Natural Interferon α/β-producing Cells Link Innate and Adaptive Immunity

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Abstract

Innate immune responses to pathogens critically impact the development of adaptive immune responses. However, it is not completely understood how innate immunity controls the initiation of adaptive immunities or how it determines which type of adaptive immunity will be induced to eliminate a given pathogen. Here we show that viral stimulation not only triggers natural interferon (IFN)-α/β-producing cells (IPCs) to produce vast amounts of antiviral IFN-α/β but also induces these cells to differentiate into dendritic cells (DCs). IFN-α/β and tumor necrosis factor α produced by virus-activated IPCs act as autocrine survival and DC differentiation factors, respectively. The virus-induced DCs stimulate naïve CD4+ T cells to produce IFN-γ and interleukin (IL)-10, in contrast to IL-3–induced DCs, which stimulate naïve CD4+ T cells to produce T helper type 2 cytokines IL-4, IL-5, and IL-10. Thus, IPCs may play two master roles in antiviral immune responses: directly inhibiting viral replication by producing large amounts of IFN-α/β, and subsequently triggering adaptive T cell–mediated immunity by differentiating into DCs. IPCs constitute a critical link between innate and adaptive immunity.

Key words: dendritic cells • interferon α/β-producing cells • innate immunity • adaptive immunity • T cells

Introduction

Innate immunity has two functions: (i) directly killing pathogens and (ii) determining the initiation and type of adaptive immune responses (1, 2). In most cases, cells in the innate immune system indirectly perform the second function by inducing maturation of dendritic cells (DCs) with proinflammatory cytokines such as IL-1 and TNF-α (3–5). The mature DCs then initiate adaptive immune responses by strongly activating antigen-specific naïve T cells (6) and may also dictate the type of Th cell response to be Th1 or Th2 (7–10). Thus, different cell types in the immune system are specialized to perform a particular function and cooperate to create the whole immune response.

IFN-α/β plays an essential role in antiviral innate immunity (11) by directly inhibiting viral replication in infected cells (12). Recent studies have shown that CD4+CD11c+ type 2 DC precursors (pre-DC2s) in human blood are identical to natural IFN-α/β-producing cells (IPCs), which produce enormous amounts of IFN-α/β in response to viruses (13–15). Thus, IPCs or pre-DC2s may represent a crucial effector cell type in antiviral innate immunity. Interestingly, unlike other effector cell types in the innate immune system such as neutrophils, macrophages, and mast cells, IPCs have the potential to differentiate into DCs when cultured with IL-3 and CD40 ligand (16). However, these factors, mainly derived from activated T cells, may not represent the earliest physiological signals for DC differentiation in antiviral immune responses. Therefore, we asked (i) whether viruses can directly induce IPCs to differentiate into DCs after triggering the antiviral innate effector function of IPCs and (ii) what kind of T cell responses virus-induced DCs would initiate.

Materials and Methods

Isolation and Culture of Cells. Monocytes, CD11c+ DCs, and IPCs were isolated from human peripheral blood as described (3, 16). To isolate monocytes, total PBMCs were centrifuged on 52% Percoll (Amersham Pharmacia Biotech). The low-density...
cells were depleted of lymphocytes with a mixture of anti-CD2, anti-CD3, anti-CD8, anti-CD19, anti-CD20, and anti-CD56 mAbs and with magnetic beads coated with goat anti-mouse IgG (Dynabeads M-450; Dynal). To isolate CD11c<sup>+</sup> DCs and IPCs, total PBMCs were depleted of lymphocytes and monocytes with a mixture of anti-CD3, anti-CD14, anti-CD16, anti-CD19, and anti-CD56 mAbs and with magnetic beads (Dynal). CD4<sup>+</sup>CD11c<sup>+</sup>G<sub>lin</sub> and CD4<sup>+</sup>CD11c<sup>+</sup>G<sub>lin</sub> cells were isolated as CD11c<sup>+</sup> DCs and IPCs, respectively, by cell sorting. The cells were cultured in RPMI 1640 containing 10% FCS at 2 x 10<sup>4</sup> cells per 200 µl in round-bottomed 96-well culture plates in the presence of 10<sup>6</sup> PFU/ml HSV-1 (KOS strain) attenuated with gamma irradiation or 10<sup>6</sup> PFU/ml intact influenza virus (PR8 strain). The concentrations of viruses that induced optimal survival and IFN production were selected. Higher concentrations of viruses induced cell death, probably due to an overwhelming cytopathic effect (data not shown).

