Interleukin-1β and Granulocyte-Macrophage Colony-Stimulating Factor Mediate Langerhans Cell Maturation Differently

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It has been reported that the in vivo maturation of Langerhans cells after hapten painting is mediated by IL-1β while Langerhans cell maturation after in vitro culture is mediated by granulocyte-macrophage colony-stimulating factor (GM-CSF). To clarify the reason for this discrepancy, we examined the expression of Ia antigen and several co-stimulatory molecules on Langerhans cells that were activated by in vitro culture, by hapten painting, or by an intradermal injection of several cytokines. Both cultured Langerhans cells and those activated by hapten painting increased the expression of Ia antigen and all the co-stimulatory molecules (i.e., intercellular adhesion molecule-1 [ICAM-1], B7-1, B7-2, and CD40). In contrast, an intradermal injection of interleukin-1β (IL-1β) or tumor necrosis factor-α (TNF-α) increased the expression of Ia antigen, ICAM-1, B7-2, and CD40, but not that of B7-1. These data indicate that IL-1β or TNF-α is not sufficient to induce B7-1 expression on Langerhans cells in vivo. Subsequently we examined the effect of anti-cytokine antibodies (Abs) on the expression of those molecules on cultured Langerhans cells. While none of the Abs to IL-1β, TNF-α, or GM-CSF changed the upregulation of Ia antigen, ICAM-1, or CD40 on cultured Langerhans cells, anti-GM-CSF Ab suppressed that of B7-1 and B7-2. Taken together, our present results suggest that IL-1β is required for the upregulation of Ia, ICAM-1, B7-2, and CD40, while GM-CSF is required for the upregulation of B7-1 and B7-2, although it still remains unclear why the injected GM-CSF could not augment B7-1 expression on Langerhans cells in vivo and why anti-IL-1β Ab did not suppress the upregulation of Ia, ICAM-1, or CD40 on cultured Langerhans cells. Key words: B7/tumor necrosis factor-α/CD40/lymphokine. J Invest Dermatol 106:441-445, 1996

Recently it has been demonstrated by several authors [1,2,14,23,24,29] that Langerhans cells can be matured both in vivo and in vitro. Maturated Langerhans cells increase their expression of major histocompatibility complex (MHC) class II antigen (Ag) and show enhanced antigen presenting function [4,21,30]. Witter-Pack et al [32] and Heufler et al [13] have demonstrated that the maturation of Langerhans cells that occurs after in vitro short-term culture is mediated by granulocyte-macrophage colony-stimulating factor (GM-CSF) and that interleukin-1 (IL-1) enhances the effect of GM-CSF. On the other hand, Enk et al [10] have found that the increase in Langerhans cell-derived IL-1 mRNA signal strength is the first event in the process of in vivo maturation of Langerhans cells after hapten painting. In addition, they showed that the cutaneous injection of IL-1β resulted in increased expression of MHC class II Ag and enhanced antigen presenting function by Langerhans cells suggesting that in vivo maturation of Langerhans cells that occurs after hapten painting is mediated by IL-1β [8]. Therefore, if Langerhans cell maturation induced by the cutaneous injection of IL-1β is the same phenomenon as that occurring on Langerhans cells that mature after hapten painting, there remains a discrepancy in the reported behavior of cytokines that mediate the maturation of Langerhans cells between in vivo and in vitro. It is also well known that accessory molecules are required for antigen-presenting cells to stimulate T cells [7,15,22,28,31]. Therefore, in this study examining the phenotypic changes in Langerhans cells matured in vivo and in vitro, we have analyzed the contribution of different cytokines in the maturation process of Langerhans cells under in vivo and in vitro situations.

MATERIALS AND METHODS

Mice Specific pathogen-free female BALB/c A/J mice, 8-10 wk of age, were obtained from the Institute of Experimental Animals, Tohoku University School of Medicine.

Antibodies We used the following monoclonal antibodies (MoAbs) for immunostaining: anti-intercellular adhesion molecule-1 (ICAM-1) (KAT-1), anti-CD40 (HM-10-3) (gifts from Dr. Yagita and Dr. Kato, Department of Immunology, Juntendo University, Tokyo, Japan), anti-B7-1 (B7/BBI), anti-B7-2 (GL-1) (PharMingen, San Diego, CA), and fluorescein isothiocyanate (FITC)-conjugated anti-IgE (d, k haplotypes) (PharMingen) MoAbs. Phycoerythrin-conjugated anti-rat IgG (TAGO, Burlingame, CA) or biotinylated anti-hamster IgG (Vector Laboratories, Burlingame, CA) followed by phycoerythrin-conjugated avidin (Becton-Dickinson, San Jose, CA) were used as second-step reagents. Isotype-matched rat control Ab (IgG2a, and IgG2b), hamster IgG and FITC-conjugated rat control IgG2b Ab (PharMingen) were used as negative controls. In order to examine the effects of anti-cytokine Abs on the upregulation of adhesion molecules or...
Both Langerhans Cells after TNCB Painting and Those Cultured in vitro Could Upregulate the Expression of MHC Class II Ag, ICAM-1, B7-1, B7-2, and CD40. Langerhans cells matured in vitro by TNCB painting (a–e) and cultured Langerhans cells (f–h) were examined for the expression of MHC class II Ag (a,j), ICAM-1 (b,g), B7-1 (c,i), BD-2 (d,k), and CD40 (e,j) after gating I-E positive cells on FITC versus side scatter contour plots. The profiles of Langerhans cells matured by TNCB painting and cultured Langerhans cells were indicated by open histograms, and those representing freshly isolated Langerhans cells are indicated by shaded ones.

