Migration of Langerhans Cells from Carcinogen-Treated Sheep Skin

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To define the mechanism(s) of carcinogen depletion of Langerhans cells (LC) from skin, the migration of LC from the skin to the regional lymph node was examined in carcinogen-treated, antigen-treated, and control sheep. This was assessed by cannulation of afferent lymphatic vessels that drain the treated areas of skin or the efferent lymphatic draining the regional lymph node. Cells draining from test or control skin were continuously collected and enumerated by indirect immunofluorescence and flow cytometry using specific anti-CD1 monoclonal antibodies.

There was a marked increase in the rate of LC migration in the 8 h following the application of the contact sensitizing antigen trinitrochlorobenzene (TNCB). The chemical carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) triggered a tenfold-greater migration of LC compared with TNCB — with the peak response at 5 d. After DMBA treatment LC were also detected in the efferent lymph of the regional lymph node.

It is concluded that the depletion of LC from carcinogen-treated skin is due to the increased LC migration and not carcinogen-induced cell death. *J Invest Dermatol 99:51S–53S, 1992*

It has been previously demonstrated that treatment of murine skin with the complete chemical carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) resulted in a marked depletion of LC within the treated area [1]. This LC depletion was evident 3–4 d after application of the carcinogen. It has been demonstrated that administration of antigen through LC depleted skin results in the induction of tolerance to the antigen [2]. This also highlights the important role that these cells normally play in presentation to the immune system of antigens that enter the skin.

The question of whether this LC depletion was due to cell death or migration of these cells from the epidermis has remained unresolved, although a detailed examination of skin sections and epidermal sheets by both histology and electron microscopy failed to show any evidence of cell death. This led to the proposal that such a depletion of LC resulted from an increase in cell migration from the epidermis [1].

We have used sheep as a model to assess the effect of DMBA on LC migration from skin. Sheep provide unique opportunities to examine these aspects of LC physiology as the relative size and accessibility of the lymphatic vessels that drain the skin of sheep allows cannulation and, therefore, direct sampling and analysis of the cells that migrate from the skin to the regional lymph node. It has previously been reported that between 20 and 30% of the cells in peripheral sheep lymph are of the macrophage/monocyte lineage, ranging from typical monocytes to those described as "veiled" or "frilly" cells [3]. Other investigators have suggested that these so-called "veiled cells" are LC [4]. Our own investigations* have shown that, under normal circumstances, approximately 4% of the cells in afferent lymph draining the skin are LC.

Recent experiments* have shown that there is an increase in the rate of migration of antigen-presenting LC from the epidermis to the regional lymph node of sheep following the topical application of either the complete chemical carcinogen DMBA or the contact-sensitizing antigen 2,4,6-trinitrochlorobenzene (TNCB). In each case, the rate of LC migration was found to increase immediately after treatment. This effect would appear to be consistent with the normal role of antigen presentation associated with these cells. When TNCB was applied to the skin, the majority of LC that migrated did so within 24 h of treatment (Fig 1). This was followed by a second smaller peak of LC migration during the following 3 d, after which the pattern of LC migration returned to the normal level. The rapid induction of the initial migration of the LC observed here is consistent with earlier reports showing a moderate increase in the number of dendritic cells (including Langerhans cells) in the draining lymph node within 30 min of the topical application of the contact-sensitizing agent fluorescein isothiocyanate (FITC) [5]. The number of dendritic cells within the draining lymph node after the topical application of FITC peaked after 4 h at a level that was maintained for 2 d, before returning to near normal by day 4.

When the complete chemical carcinogen DMBA was applied to the skin the rate of LC migration showed an immediate though moderate increase. This increase was approximately one third the size of that which followed the application of TNCB (i.e., 1×10^6 LC/h and 3×10^6 LC/h, respectively). The great majority of LC

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Abbreviations:
DC: dendritic cells
DMBA: 7,12-dimethylbenz(a)anthracene
FITC: fluorescein isothiocyanate
GM-CSF: granulocyte/macrophage colony-stimulating factor
IL: Interleukin
IU: International Units
LC: Langerhans cells
TNCB: 2,4,6-trinitrochlorobenzene
TNF: tumor necrosis factor

* Dandie GW, Watkins FY, Ragg SJ, Holloway PE, Muller HK: The migration of Langerhans cells into and out of lymph nodes draining normal, carcinogen and antigen treated sheep skin (submitted).

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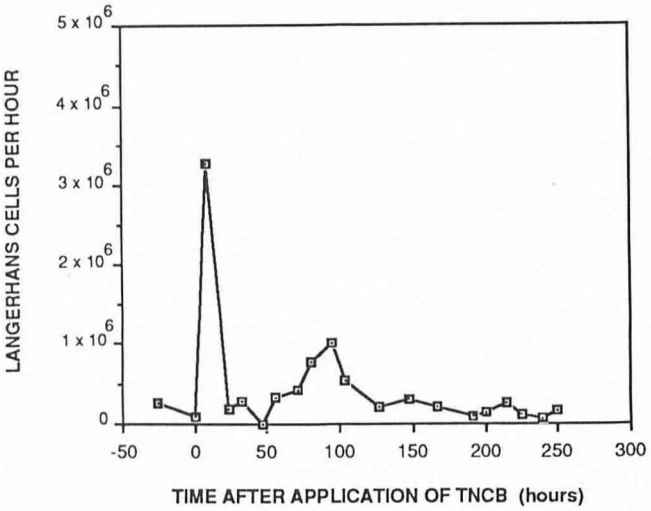

Figure 1. Number of Langerhans cells migrating per hour via afferent lymphatics from TNCB-treated sheep flank skin.

