STIMULATION OF LANGERHANS CELL MIGRATION IN MICE BY TUMOUR NECROSIS FACTOR α AND INTERLEUKIN 1β

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1. INTRODUCTION

Following contact sensitization or other forms of cutaneous trauma epidermal Langerhans cells (LC) are stimulated to migrate from the skin and to travel, via afferent lymphatics, to draining lymph nodes. We have demonstrated previously that tumour necrosis factor α (TNF-α), a keratinocyte-derived epidermal cytokine, provides one stimulus for LC migration. It has been shown that intradermal injection of mice with homologous recombinant TNF-α induces a rapid reduction in the frequency of epidermal LC local to the site of exposure and results, somewhat later, in the accumulation of dendritic cells (DC) in draining lymph nodes. Systemic (intraperitoneal) treatment of mice with a neutralizing anti-TNF-α antibody prior to topical sensitization with oxazolone, a potent contact allergen, was found to inhibit almost completely the accumulation of DC in draining nodes normally provoked by exposure to this chemical. Such treatment was found also to suppress the development of contact sensitization. In the present investigations we have examined whether another epidermal cytokine interleukin 1β (IL-1β), a product of LC, also plays a role in LC migration. Attention has focused on IL-1β for three reasons. First, it has been shown that the development of contact hypersensitivity is compromised in IL-1β gene deletion transgenics, or in mice that have been treated with an anti-IL-1β antibody. Second, there is evidence suggesting that IL-1β, a cytokine which is upregulated very rapidly following contact sensitization, is able to provoke the increased expression of TNF-α by keratinocytes. Third, it has been claimed on the basis of experiments performed with human skin explants that IL-1β may affect LC migration in vitro. We here describe investigations in which the influence of IL-1β and TNF-α on LC migration and DC accumulation were examined.

2. RESULTS

Groups of BALB/c strain mice (n=3 for measurement of LC frequency, or n=10 for evaluation of DC accumulation in draining lymph nodes) were treated, by intradermal injection (30μl) into both ear pinnae, either with 50ng of recombinant murine TNF-α (specific activity 2x10^6U/mg) or with the same amount of recombinant murine IL-1β (specific activity 1–2×10^6U/mg). Additional groups of mice were exposed in the same way to 30μl of 0.1% bovine serum albumin (BSA), the protein in which the cytokines were suspended. Further control animals were untreated.

The frequency of epidermal LC local to the site of exposure was measured 30 minutes and 2 hours following treatment. Epidermal sheets were prepared and LC identified as described previously using immunocytochemical assessment of MHC class II (Ia) antigen expression. In untreated mice the frequency of LC in epidermal sheets remained relatively constant at approximately 900 LC/mm². LC density in mice exposed for either 30 minutes or 2 hours to BSA alone did not differ significantly from control values. Consistent with previous observations, intradermal treatment of mice with homologous TNF-α induced a rapid reduction in the frequency of epidermal LC that was apparent within 30 minutes of exposure. In contrast, 30 minutes following treatment with IL-1β the density of LC in epidermal sheets was unchanged. Within 2 hours of treatment with either cytokine there was a clear and comparable reduction in LC numbers (Figure 1a).

In parallel experiments the ability of TNF-α and IL-1β to induce the accumulation of DC in draining lymph nodes was measured. Lymph nodes were excised 2 and 4 hrs after exposure, single cell suspensions prepared and DC enriched and enumerated as described previously. In this series of experiments lymph nodes taken from untreated control mice were found to contain between 1,500 and 2,200 DC. Treatment of mice with BSA alone failed to influence DC numbers. It has been reported previously that intradermal exposure of mice to homologous TNF-α induces within 2 hours an increase in the number of DC found within draining lymph nodes. In accord with those data such treatment was shown here to be associated with an approximate 2-fold increase in DC numbers by 2 hours and a further increase to over 5,000 DC/node when measurements were made at 4 hours. The frequency of DC in draining lymph nodes was increased by 4 hours following exposure to IL-1β, but not at 2 hours (Figure 1b).

3. DISCUSSION

The data presented here demonstrate that dermal exposure of mice to homologous TNF-α induces a rapid movement of a proportion of LC away from the epidermis and the accumulation, somewhat later, of DC in draining lymph nodes. As such these results are in agreement with those of previous investigations. It is apparent, however, that IL-1β also has the ability to stimulate LC migration and DC accumulation, albeit with somewhat slower kinetics. One possibility is that IL-1β serves to induce or upregulate the production by keratinocytes of TNF-α that then acts on neighbouring LC to stimulate their migration. Certainly it has been shown that intradermal injection of mice with concentrations of IL-1β comparable to those used here caused a very substantial increase in the epidermal expression of mRNA for TNF-α. The need for IL-1β to first induce the production of TNF-α would be consistent with the delayed kinetics of both LC migration and DC accumulation compared with those observed in mice exposed to TNF-α itself. Alternatively, or additionally, IL-1β may influence directly the activity of LC; this being a possibility since