Langerhans Cells Do Not Produce Interferon-γ

To the Editor:

Interferon-γ (IFN-γ) is the predominant cytokine during Th1-dominated immune reactions, participates importantly during antigen presentation, and is the prototypical macrophage-activating cytokine. Consequently, a pivotal role of IFN-γ in the clearance of various intracellular pathogens has been demonstrated (Boehn et al, 1997). IFN-γ had historically been regarded as a product solely of T cells and natural killer cells; however, it was discovered recently that, with appropriate stimulation, B cells, macrophages (Mφ), and even dendritic cells (DC) could produce IFN-γ (Di Marzio et al, 1994; Yoshimoto et al, 1997; Ohteki et al, 1999). In the case of DC, IL-18 acts in synergy with IL-12 on IFN-γ production (Fukao et al, 2000). In addition, IL-4 unexpectedly enhances DC IFN-γ production, synergistically with IL-12 or with DC stimulation through CD40 or major histocompatibility complex class II molecules triggering endogenous IL-12 production (Fukao et al, 2000). Therefore, we were interested in examining whether Langerhans cells, a special subset of DC localized in the epidermis, could produce IFN-γ in response to these stimulations. Recently, we succeeded in preparing high purcity Langerhans cells (> 95%) from BALB/c mouse skin (Tada et al, 2000), and this allowed us to examine direct effects of several cytokines on Langerhans cell IFN-γ production, without interference of keratinocytes and keratinocyte-derived cytokin.

First, we employed reverse transcription polymerase chain reaction (RT-PCR) to assess IFN-γ gene expression in Langerhans cells exposed to various combined stimulations including IL-12 (2 ng per ml), IL-18 (20 ng per ml), IL-4 (10 ng per ml), and IFN-γ (100 ng per ml), as shown in Fig 1. These stimulations were formerly reported to induce IFN-γ production in both spleen- and bone-marrow-derived DC or Mφ (Di Marzio et al, 1994; Fukao et al, 2000; Stober et al, 2001). As shown in Fig 1, no IFN-γ mRNA was detected in any of the RNA extracted from fresh Langerhans cells and 24 h incubated Langerhans cells with and without stimulation. As expected, IFN-γ mRNA was detected in spleen-derived CD11c+ DC stimulated with IL-12 and IL-18.

Next, supernatants of 48 h cultured Langerhans cells and DC were subjected to the measurement of IFN-γ protein levels using enzyme-linked immunosorbent assay (ELISA). The synergistic effects of IL-4 and IL-18 on IL-12-dependent IFN-γ production of spleen-derived CD11c+ DC previously reported by Fukao et al (2000) were confirmed (Fig 2A). In contrast to spleen-derived CD11c+ DC, simultaneous addition of IL-12 and IL-18 failed to induce Langerhans cells IFN-γ secretion (Fig 2B). Further administration of IL-4 to the combination of IL-12 and IL-18 did not affect this Langerhans cell unresponsiveness (Fig 2B). IL-15, recently characterized as another IFN-γ-inducing cytokine from DC (Mattei et al, 2001), also failed to stimulate Langerhans cells for IFN-γ production (data not shown). We also evaluated the effect of other stimulations closely related to Langerhans cell maturation and IL-12 production (Tada et al, 2000), such as anti-CD40 monoclonal antibody (20 µg per ml), granulocyte-macrophage colony stimulating factor (1 ng per ml), tumor necrosis factor α (10 ng per ml), IFN-β (1000 U per ml), anti-IL-10 monoclonal antibody (20 µg per ml), transforming growth factor β (TGF-β; 1 ng per ml), and the combination of anti-CD40 monoclonal antibody and TGF-β. None of these reagents caused Langerhans cell IFN-γ production, however, even when added together with IL-12, IL-18, and IL-4 (data not shown). The same results were obtained with both 48 h and 72 h cultured Langerhans cells.

