

**SONY**

Watch and learn how the ID7000 software enables users at all expertise levels to acquire and analyze high parameter data

[View Tutorial Videos](#)

*The Journal of*  
**Immunology**

RESEARCH ARTICLE | APRIL 01 2004

## Plasmacytoid Dendritic Cells Regulate Th Cell Responses through OX40 Ligand and Type I IFNs<sup>1</sup> **FREE**

Tomoki Ito; ... et. al

*J Immunol* (2004) 172 (7): 4253–4259.

<https://doi.org/10.4049/jimmunol.172.7.4253>

### Related Content

OX40 Cooperates with ICOS To Amplify Follicular Th Cell Development and Germinal Center Reactions during Infection

*J Immunol* (January,2017)

Central Roles of OX40L–OX40 Interaction in the Induction and Progression of Human T Cell–Driven Acute Graft-versus-Host Disease

*Immunohorizons* (March,2019)

Constitutive OX40/OX40 Ligand Interaction Induces Autoimmune-Like Diseases

*J Immunol* (October,2002)

# Plasmacytoid Dendritic Cells Regulate Th Cell Responses through OX40 Ligand and Type I IFNs<sup>1</sup>

Tomoki Ito,\* Ryuichi Amakawa,<sup>2\*</sup> Muneo Inaba,<sup>†</sup> Toshiyuki Hori,<sup>‡</sup> Maiko Ota,\* Kengo Nakamura,\* Masashi Takebayashi,\* Michihiko Miyaji,\* Tomoo Yoshimura,<sup>§</sup> Kayo Inaba,<sup>¶</sup> and Shirou Fukuhara\*

Dendritic cells (DCs) show a functional plasticity in determining Th responses depending on their maturational stage or on maturational signals delivered to the DCs. Human plasmacytoid DCs (PDCs) can induce either Th1- or Th2-type immune responses upon exposure to viruses or IL-3, respectively. In this study we have investigated the Th-polarizing capacity of PDCs after short (24-h) or long (72-h) culture with stimuli and have assessed the expression and function of OX40 ligand (OX40L) in PDC-mediated Th polarization in addition to type I IFN-dependent responses. IL-3-treated PDCs expressed OX40L, but produced almost no IFN- $\alpha$  in response to T cell stimulation (CD40 ligand or T cell interaction), resulting in the preferential priming of Th2 cells through OX40L-dependent mechanisms. Meanwhile, PDCs were rapidly endowed by viral infection (Sendai virus) with a high potency to develop IFN- $\gamma$ -producing Th cells depending on their capacity to residually produce IFN- $\alpha$ . Although Sendai virus-stimulated PDCs simultaneously expressed OX40L in their maturational process, the Th1-inducing effect of endogenous type I IFNs may overcome and thus conceal the OX40L-dependent Th2 responses. However, during maturation in response to Sendai virus over the longer 72-h period, the expression level of OX40L was up-regulated, whereas the residual IFN- $\alpha$ -producing ability was down-regulated, and consequently, the PDCs with prolonged Sendai virus stimulation induced Th2 responses to some extent. Thus, PDCs have the distinct means to dictate an appropriate response to environmental stimuli. *The Journal of Immunology*, 2004, 172: 4253–4259.

Several lines of evidence indicate that plasmacytoid cells (PCs),<sup>3</sup> referred to as natural IFN- $\alpha\beta$ -producing cells (1, 2), are dendritic cell (DC) precursors (3, 4). IL-3 or viral infection can trigger PCs to develop into DCs, which can initiate Th cell responses (5–7). In immune responses, the functional properties of PCs are altered during their maturational process; PCs act as type I IFN producers in enhancing innate immunity at microbial recognition and develop into DCs (plasmacytoid DCs (PDCs)), which eventually act as APCs in adaptive immunity (2, 5, 6). To provide the essential functions in priming naive T cells in lymphoid tissues, PDCs require CD40 ligands (CD40L), which are delivered from T cells and cause the terminal maturation of DCs (3, 4).