The Intradermal Injection of Cytokines Could Not Upregulate B7-1 on Langerhans Cells in vitro. IL-1β and GM-CSF are reported to be involved in Langerhans cell maturation in vivo and in vitro, respectively [8,13,32]. In addition, according to Enk et al [8], the cutaneous injection of IL-1β induces the phenotypic as well as functional maturation in Langerhans cells in vivo similar to that in these cells after hapten painting. We examined the expression of MHC class II Ag, ICAM-1, B7-1, B7-2, and CD40 on Langerhans cells 16 h after intradermal injection of GM-CSF, Tnf-α, or IL-1β, and compared their expression with those on Langerhans cells after TNCB painting (Fig 2). Langerhans cells from the mice injected with IL-1β or Tnf-α enhanced the expression of MHC class II Ag, ICAM-1, B7-2, and CD40 compared with those injected with 0.2% bovine serum albumin in PBS as control, while significant changes were recognizable on their expression of B7-1. Intradermal injection of GM-CSF in two different doses, either 50 or 250 ng/ear, could not affect the expression on Langerhans cells of all the molecules we studied. Furthermore, we performed a kinetic study in which we prepared epidermal cell suspensions at the various time intervals (i.e., 1, 4, 8, 16, and 48 h) after GM-CSF, IL-1β injection, or TNCB treatment (Fig 3). We found that the most remarkable phenotypic changes on Langerhans cells were observed at 16 h after the treatments and that the phenotype of Langerhans cells after GM-CSF injection was not different from that after PBS injection at each time point. Recently, Kampgen et al [16] have reported that IL-1 induces GM-CSF receptor expression on Langerhans cells. Therefore, it is conceivable that the simple injection of GM-CSF cannot affect the phenotypes of Langerhans cells because of insufficient numbers of expressed GM-CSF receptors on freshly isolated
Figure 2. The intradermal injection of cytokines cannot upregulate B7-1 on Langerhans cells in vivo. Langerhans cells treated by the intradermal injection of GM-CSF, TNF-α, IL-1β, or by TNCB painting were examined for the expression of MHC class II Ag (a), ICAM-1 (b), B7-1 (c), B7-2 (d), and CD40 (e) after gating I-E-positive cells on FITC versus side scatter contour plots. MFI was determined for each surface molecule, and the effects of cytokines were evaluated by percentage MFI as calculated using the following formula:

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\% \text{ MFI} = \left( \frac{\text{MFI of Langerhans cells treated with cytokine(s) or TNCB}}{\text{MFI of Langerhans cells treated with 0.2% BSA/PBS}} \right) \times 100\%
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Langerhans cells. To exclude such a possibility, we injected at first IL-1β, and at different time intervals (0, 4, 8, 16 h) thereafter we examined the effect of GM-CSF; however, these combined injections did not alter the effects of injecting IL-1β alone (data not shown).

The Upregulation of B7-1 Expression on Cultured Langerhans Cells Was Downregulated by Anti-GM-CSF Ab and Anti-TNF-α Ab 

From in vitro study, we could not specify the cytokine(s) that mediate B7-1 upregulation. Thus, we examined the effects of Abs to several cytokines on the upregulation of B7-1, MHC class II Ag, and other accessory molecules on Langerhans cells cultured for 72 h (Fig. 4). When we added 20 μg/ml of anti-GM-CSF or anti-TNF-α Ab to the culture of epidermal cells, the expression of B7-1 on Langerhans cells was inhibited, whereas the addition of 20 μg/ml of anti-IL-1β Ab could not suppress its expression at all. In addition, only anti-GM-CSF Ab exerted a partial inhibitory effect on the upregulation of B7-2 expression. None of the Abs to GM-CSF, TNF-α, or IL-1β changed the expression of MHC class II Ag, ICAM-1, or CD40.

DISCUSSION

In this study, we have demonstrated that Langerhans cells matured in vivo by hapten painting also upregulate the co-stimulatory molecules as well as MHC class II Ag. In other words, Langerhans cells that mature in vivo seem to be almost identical with those that mature in vitro, although the latter express the co-stimulatory molecules much more than the former.