We used only dispase for epidermal sheet separation from the mouse skin, and did not use trypsin throughout the process of Langerhans cell isolation. With this isolation technique, receptors of cytokines are less likely to be degraded on Langerhans cells. The whole procedure may have affected expression of cell surface receptors, however, causing Langerhans cells to be unresponsive to several cytokines in comparison to spleen-derived CD11c+ DC. To address this issue, we treated DC in the same condition that was employed for Langerhans cell isolation, but the viability of treated DC then fell significantly and an exact comparison was not possible (Tada et al, 2000). As another approach, we left isolated Langerhans cells untreated in culture medium for 24 h before the addition of stimuli, in order to allow reappearance of cell surface receptors putatively degraded by the isolation procedure. This experiment also failed to stimulate Langerhans cells for IFN-γ production (data not shown). Finally, we have recently found out that IL-12, an important IFN-γ inducer of splenic DC, strongly enhances the level of IFN-γ-inducible protein 10 (IP-10) production of isolated Langerhans cells prepared in the same way (Fujita et al, submitted), indicating that IL-12 indeed has a biologic activity on Langerhans cells.

![Figure 1](image-url) IFN-γ mRNA was not detected in any of the stimulated Langerhans cells. IFN-γ mRNA expressions of stimulated Langerhans cells and spleen-derived CD11c+ DC of BALB/c mice were analyzed using RT-PCR, as previously described (Tada et al, 2000). Messenger RNA was obtained from Langerhans cells and DC at 0 h and 24 h after stimulation as indicated. The primers used were: IFN-γ (227 bp) sense, GCTCTGAGA-CATTGACCGT; IFN-γ antisense, AAAGAGATACTTGCTCTGC; glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 659 bp) sense, GAGGGACAGACCCCACTAA; GAPDH antisense, GGACAGAGG-GATGGAAGAGT. The denaturing, annealing, and extension were performed for 35 cycles at 95 °C for 1 min, 58 °C for 1 min, and 72 °C for 2 min, respectively.

Abbreviations: DC, dendritic cells; Mφ, macrophages.

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It is likely that IFN-γ production by DC plays an important role in innate immunity and subsequent Th1 responses, and IFN-γ also primes and activates Mφ and DC themselves to produce IL-12, which act on DC and Mφ to further induce IFN-γ production. In this context, it is rather surprising that Langerhans cells do not respond to such stimulations that are known to induce IFN-γ production of DC and Mφ, although the possibility still remains that Langerhans cells may secrete IFN-γ under some other conditions. It has also been shown, however, that splenic CD8+ DC produce more IFN-γ than do CD8- DC after stimulation with IL-12 (Ohteki et al., 1999). Another report argues that splenic CD4+CD8- DC, but not CD4 CD8+ DC or CD4+CD8+ DC, produce the highest amount of IFN-γ (Hochrein et al., 2001). The reason for the discrepancy between these two results is uncertain, but the results at least indicate that the ability of DC to produce IFN-γ depends on their subsets.

The skin covers the surface of the body, and it is frequently exposed to various pathogens. Therefore, our finding may be reasonable in preventing excessive IFN-γ production and exaggerated skin inflammation leading to skin damage. Otherwise, other cell types may substitute for Langerhans cells as a source of IFN-γ in the skin. Similarly to Langerhans cells, keratinocytes have been shown not to produce IFN-γ by any means (Akiba et al., 2001). In mouse skin, however, we have previously reported that dendritic epidermal T cells produce IFN-γ by the synergistic effect of IL-12 and IL-18 (Sugaya et al., 1999). In addition, these cells are known to possess a capacity to kill relevant skin-derived tumor targets (Kaminski et al., 1993). It is very likely that dendritic epidermal T cells play an important role in protecting the mouse skin together with Langerhans cells in the innate immunity. In human skin, however, the cells corresponding to mouse dendritic epidermal T cells remain to be elucidated.

Figure 2. Effect of cytokines on IFN-γ production of DC and Langerhans cells. Spleen-derived CD11c+ DC (A) and Langerhans cells 1.5 x 10⁶ per ml (B) were cultured with IL-12 (0.1, 1 ng per ml), IL-18 (0.1, 1, 10 ng per ml), and/or IL-4 (1, 10 ng per ml) for 48 h. The supernatants were collected and IFN-γ contents were measured by a mouse IFN-ELISA kit. Results are mean ± SE (n = 3).

This work was supported by grants from the Cell Science Research Foundation (AA), Ministry of Education of Japan (AA, KT), and Ministry of Health and Welfare of Japan (KT).

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Yayoi Tada, Akihiko Asahina, Hideki Fujita, Makoto Sugaya, and Kunihiko Tamaki

Department of Dermatology, Faculty of Medicine, University of Tokyo, Japan


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Yayoi Tada, Akihiko Asahina, Hideki Fujita, Makoto Sugaya, and Kunihiko Tamaki

Department of Dermatology, Faculty of Medicine, University of Tokyo, Japan