Recent research has clarified the functional plasticity of DCs in determining T cell responses (8, 9). Most myeloid DC subsets have some capacity to produce IL-12 and thereby to induce Th1 devel-

opment. However, this capacity varies with their stage of maturation and the environmental stimuli delivered to them (10–12). In contrast, when PCs sense viruses, they rapidly produce a large amount of IFN- $\alpha\beta$  and differentiate into DCs, which can facilitate Th1 cell development (6, 7). Meanwhile, IL-3-treated PDCs favor Th2 cell responses (4, 6, 13). However, it remains largely unknown how PDCs dictate the type of Th cell response. Upon viral infection, PCs produce IFN- $\alpha\beta$ , which is responsible for IFN- $\gamma$  production by T cells (6, 7). In contrast, we still do not know which PDC-derived factors actively induce Th2 development. One possible mechanism underlying the Th2 development by PDCs is the default mechanism due to the lack of Th1-inducing cytokines such as IL-12 or IFN- $\alpha\beta$ . However, a previous study has shown that the addition of IL-12 to the coculture of Th cells and PDCs preincubated with IL-3 plus CD40L increases the frequency of IFN- $\gamma$ -producing Th cells, but does not decrease the frequency of IL-4-producing Th cells (4). Thus, the default mechanism mentioned above cannot completely explain this issue.

Several studies have pointed to the role of the OX40-OX40 ligand (OX40L) pathway, which selectively regulates Th2 cell development in mice (14–19) and humans (20, 21). OX40 is expressed on activated CD4<sup>+</sup> T cells (22, 23), whereas OX40L is induced on activated mouse CD11c<sup>+</sup> DCs (24, 25) and human monocyte-derived DCs (23, 26). The stimuli triggered by OX40L on these DCs elicit a costimulatory role in preferential priming of Th2 cells (24, 25, 27). However, it is not clear at present whether OX40L is induced on human PDCs or whether it exhibits costimulatory functions in the PDC-mediated Th2 responses.

Monocyte-derived DCs can produce IL-12 for a limited time soon after activation (10). Similarly, PCs/PDCs can actively produce IFN- $\alpha\beta$  only within a limited time after microbial stimulation (1, 28, 29), suggesting that the Th1-polarizing capacity of PDCs is

\*First Department of Internal Medicine, Kansai Medical University, Osaka, Japan; <sup>†</sup>First Department of Pathology, Kansai Medical University, Osaka, Japan; <sup>‡</sup>First Department of Internal Medicine, Kyoto University, Kyoto, Japan; <sup>§</sup>Department of Obstetrics and Gynecology, Kansai Medical University, Osaka, Japan; and <sup>¶</sup>Department of Animal Development and Physiology, Graduate School of Biostudies, Kyoto University, Kyoto, Japan

Received for publication May 14, 2003. Accepted for publication January 22, 2004.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup> This work was supported by research fellowships from the Japan Society for the Promotion of Science for Young Scientists.

<sup>2</sup> Address correspondence and reprint requests to Dr. Ryuichi Amakawa, First Department of Internal Medicine, Kansai Medical University, 10-15 Fumizono-cho, Moriguchi City, Osaka 570-8506, Japan. E-mail address: amakawa@takii.kmu.ac.jp

<sup>3</sup> Abbreviations used in this paper: PC, plasmacytoid cell; CD40L, CD40 ligand; 1d-SV-PDC, PDC prestimulated with Sendai virus for 1 day; DC, dendritic cell; OX40L, OX40 ligand; PDC, plasmacytoid DC; sCD40, soluble CD40.

dependent on the duration of the stimulus. In this study we investigate the kinetics of OX40L expression and IFN- $\alpha$  production in PDCs in response to the two major PDC-stimulating factors, IL-3 and viruses, and assess their functional roles in the regulation of the quality of Th responses.

## Materials and Methods

### Media and reagents

RPMI 1640 supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 ng/ml streptomycin, and heat-inactivated 10% FCS (Irvine Scientific, Santa Ana, CA) was used for the cell culture throughout the experiments. Recombinant human IL-3 (10 ng/ml; PeproTech EC, London, U.K.), baculovirus-expressed soluble CD40 ligand (sCD40L: trimer form, 1/100 dilution) (30, 31), and UV-irradiated Sendai virus (at 5 hemagglutinating units/ml; HVJ: Cantell strain, provided by Sumitomo Pharmaceuticals, Ehime, Japan) were used for cell cultures. The mAb against human OX40L/gp34, ik-1 (mouse IgG1) (23, 32), was used for analysis of surface expression on cells and for neutralization of the OX40/OX40L pathway in DC-T cell coculture.

