole

containing Langerhans cell granules shown at the arrow. Stained with uranyl acetate and lead citrate. $\times 15\,500$; inset magnification $\times 104\,000$.

lated or partially degranulated, are also present unassociated with mononuclear cells and Langerhans cells.

Some Langerhans cells in all DNCB-sensitive animals show signs of damage in the 19 hour biopsy. These changes were also evident in the few animals



Fig. 6. Contact-allergic reaction at 48 hours. A cell morphologically compatible with a Langerhans cell is shown. A Langerhans cell granule is shown at the arrow and at higher

magnification in the inset. Several phagocytic vacuoles (V) are present in the cytoplasm. Stained with uranyl acetate and lead citrate. $\times 19\,500$; inset magnified $\times 32\,500$.

biopsied at 12 hours and rarely earlier after application of DNCB. Fig. 4 shows such damage to a Langerhans cell, as evidenced by poorly demarcated cell borders. Still other Langerhans cells (not shown) ex-

hibit rarefaction of their cytoplasm. At 48 hours, macrophages containing Langerhans cell granules in phagocytic vacuoles are occasionally present (Fig. 5). Some cells which are morphologically com-

Table I. Langerhans cells and mononuclear cells in the epidermis in different groups of guinea pigs^a

Group of Guinea pigs	Av. no. recognizable Langerhans cells/animal	Av. no. mononuclear cells/animal	Av. no. mononuclear cells apposed to Langerhans cells/animal (%)
Normal, untreated (6 animals)	23	1	0 (0%)
Normal, treated with 0.3% DNCB (6 animals)			
24 h	22	1	0 (0%)
DNCB-sensitized, treated with 0.3% DNCB (10 animals)			
0 h	23	1	0 (0%) ^b
3 h	18	13	3 (23%)
6 h	16	36	4 (11%)
19 h	12	63	4 (6%)
24 h	11	58	4 (6%)
PDC-sensitized, treated with 0.3% DNCB (1 animal)			
0 h	24	1	0 (0%) ^b
3 h	26	3	0 (0%)
24 h	25	10	1 (10%)
CA-sensitized, treated with 0.3% DNCB (2 animals)			
0 h	22	1	0 (0%) ^b
3 h	22	2	0 (0%)
24 h	19	7	0 (0%)
Normal, treated with 5% DNCB (10 animals)			
6 h	23	9	0 (0%)
19 h	24	8	0 (0%)
24 h	15	10	0 (0%)

^a Average cells counts per biopsy were calculated from six areas in the epidermis, each approximately 0.1 mm². The areas were randomly selected from six different blocks of every skin biopsy obtained.

^b In animals sensitized by using Freund's adjuvant, mononuclear cell to Langerhans cell apposition was rarely seen in the dermis but not in the epidermis at any site which was not challenged with DNCB.

patible with Langerhans cells also may contain phagocytic vacuoles (Fig. 6).

D. Non-sensitized guinea pigs after DNCB challenge

Normal animals which were skin-tested with 0.3% DNCB showed only occasional mononuclear cells

and no apposition of mononuclear cells to Langerhans cells either in the epidermis or in the dermis. There was no marked change in the morphology of Langerhans cells.

E. Animals sensitized to PDC and CA and challenged with DNCB

In 1 of 3 animals and only at 24 hours after challenge with DNCB, was a single mononuclear cell to Langerhans cell apposition seen in the epidermis (Table I). None of the other previously described morphologic changes in Langerhans cells at sites of contact-allergic reactions were noted.

F. Contact-irritant reactions to DNCB

In contrast to the findings at sites of contact-allergic reactions, no selective damage to Langerhans cells was observed at contact-irritant reaction sites (normal animals skin-tested with 5% DNCB). Despite general cell damage, Langerhans cells were still recognizable (Fig. 7). No mononuclear cell to Langerhans cell apposition was found in the epidermis at sites of contact irritant reactions. This corresponds to our findings in contact-irritant reactions in the epidermis of man (12).

On rare occasions mononuclear cells were seen apposed to Langerhans cells in the dermis. The morphology of these dermal mononuclear cells did not resemble that of lymphocytes. Instead, they had much cytoplasm which contained prominent channels of endoplasmic reticulum. Basophils were occasionally seen in some of these reactions.

G. Quantitation of observations

In contact-allergic reactions, 23% of all mononuclear cells seen were found in apposition to Langerhans cells 3 hours after DNCB application (Table I). The percentage of mononuclear cells apposed to Langerhans cells decreased with time and at 19 hours it was only 6%. There was a marked decrease in the number of recognizable normal Langerhans cells in sensitized animals from 23, seen at the time just prior to application of DNCB, to only 12, seen 19 hours after DNCB application (Table I). A similar decrease in recognizable normal-appearing Langerhans cells did not occur by 19 hours in normal guinea pigs treated with 5% DNCB. However, by 24 hours some decrease in the number of recognizable Langerhans cells was seen at these sites of irritant reactions.

