Stimulation of Langerhans Cell Migration by Tumor Necrosis Factor α (TNF-α)

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Following topical exposure of mice to skin-sensitizing chemicals, Langerhans cells (LC), many of which bear antigen, are stimulated to migrate via the afferent lymphatics to draining lymph nodes. Consistent with the acquisition of potent immunostimulatory activity, LC while in transit to lymph nodes, are subject to a functional and phenotypic maturation thought to be mediated by granulocyte/macrophage colony-stimulating factor (GM-CSF) and possibly other epidermal cytokines. An interesting question is the nature of the stimulus that initiates the migration of LC from the epidermis. We have examined the influence of intradermal tumor necrosis factor α (TNF-α), another epidermal cytokine, on the accumulation of dendritic cells (DC) in draining lymph nodes. Murine, but not human, recombinant TNF-α caused a rapid and concentration-dependent increase in the frequency of DC in draining nodes. The conclusion drawn is that local production of TNF-α provides one signal for LC migration during cutaneous immune and inflammatory responses.
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Langerhans cells (LC) are considered to play an important, and in normal circumstances an essential, role in the induction phase of contact sensitization. Some 15 years ago it was proposed that LC form a reticuloepithelial trap for external antigen and transport this antigen, via afferent lymphatics, to the draining lymph nodes [1,2]. Consistent with this is the fact that following skin sensitization of mice there is an accumulation of dendritic cells (DC) in lymph nodes draining the site of exposure [3–7]. Studies with skin-sensitizing fluorochromes, such as fluorescein isothiocyanate (FITC), have demonstrated that a significant proportion of the DC that arrive in the draining node bear antigen, and that, initially at least, all cell-associated antigen within draining lymph nodes is bound to dendritic cells [4–7]. Compelling evidence that these antigen-bearing lymph node DC derive from epidermal LC is provided by the recent investigations of Kripke et al [8]. Nude mice were sensitized with FITC at the site of an allogeneic skin graft. It was observed that the antigen-bearing DC found subsequently within draining lymph nodes were of graft donor origin [8].

Although a variety of cells are able to stimulate secondary immune responses, it would appear that DC are essential antigen-presenting cells for primary T-cell activation [9]. Certainly the antigen-bearing DC that arrive in the draining nodes of mice following initial exposure to chemical are highly immunogenic and, by implication, induce the primary response to allergen [3,4,6,10]. It is of some interest therefore that epidermal LC, from which these DC derive, are relatively inefficient stimulators of T-lymphocyte activation [11]. However, this property develops during short-term culture of freshly-isolated LC, and is associated with an elevated expression of membrane major histocompatibility complex (MHC) class II (Ia) antigen [11–13]. We speculated that the potent immunostimulatory activity of antigen-bearing DC isolated from draining nodes was consistent with LC being subject to a similar phenotypic and functional maturation while in transit from the skin to the nodes. Recent evidence suggests that this is the case. Thus, compared with freshly-isolated LC, lymph node DC, including antigen-bearing lymph node DC, exhibit increased membrane Ia expression [14]. It is now apparent that the maturation of LC in vitro is effected by granulocyte/macrophage colony-stimulating factor (GM-CSF) [15,16]. Because this cytokine is produced by keratinocytes [17], it is not unreasonable to speculate that GM-CSF is also responsible for the observed changes in LC phenotype and function in vivo. The migration of LC is, in addition, associated with a markedly elevated expression of intercellular adhesion molecule-1 (ICAM-1),* which may, at least in part, explain why, unlike LC, antigen-bearing lymph node DC form stable clusters with T lymphocytes [18].

A question that remained was the nature of the signal following skin sensitization that provides the stimulus for LC to migrate from the epidermis in the first place. Keratinocytes can be induced to produce a variety of cytokines in addition to GM-CSF including interleukins 1α and β, 3, 6, and 8 and tumor necrosis factor α (TNF-α) [19]. We questioned whether epidermal cytokine production may, in addition to effecting their maturation, induce LC migration. We chose to examine the influence of TNF-α. Mice (BALB/c strain) received murine recombinant TNF-α (50 ng) intradermally in the dorsum of both ear pinnae. Control mice were untreated or received 30 μg of bovine serum albumin (BSA); the

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Abbreviations:
BSA: bovine serum albumin
DC: dendritic cells
FITC: fluorescein isothiocyanate
GM-CSF: granulocyte/macrophage colony-stimulating factor
LC: Langerhans cells
TNF-α: tumor necrosis factor α