Flow Cytometric Analysis. IPCs were cultured for 3 d with 10<sup>6</sup> PFU/ml HSV, 10 ng/ml IL-3 (R&D Systems), 500 IU/ml IFN-a<sub>2b</sub> (Schering-Plough), 10 ng/ml TNF-α (R&D Systems), 10 ng/ml IL-6 (DNAX), 20 µg/ml mouse anti-human TNF-α mAb (MP9-20A4; a gift from J. Abrams, DNAX), or 20 µg/ml mouse anti-human IL-6 mAb (MQ2-39C3; DNAX). The resulting cells were stained with FITC-conjugated anti-HLA-A,B,C (G46-2.6; PharMingen), FITC-conjugated anti-HLA-DR (L243; Becton Dickinson), PE-conjugated anti-CD80 (L307.4; Becton Dickinson), PE-conjugated anti-CD86 (2331; PharMingen), or an isotype control Ab and were analyzed with a FACScan™ flow cytometer (Becton Dickinson). Dead cells were excluded by staining with propidium iodide.

Figure 1. HSV induces differentiation of IPCs into DCs. (A) Cell numbers of IPCs cultured in different conditions. Viable cells were counted by trypan blue exclusion. The data shown are representative of three experiments. (B) Phenotype of fresh IPCs, HSV-stimulated IPCs, and IL-3-stimulated IPCs. Open histograms represent cells stained with isotype-matched control mAbs. The data shown are representative of four experiments. (C) Allogeneic mixed lymphocyte reaction. Naive CD4<sup>+</sup> T cells from cord blood were cocultured with different numbers of fresh IPCs, HSV-DCs, or IL-3-DCs for 6 d. Error bars indicate SD. The data shown are representative of five experiments.
L293.1 (Becton Dickinson). After 6 d of priming, T cells were washed and restimulated at 10⁶ cells/ml with anti-CD3 and anti-CD28 for 24 h for ELISA or for 5 h for intracellular cytokine staining. 10 μg/ml brefeldin A (Epicentre Technologies) was added at 2.5 h for intracellular staining.

Quantitation of Cytokine Secretion by ELISA. ELISA kits from the following companies were used to analyze cytokine production: IFN-α (Biosource International), IFN-β (FUJIREBIO), IL-18 (MBL Medical and Biological Lab. Co.), IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-10, IL-12, IL-15, IFN-γ, lymphotoxin α, and GM-CSF (R&D Systems).

Flow Cytometric Analysis of Intracellular Cytokines. Intracellular cytokines produced by T cells were analyzed as described (8).

**Results**

Viruses Directly Induce IPCs to Differentiate into DCs. We and others previously showed that IPCs die rapidly and that few viable cells are found after 3 d of culture in medium alone (reference 16, 20, 21; Fig. 1 A). However, ~50% of the initial numbers of the cells were viable after 3 d of culture with either HSV or IL-3. IPCs differentiate into immature DCs when cultured with IL-3 (16). Similarly, IPCs acquired DC morphology after 3 d of culture with HSV (data not shown). They also increased the expression of MHC class I and class II antigens as well as co-stimulatory molecules CD80 and CD86 (Fig. 1 B). IPCs stimulated with HSV for 3 d (HSV-DCs) were as potent as IPCs stimulated with IL-3 for 3 d (IL-3–DCs) in inducing proliferation of allogeneic naive CD4⁺ T cells (Fig. 1 C). Influenza virus also induced IPCs to differentiate into DCs (data not shown). These data demonstrate that viruses can directly induce differentiation of the DC precursors into DCs in the absence of exogenous cytokines.