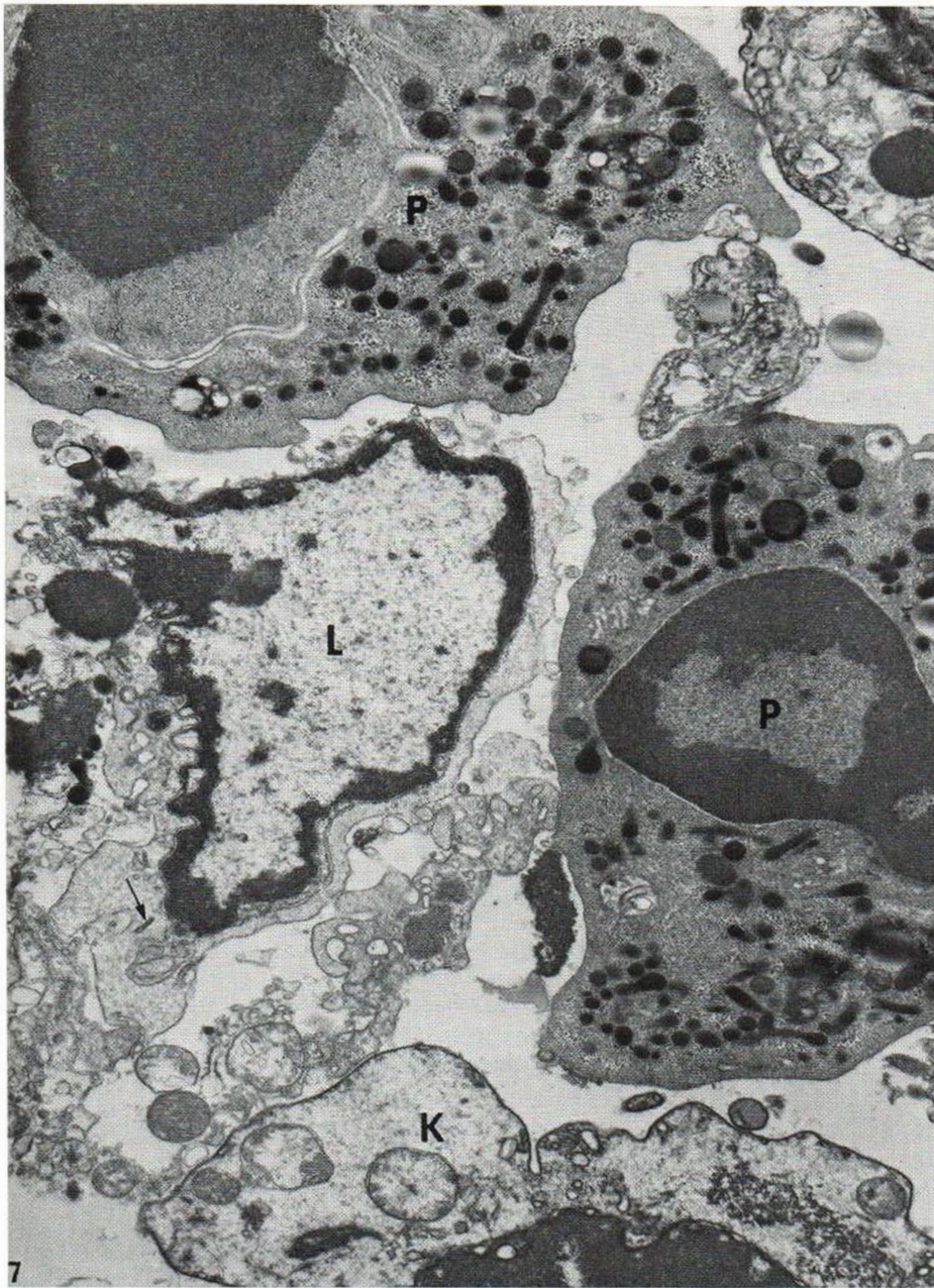


Fig. 7. Contact irritant reaction at 12 hours. A Langerhans cell (*L*) containing Langerhans cell granules (arrow) is surrounded by two polymorphonuclear leukocytes (*P*). A badly

damaged keratinocyte (*K*) is also shown. Stained with uranyl acetate and lead citrate. $\times 14\,000$.

With the exception mentioned above, apposition of mononuclear cells to Langerhans cells was not seen in the epidermis of PDC- and CA-sensitized animals challenged with DNCB.