### Isolation of blood PCs

Peripheral blood PCs were isolated according to the modified protocol described previously (13, 33, 34). Briefly, the DC-enriched population (CD4<sup>+</sup>/CD3<sup>-</sup>/CD14<sup>-</sup> cells) was obtained from PBMCs by negative and subsequent positive immunoselection. The CD11c<sup>-</sup>/lineage<sup>-</sup>/DR<sup>+</sup> cells (PCs) were sorted by an EPICS ALTRA flow cytometer (Coulter, Hialeah, FL) using PE-labeled anti-CD11c (Leu-M5; BD Biosciences, Sunnyvale, CA); a mixture of FITC-labeled mAbs against lineage markers, CD3 (M2AB; Exalpa, Boston, MA), CD14 (FWKW-1; Exalpa), CD15 (Leu-M1; BD Biosciences), CD16 (J5511; Exalpa), CD19 (SJ25C1; BD Biosciences), and CD56 (NCAM16.2; BD Biosciences); and PE-cyanin 5.1 (PC5)-labeled HLA-DR (Immu-357; ImmunoTech, Marseilles, France). The purity of PCs was >98%.

### Analyses of T cell polarization stimulated by PDCs

CD4<sup>+</sup>/CD45RA<sup>+</sup> naive T cells were obtained from allogeneic healthy volunteers using a CD4<sup>+</sup> T cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany), followed by positive selection with CD45RA-conjugated microbeads (Miltenyi Biotec) or sorting with staining of PE-labeled CD45RA mAb (HI100; BD Biosciences). The purity of the cells was 95% or greater by reanalysis. PDCs preincubated with IL-3 or Sendai virus for 24 or 72 h were washed and then cocultured with allogeneic CD4<sup>+</sup>/CD45RA<sup>+</sup> naive T cells (10<sup>5</sup> cells/well; DC-T ratio, 1:5) in 96-well, round-bottom, tissue culture plates. In some experiments a mixture of neutralizing rabbit polyclonal anti-human IFN- $\alpha$  Ab (2000 neutralization units/ml), neutralizing rabbit polyclonal anti-human IFN- $\beta$  Ab (1000 neutralization units/ml), mouse anti-human IFN- $\alpha$  receptor mAb (20  $\mu$ g/ml; PBL Biomedical Laboratories, New Brunswick, NJ), and/or anti-OX40L mAb (ik-1; 50  $\mu$ g/ml) was added to the coculture. Heat-inactivated rabbit serum (Vector Laboratories, Burlingame, CA) and mouse IgG1 (R&D Systems, Minneapolis, MN) were used as a control. Seven days after coculture, T cells were washed and restimulated with PMA (50 ng/ml) and ionomycin (2  $\mu$ g/ml) at 10<sup>6</sup> cells/ml for 24 h for ELISA or 6 h for intracellular cytokine staining. ELISA kits for IFN- $\gamma$ , IL-4, IL-5, and IL-13 (R&D Systems) were used to analyze T cell cytokine production. For intracellular cytokine analyses, brefeldin A (10  $\mu$ g/ml) was added during the last 2 h. The cells were stained with PE-cyanin 5.1 (PC5)-labeled anti-CD4 (ImmunoTech), then with PE-labeled anti-IL-4 (BD Biosciences) plus FITC-labeled anti-IFN- $\gamma$  mAb (BD Biosciences) using FIX and PERM kit (Caltag Laboratories, Burlingame, CA).

### Measurements of IFN- $\alpha$ production from PDCs

The sorted blood PCs were cultured with IL-3 or Sendai virus for 24 or 72 h in 96-well, round-bottom, tissue culture plates at 5  $\times$  10<sup>4</sup> cells in 200  $\mu$ l of medium/well. After removal of each supernatant and washing, the adjusted number of cells were recultured with medium alone or restimulated with sCD40L or allogeneic CD4<sup>+</sup>/CD45RA<sup>+</sup> cells (5  $\times$  10<sup>4</sup> PDCs/5  $\times$  10<sup>4</sup> T cells/200  $\mu$ l) for an additional 24 h, respectively, and the supernatants were collected. The production of IFN- $\alpha$  and IL-12 p70 in the supernatants was determined by ELISA (Endogen, Woburn, MA).