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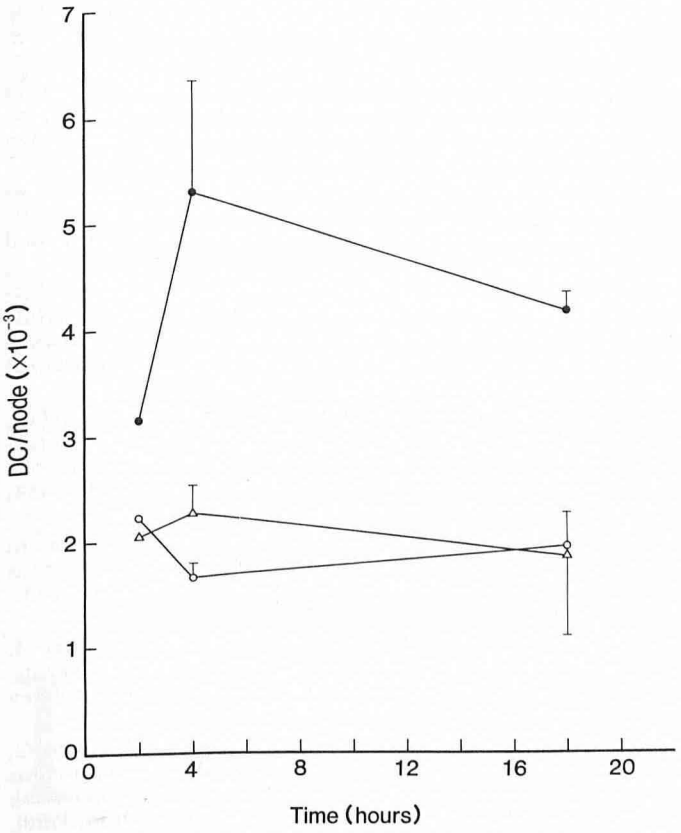

Figure 1. Increases in lymph node DC following exposure to murine rTNF-α. Groups of mice (n = 10) received 30 μl intradermal injections in both ear pinnae of either 50 ng murine TNF-α in 0.1% BSA (●), or 0.1% BSA alone (○). Control mice were untreated (△). The number of DC in draining nodes 2, 4, and 18 h following exposure was measured. The data illustrated represents the results of a single experiment (2 h) or the mean ± SE of three independent experiments (4 and 18 h).

amount of carrier protein in which TNF-α was suspended. Draining auricular lymph nodes were examined 18 h later and the frequency of DC per node estimated by direct morphologic examination using phase-contrast microscopy and/or by indirect immunofluorescence. TNF-α, but not BSA alone, caused a significant increase in the number of DC in draining nodes [20]. Under the same conditions of exposure mouse rGM-CSF (10 ng/ear) and IL-1α (25 u/ear) were without effect. Previous studies have shown that DC arrival in draining nodes is maximal approximately 24 h following topical sensitization [6]. We speculated that if DC migration following skin sensitization results from the production by keratinocytes of TNF-α, then the direct injection of the cytokine might cause an accelerated accumulation of DC in draining nodes. An increased frequency of DC in draining nodes was first detectable 2 h following exposure to TNF-α, and maximal at 4 h (Fig 1). The effect of TNF-α was dose dependent, with as little as 12.5 ng causing a significant increase in node DC within 4 h of exposure. Interestingly, treatment of mice with equal amounts of human rTNF-α of the same specific activity failed to induce any alteration in the number of DC in draining nodes.

The conclusion drawn from these investigations is that TNF-α provides one signal for the migration of LC from the skin. An assumption is that the DC that arrive in the draining nodes following TNF-α treatment derive from epidermal LC. Such an assumption appears justified because, under normal circumstances, the major route of entry of DC into peripheral lymph nodes is via the afferent lymphatics [21]. There exists a precedent for a direct influence of TNF-α on LC. Koch et al [22] demonstrated that addition to culture of TNF-α would maintain the viability of mouse LC without effecting their functional maturation. It is of interest that in those investigations also it was observed that the same concentration of human TNF-α was without influence [22]. Mice possess two receptors for TNF-α, designated TNF-R1 and TNF-R2, which differ in terms of both extracellular nucleotide sequence and species specificity [23]. Whereas TNF-R1 binds human and mouse rTNF-α with comparable affinity, TNF-R2 exhibits strong species specificity for the mouse cytokine [23]. The available evidence suggests therefore that it is TNF-R2 that is expressed selectively by murine LC.

An induction by TNF-α of LC migration is consistent with data reported by Streilein and colleagues, who found that intradermal injection of mice with comparable amounts of this cytokine caused a rapid reduction in the density of Ia^+ LC [24,25]. Although these authors also propose that dermal TNF-α influences the behavior of LC, they suggest a different mode of action, contending that the cytokine immobilizes LC within the epidermis [24,25]. As it has been reported that ultraviolet (UV) light promotes the synthesis and secretion of TNF-α by keratinocytes [26], the implication is that the immunosuppressive effects of UVB are mediated, at least in part, through the inhibition by TNF-α of antigen transport to the lymph nodes [24,25]. Our own hypothesis, based upon the information summarized in this report, is that TNF-α has quite the opposite effect and rather than immobilize LC in the skin it in fact stimulates their migration to the draining lymph nodes. Such may also be compatible with TNF-α being an important mediator of the immunosuppressive effects of UVB. Impaired sensitization following local UVB treatment would, we speculate, be a consequence of the prior migration of responsive LC from the epidermis. Certainly this would be consistent with the fact that UVB (like TNF-α) causes a reduction in the number of recognizable Ia^+ epidermal LC [24,25].

In summary we propose that the migration and maturation of LC, which is essential for the induction of contact sensitization and presumably for the initiation of responses to other cutaneous antigens, is effected by epidermal cytokines. TNF-α provides one signal for migration, and while in transit to the lymph nodes LC are subject to maturational changes mediated by GM-CSF and possibly other cytokines.

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