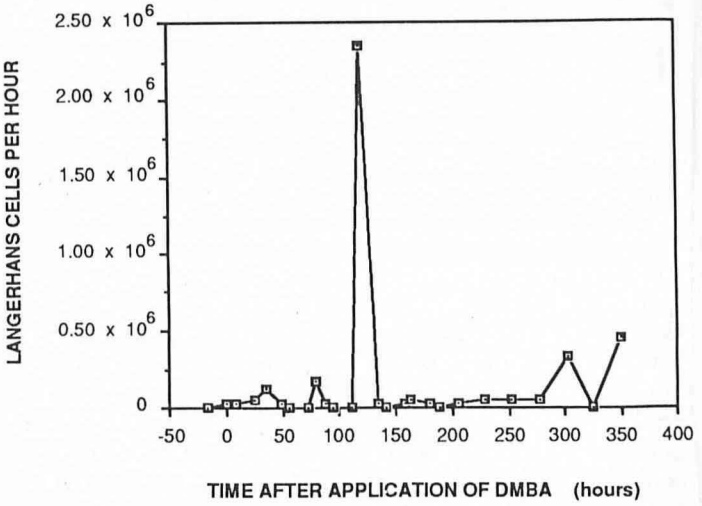

Figure 3. Number of Langerhans cells migrating per hour through the draining lymph node and into the efferent lymph after topical application of DMBA.

that migrated away from DMBA-treated skin did so approximately 4–5 d after application of the carcinogen, when up to 3.5×10^7 LC/h migrated from the treated skin to the regional lymph node (Fig 2). The time of greatest LC migration was similar to that at which LC depletion had been previously observed in the dorsal skin of DMBA-treated mice [1]. This peak in LC migration was also associated with the detection of large numbers of LC in the efferent lymph draining the regional lymph node (Fig 3).

It is evident that the effects of DMBA application to skin differ from those that follow treatment with TNCB not only in relative magnitude, but also in the times at which the majority of LC are triggered to migrate away from treated skin. As both agents induce a rapid increase in LC migration that is followed by a second peak some days later this indicates that similar, as yet unidentified, regulatory mechanisms may be initially involved in each case. This is supported by the similar size of the initial increase in LC migration that follows the application of either DMBA or TNCB.

The profound difference in the magnitude of the major DMBA-induced peak at 4–5 d indicates that there are important differences in the effects of DMBA and antigen on LC metabolism and/or function. The time at which the greatest number of LC migrated away from the skin after application of DMBA suggests some direct action of the carcinogen on LC triggering their enhanced rate of migration. Alternatively, DMBA may affect some other cell type(s) within the skin that in turn signal the LC to migrate.

The initial signal that triggers LC migration may result from direct activation of these cells by antigen or what is initially perceived as antigen in the case of DMBA. The different migration patterns may reflect different cytokine production/secretion profiles from other cells in the skin, such as keratinocytes, which are known to produce IL-1 [6], IL-6 [7], TNFα [8], and GM-CSF [9].

The disruption of the epidermal LC network by DMBA-induced depletion of these cells, like UV irradiation, has already been shown to cause a functional impairment of the skin immune system [2,10,11]. As the magnitude of the LC migration response is sufficient to cause depletion of these cells, any foreign antigen administered via DMBA-treated skin at or shortly after the time of maximum LC migration (i.e., 4–5 d post-treatment) would result in the induction of tolerance and not immunity.

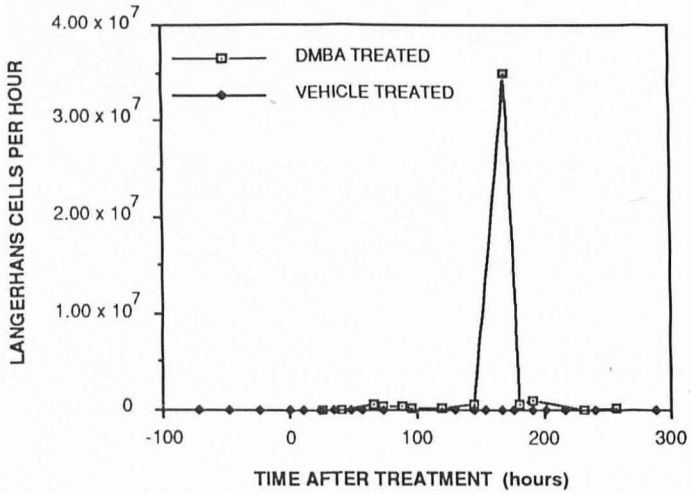

Figure 2. Number of Langerhans cells migrating per hour via afferent lymphatics from DMBA- or lanolin/paraffin vehicle-treated sheep flank skin.

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