### Analyses of expression of OX40L and costimulatory molecules

Total RNA was prepared from freshly isolated PCs and PDCs after culture using TRIzol reagent (Life Technologies, Grand Island, NY). RT reactions

were performed on 2  $\mu$ g of total RNA using an oligo(dT) primer with SuperScript II (Life Technologies). One-microliter aliquots of the DNA resulting from each RT reaction were subjected to PCR. The temperature profiles of PCR were as follows: an initial denaturation step of 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, and a final elongation step of 72°C for 7 min. The sequences of primers were as follows: OX40L: forward, 5'-CCCAGATTGTGAAGATGGAA-3'; and reverse, 5'-GCCTGGTTTTAGATATTGCC-3'; and  $\beta$ -actin: forward, 5'-GACCCAGATCATGTTTGGACCTTCAACAC-3'; and reverse, 5'-TACTTGGCGCTCAGGAGGAGCAATGATCTTG-3'. A  $\Phi$ X174 DNA-HaeIII digest (New England Biolabs, Beverly, MA) was used as a DNA size marker. To evaluate surface OX40L expression, the cells were stained with anti-OX40L mAb (ik-1) or isotype-matched control mAb, followed by PE-labeled goat anti-mouse IgG (BD Biosciences). To analyze the expression of costimulatory molecules, the cells were stained with FITC-labeled anti-CD40 (5C3; BD Biosciences), CD86 (2331; BD Biosciences), or CD80 (BB-1; Ancell, Bayport, MN) and analyzed with a FACScan (BD Biosciences).