DISCUSSION

Previous electron microscopic studies of allergic contact-type reactions man strongly suggested a functional relationship between mononuclear cells and

Langerhans cells (12, 13, 14). The present study shows that apposition of mononuclear cells to Langerhans cells also occurs regularly in contact-allergic reactions to DNCB in guinea pigs. At such allergic reaction sites some Langerhans cells in both species show signs of much activity in their cytoplasm, as evidenced by numerous multivesicular bodies, membrane-limited, electron-dense bodies (probably lysosomes), prominent Golgi regions, prominent endoplasmic reticulum and numerous Langerhans cell granules. The appearance of prominent channels of partially rough and smooth endoplasmic reticulum as shown in Fig. 2 suggests that some of these cells may be engaged in protein synthesis.

While these changes were seen in the early hours after challenge, some Langerhans cells showed signs of damage somewhat later in the reaction. Figs. 5 and 6 indicate that the fate of some of the injured Langerhans cells is phagocytosis by macrophages and that other Langerhans cells may autophagocytose their injured cell components. By 19 hours, the number of normal-appearing Langerhans cells was decreased. Some cells were found with prominent endoplasmic reticulum and occasional tubular structures, but no typical Langerhans cell granules were seen.

Since no other reliable marker is known, identification of Langerhans cells rests on the intracellular demonstration of Langerhans cell granules. Because of this we were unable to ascertain with certainty whether there was a true decrease in the number of Langerhans cells in the epidermis or whether the Langerhans cells merely had lost their identifying granules. Indeterminate dendritic cells resemble Langerhans cells, except for the fact that they lack Langerhans cell granules (18). In normal skin, where only rare mononuclear cells are present, it is possible to identify indeterminate dendritic cells as such. However, in the presence of a mononuclear cell infiltrate, these dendritic cells become difficult to differentiate from mononuclear cells.

Statistical analysis of the data showed that the differences in the incidence of mononuclear cell to Langerhans cell apposition between the contact-allergic and contact-irritant reactions could perhaps be explained by differences in the number of mononuclear cells present: the number of these cells in the epidermis in contact-irritant reactions is considerably less than in contact-allergic reactions. However, this apposition would appear not to be a coincidence due to the large numbers of mono-

nuclear cells present in the skin: guinea pigs which had been passively sensitized to DNCB by transfer of lymph node, peritoneal exudate or spleen cells from DNCB sensitized donor animals, in spite of the lower number of mononuclear cells, showed apposition of mononuclear cells to Langerhans cells at positive reaction sites. (Unpublished observations.)

We considered the question of whether the apposition of mononuclear cells to Langerhans cells is a specific phenomenon. This was investigated by testing with DNCB guinea pigs which had been contact-sensitized to PDC or CA. No apposition of mononuclear cells to Langerhans cells occurred in the epidermis, with a single exception in an animal which had been sensitized using Freund's adjuvant. Mononuclear cell-Langerhans cell apposition, as noted above was seen also before challenge in the dermis of a few animals after DNCB-sensitization. In them, the sensitization procedure had involved the use of Freund's adjuvant. It is known that Freund's adjuvant may spread from the site of original injection to many other sites (4). It appears possible that the mononuclear cells found in apposition to the Langerhans cells may have been cells which had become sensitized to components of Freund's adjuvant. Passive transfer studies in which sensitivity to two different antigens is transferred to a single recipient (9) are presently in progress to determine whether the mononuclear cells apposed to Langerhans cells are immunologically specific.

Our findings in guinea pigs reinforce our previous observation in man that Langerhans cells, for which no function had hitherto been found, serve a function in contact-allergic reactions (12). Previously it has been suggested that Langerhans cells may have a role in processing antigen (7, 11). Our observation tend to support the concept that Langerhans cells may have a function in someways analogous to macrophages, i.e., in their ability to retain antigen. We propose the following hypothesis. Some of the mononuclear cells apposed to Langerhans cells are specifically sensitized lymphocytes which interact with antigen on or near the surface of Langerhans cells. In response to factors (lymphokines) which are released when sensitized lymphocytes contact antigen, Langerhans cells may undergo ultrastructural changes. As the reaction proceeds, damage to some Langerhans cells occurs. The Langerhans cells thus perhaps act as target cells for the specifically sensitized lymphocytes. Substances contained in these cells may be released, producing further inflamma-

tory changes. Previously, we have shown that in a positive contact allergic reaction, Langerhans cells may circulate (15). This finding raises the possibility that in these contact allergic reactions a variable percentage of the Langerhans cells participating may represent an unmasked or mobilized population. If indeed Langerhans cells retain antigen on their surfaces, their presence within a vessel resembling a lymphatic may indicate that they could reach lymph nodes and eventually be involved in the immunologic response there as well. The guinea pig furnishes a suitable model for further studies of the role of Langerhans cells in contact allergy.

In this report the apposition of Langerhans cells and mononuclear cells at sites of contact-allergic reactions has been stressed. This should not detract from the role played in these reactions by other cell types such as macrophages and monocytes (10, 16). Basophils also have been found in the blister fluid (3, 1, 17) and recently were found in large numbers in allergic contact dermatitis in man and guinea pigs (5, 6).

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