IFN-α and TNF-α Function as Autocrine Survival and Differentiation Factors of IPCs, Respectively. Next we tested whether endogenous cytokines from IPCs induced by viruses are responsible for the survival and differentiation of the cells. Consistent with previous reports (14, 15), IPCs produced large amounts of IFN-α and IFN-β within 24 h in response to HSV, whereas monocytes and CD11c⁺ DCs produced only small or undetectable amounts (Fig. 2). IPCs also produced significant amounts of TNF-α and IL-6 (Fig. 2), but IL-1α, IL-1β, IL-3, IL-10, IL-12, IL-15, IL-18, IFN-γ, lymphotoxin α, and GM-CSF were undetectable (data not shown). IFN-α, but not TNF-α (Fig. 3 A) or IL-6 (data not shown), was found to maintain the survival of IPCs during 3 d of culture, suggesting that IFN-α is a virus-induced autocrine survival factor for IPCs. IFN-α did not upregulate CD80 and CD86 on IPCs (Fig. 3 B). However, TNF-α was found to upregulate CD80 and to a lesser extent CD80 on IPCs when added with IFN-α (Fig. 3 B). Anti–TNF-α diminished the expression of CD80 and CD86 on HSV-stimulated IPCs (data not shown). On the other hand, IL-6 or anti–IL-6 did not affect the expression of CD80 and CD86 (data not shown). Thus, IFN-α/β and TNF-α may represent virus-induced autocrine survival factors and a partial differentiation factor, respectively, for IPCs to become DCs.

IL-3–DCs and HSV-DCs Induce Distinct T Cell Differentiation. To examine the role of HSV-DCs in naive T helper cell differentiation, allogeneic naive CD4⁺ T cells were cultured for 6 d with IL-3–DCs, HSV-DCs, or anti-

![Figure 2: Cytokine production by different populations of blood cells stimulated with HSV. Cells were stimulated with HSV for 24 h, and cytokine concentrations in the supernatants were measured by ELISA. PBMC, total PBMCs; Mono, monocytes; CD11c⁺ DC, FACS-sorted CD11c⁺lin immature DCs; IPC, FACS-sorted CD4⁺ CD11c⁻lin cells. Error bars indicate SD. The data shown are representative of four experiments.](image-url)
CD3 and anti-CD28. The activated T cells were restimulated with anti-CD3 and anti-CD28 for either 5 h for intracellular cytokine staining or 24 h for cytokine ELISA of the culture supernatants. Whereas IL-3–DCs induced naïve CD4+ T cells to produce high levels of IL-4, IL-5, and IL-10 and a low level of IFN-γ as reported (8), HSV-DCs induced naïve CD4+ T cells to produce high levels of IFN-γ and IL-10 and a low level of IL-4 (Fig. 4 A). Intracellular cytokine staining showed that HSV-DCs induced CD4+ T cells to differentiate into three subpopulations with respect to IFN-γ and IL-10 expression: (i) IFN-γ+/IL-10−, (ii) IFN-γ+/IL-10+, and (iii) IFN-γ−/IL-10− (Fig. 4 B). IL-12 (22) and IFN-α (de Waal-Malefyt, R., personal communication and reference 23) were shown to induce T cells to produce IFN-γ and IL-10. However, IPCs did not produce a detectable level of IL-12 during 3 d of culture with HSV (data not shown), and anti-IL-12 mAb did not inhibit IFN-γ or IL-10 production by T cells cultured with HSV-DCs (Fig. 4 C), suggesting that IL-12 is not involved in IFN-γ and IL-10 induction in this system. A mixture of anti-IFN-α, anti-IFN-β, and anti-IFN-α/β receptor Abs significantly diminished the induction of IFN-γ but not IL-10 in the T cell–HSV-DC coculture (Fig. 4 C), indicating that IFN-α/β from HSV-DCs is responsible for T cell production of IFN-γ but not IL-10. HSV-DCs may stimulate T cells to produce a high level of IL-10 by an IL-12- and IFN-α/β-independent mechanism.

**Discussion**

DC precursors develop into mature DCs in two steps: first, differentiation into immature DCs, and second, development from immature into mature DCs (6). Recent studies have shown that whereas microbial stimuli trigger the final maturation of DCs (24), differentiation of DC precursors into immature DCs is dependent on cytokines such as GM-CSF, TNF-α, and IL-4 (3, 25, 26). However, the physiological relevance of these cytokines in DC differentiation in vivo remains to be established (27). Our study demonstrated that viral stimulation alone is capable of inducing DC precursors to differentiate into DCs in the absence of exogenous cytokines. This, together with the ability of monocytes to differentiate into DCs after undergoing transendothelial migration and phagocytosis (28, 29), suggests that microbes, as well as cytokines, may directly trigger differentiation of DC precursors into DCs.