We did demonstrate that the phenotype of Langerhans cells after intradermal injection of IL-1β was different from that of Langerhans cells after hapten painting. Langerhans cells obtained after intradermal injection of IL-1β did not upregulate B7-1, whereas in vitro hapten treatment definitely increased B7-1. This observed difference indicates that the simple injection of IL-1β could not be a substitute for the mediators involved in Langerhans cell maturation after hapten painting and that other signals are required for the full simulation of Langerhans cells. Therefore, we next examined whether TNF-α or GM-CSF, which were suggested to affect Langerhans cell function in vitro [13,18,32], could upregulate the expression of B7-1. Unexpectedly, we could not recognize the upregulation of B7-1 expression either by the intradermal injection of 50 or 250 ng/ear of GM-CSF, or by that of TNF-α.

On the other hand, anti-GM-CSF Ab and, although less efficiently, anti-TNF-α Ab could suppress the upregulation of B7-1 on cultured Langerhans cells, suggesting that GM-CSF and TNF-α mediate the increase in expression of B7-1 on Langerhans cells in vitro. These in vitro and in vivo data suggest that Langerhans cells require both IL-1β and GM-CSF for their full maturation; the former is responsible for the upregulation of MHC class II Ag, ICAM-1, B7-2, and CD40, while the latter is for that of B7-1.

When we assume, however, that GM-CSF upregulate the expression of B7-1, while IL-1β increase the expression of MHC class II Ag, ICAM-1, B7-2, and CD40, at least two questions arise. The first is why we could not recognize the upregulation of B7-1 after GM-CSF injection. We can think of three possible explanations. The first one is that the amount of GM-CSF we injected is too small to induce the phenotypic changes of Langerhans cells. To exclude this possibility, we measured GM-CSF concentration of the culture supernatant of epidermal cells, in which B7-1 upregulation on Langerhans cells was induced. The average GM-CSF concentration in repeated experiments ranged from 400 to 500 pg/ml (data not shown), whereas we injected 1 or 5 μg/ml far exceeding amounts of GM-CSF in our experiments. The second possible explanation is that GM-CSF actually cannot reach or bind the receptors on epidermal Langerhans cells. It is well known that glycosaminoglycans bind to GM-CSF [11,25]. The glycosaminoglycan that is abundantly present in the dermis as well as at the dermo-epidermal junction may bind to GM-CSF to inhibit its further diffusion to
could not enhance their antigen-presenting function. Heufler et al. [13] demonstrated that none of anti-cytokine Abs could suppress the upregulation of MHC class II Ag that occurs on cultured Langerhans cells. Our present observation is consistent with these data, but it is not proper to judge that IL-1β is not involved in the upregulation of MHC class II Ag, ICAM-1, B7-2, or CD40 in cultured Langerhans cells only based on these findings. Enk et al. [9] have found that increased IL-1β mRNA signal is accumulated in Langerhans cells as early as 15 min after exposure to contact allergens. Therefore, it is speculated that the production of IL-1β also starts in Langerhans cells at an extremely early stage in culture. It took about 3 h for us to prepare Langerhans cell-enriched population and to start their culture with anti-IL-1β Ab. Thus, we cannot exclude the possibility that during this time interval, Langerhans cells are already stimulated to increase MHC class II Ag, ICAM-1, B7-2, and CD40 expression.

As for the regulation of B7-2 expression, we found that anti-GM-CSF Ab slightly but clearly increased its augmentation in our in vitro culture study of Langerhans cells. Larsen et al. [20] recently demonstrated that Langerhans cells from cultures containing anti-GM-CSF expressed decreased levels of CTLA-4-counter receptor and that GM-CSF added in the culture can upregulate the expression of both B7-1 and B7-2 on splenic dendritic cells. These data suggest that GM-CSF regulate both B7-1 and B7-2 expression of dendritic cells including Langerhans cells.

Our present experiments showed that the intradermal injection of TNF-α increased the expression of MHC class II Ag, ICAM-1, B7-2, and CD40, and that anti-TNF-α Ab suppressed the upregulation of B7-1 on cultured Langerhans cells. Recently, Sallusto et al. [26] have also demonstrated that TNF-α increased the expression of MHC class II Ag, ICAM-1, and B7-1 on human dendritic cells that were obtained by the culture of peripheral blood mononuclear cells with GM-CSF and IL-4. TNF-α also play a role in Langerhans cell maturation which is distinct from that of IL-1β and GM-CSF.

Human Langerhans cells, which are potent antigen-presenting cells in the skin, have been shown to express CD40 [23]. It has not yet been reported, however, whether murine Langerhans cells express CD40 or whether they upregulate it when they become mature in vitro or in vivo. In this experiment, we demonstrated that murine freshly isolated Langerhans cells also express CD40 molecule and they upregulate CD40 during in vitro culture or after hapten painting. The expression of CD40 on Langerhans cells may play a crucial role in their antigen presentation.

Finally, because we did not examine the production of other cytokines after the injection of IL-1β, TNF-α, or GM-CSF, we could not completely exclude the possibility that the cytokines induced by IL-1β injection may affect the phenotypic changes on Langerhans cells in vitro.

**REFERENCES**