The finding that IL-3–DCs and HSV-DCs induce distinct types of T cell differentiation illustrates the critical role of innate immunity in determining the type of adaptive immune responses, depending on the nature of pathogens (30). Upon invasion of certain parasites or allergens, IL-3 produced by activated mast cells (31) may cause IPCs to differentiate into Th2-inducing DCs, which may contribute to the establishment of T cell-mediated allergic responses. On the other hand, HSV-DCs induce naïve CD4+ T cells to produce IFN-γ and IL-10, different from a classical Th1 or Th2 type. Existence of a T cell population producing IFN-γ and IL-10 during intracellular infection with viruses (32), bacteria (33), and parasites (34) suggests that IFN-γ- and IL-10-producing T cells may play an important role in immune responses to intracellular patho-

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**Figure 3.** IFN-α and TNF-α function as autocrine survival and differentiation factors of IPCs, respectively. (A) Cell numbers of IPCs cultured without stimulation or with HSV, IFN-α, or TNF-α. Viable cells were counted by trypan blue exclusion. The data shown are representative of three experiments. (B) Expression of CD80 and CD86 on fresh IPCs and IPCs cultured with IFN-α, IFN-α and TNF-α, or HSV for 3 d. Few cells remained viable in culture with TNF-α alone, as shown in A. Open histograms represent cells stained with isotype-matched control mAbs. The data shown are representative of four experiments.
In fact, it has been suggested that IL-10 protects the host against detrimental effects of excessive cellular immune responses elicited during acute infection (35). This study, together with previous studies (8, 14, 15), indicates that IPCs represent a unique cell lineage within the immune system, which performs the two master functions of innate immunity during their lifetime: (i) killing of viruses and (ii) initiation and dictation of adaptive immune responses. Importantly, the functional transition from IPCs to DCs occurs with viral stimulation alone in the absence of any exogenous factors. In addition to viruses, bacterial stimuli such as heat-killed Staphylococcus aureus also induce IPCs to produce large amounts of IFN-α/β (36) and to upregulate CD80 and CD86 (data not shown). These findings, together with pleiotropic effects of IFN-α/β on various cell types in the immune system such as macrophages (37), NK cells (38), and T cells (39–41), suggest that IPCs represent a crucial cell type in the immune system that, upon recognizing various types of pathogens, promptly alerts the immune system to “dangers” (42) by producing vast amounts of IFN-α/β and subsequently initiates adaptive immune responses by differentiating into DCs. Furthermore, IPCs may be involved in allergic responses by differentiating into Th2-inducing DCs in response to IL-3. This marked versatility of IPCs distinguishes them from other cell types in the immune system that have only limited functions and suggests that IPCs may play a key role in integrating the innate and adaptive aspects of various immune responses.

In conclusion, this study offers an account of the cellular and molecular mechanisms by which innate immunity connects with and shapes adaptive immunity. Owing to the

![Figure 4](image-url)

**Figure 4.** IL-3–DCs and HSV-DCs induce different types of differentiation of naive CD4+ T cells. Allogeneic naive CD4+ T cells were cultured for 6 d with IL-3–DCs, HSV-DCs, or anti-CD3 and anti-CD28. T cells were restimulated with anti-CD3 and anti-CD28 for 24 h (A) or 48 h (C) for ELISA or for 5 h for intracellular staining (B). (A) Quantitation of IFN-γ, IL-4, IL-5, and IL-10 by ELISA. Error bars indicate SD. The data shown are representative of four experiments. (B) Intracellular IFN-γ, IL-4, and IL-10 staining of T cells cultured with HSV-DCs. The percentages in each quadrant are indicated on the plot. The data shown are representative of three experiments. (C) IFN-γ and IL-10 production by T cells cultured with HSV-DCs in the presence of neutralizing anti–IL-12 mAb and/or a mixture of anti–IFN-α, anti–IFN-β, and IFN-α/β receptor Abs. The same concentration of anti–IL-12 mAb inhibited IFN-γ production by T cells cultured with anti-CD3, anti-CD28, and 10 ng/ml IL-12 (data not shown). Error bars indicate SD. The data shown are representative of three experiments.
apparent involvement of IPCs in a variety of immune responses, enhancing or suppressing functions of these cells may serve as potential therapies for infectious diseases, cancers, and allergic diseases.

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