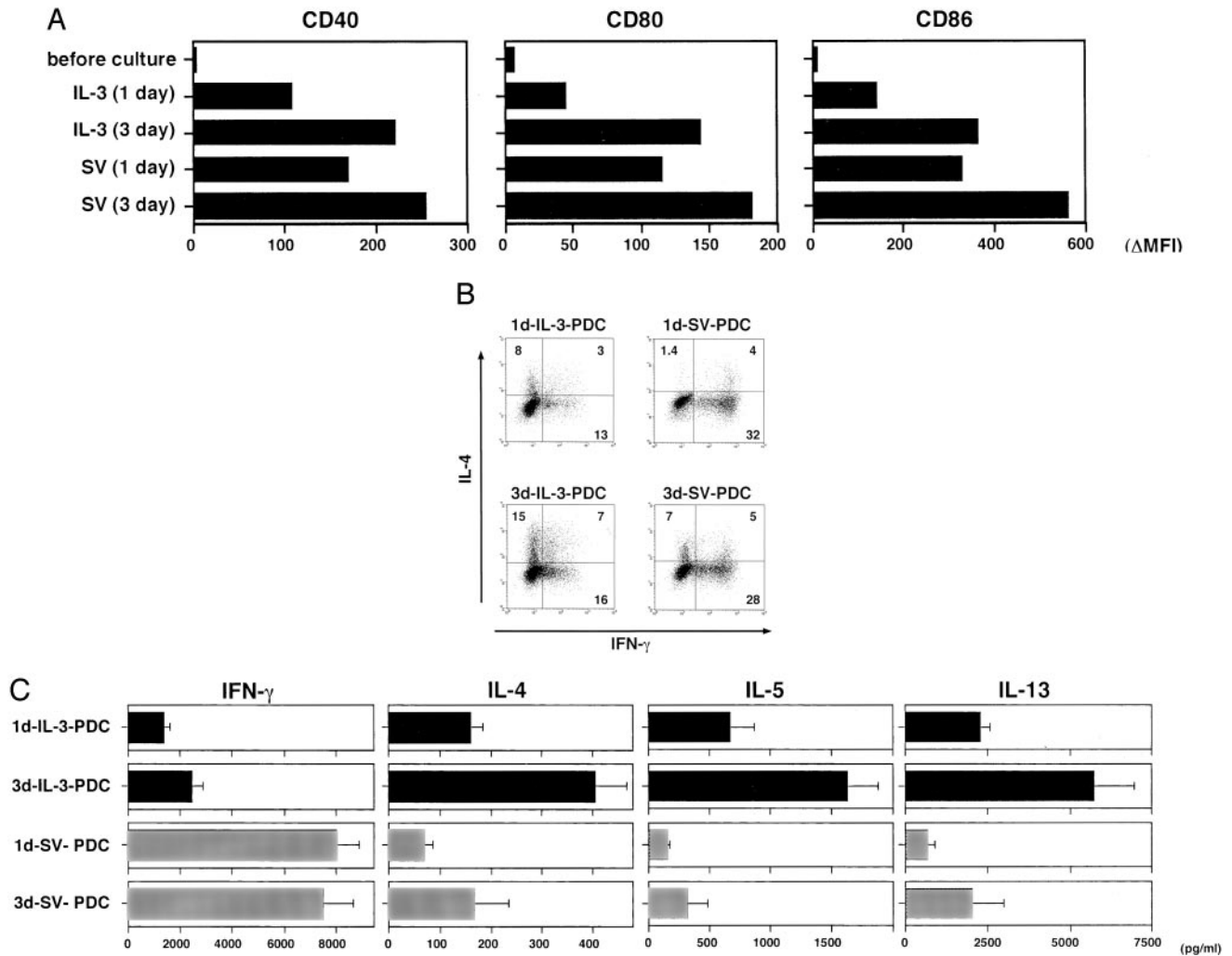
## Results

### PDCs induce different Th responses depending on the developmental stimuli and the duration of stimulation

We first analyzed PDC functions in polarizing Th cells when PDCs were stimulated with IL-3 or Sendai virus for 1 or 3 days. The costimulatory molecules (CD40, CD80, and CD86), which are the hallmarks of DC differentiation, were up-regulated modestly on PDCs by IL-3 during 1-day culture and were further up-regulated during 3-day culture (Fig. 1A). Sendai virus readily up-regulated all markers after the 1-day culture, indicating that Sendai virus rapidly induces PDCs to differentiate into mature DCs. These PDCs were cocultured with allogeneic CD4<sup>+</sup> naive T cells for 7 days, and the cytokine profiles of the activated Th cells were examined by intracellular staining (Fig. 1B) and ELISA analysis of the culture supernatants (Fig. 1C). PDCs pretreated with IL-3 for 1 day (1d-IL-3-PDCs) could stimulate naive Th cells to develop into the polarized (IL-4- or IFN- $\gamma$ -producing) Th cells to a certain extent (Fig. 1B). Compared with 1d-IL-3-PDCs, 3d-IL-3-PDCs increased the frequency of IL-4-producing cells, as determined by intracellular staining (Fig. 1B), and also induced the higher amounts of IL-4, IL-5, and IL-13 production (Fig. 1C) in Th cells. In contrast, PDCs prestimulated with Sendai virus for 1 day (1d-SV-PDCs) showed a high potency to preferentially induce IFN- $\gamma$ -producing T cell development, but induced low levels of IL-4, IL-5, and IL-13 production in Th cells. Compared with 1d-SV-PDCs, 3d-SV-PDCs developed a comparable level of IFN- $\gamma$  production in Th cells. However, they concomitantly induced an increased number of IL-4-producing T cells (Fig. 1B) and moderate levels of IL-4, IL-5, and IL-13 production (Fig. 1C) in Th cells. These findings indicate that PDCs cultured with IL-3 can preferentially induce Th2 cells, and that viral infection rapidly equips PDCs with the ability to evoke IFN- $\gamma$  production in Th cells. In addition, as a result of prolonged stimulation with Sendai virus, PDCs acquire a moderate ability to induce Th2 cells.

### Residual capacity to produce IFN- $\alpha$ in PDCs in response to Sendai virus

PCs are the major source of IFN- $\alpha$ , which not only plays a central role in inducing innate immunity against microbial infections but can also facilitate Th1 development in adaptive immunity (35–37). However, it is likely that the capacity of PCs to produce IFN- $\alpha$  is transient (1, 28, 29). Therefore, to clarify whether PDC-derived IFN- $\alpha$  actually participates in the Th cell priming, we next investigated whether the Sendai virus-infected PDCs have a residual capacity to produce IFN- $\alpha$  when subsequently stimulated with sCD40L or allogeneic naive CD4<sup>+</sup> T cells. Freshly isolated PCs can produce a large amount of IFN- $\alpha$  in response to Sendai virus during the first 24 h (14,851  $\pm$  1,225 pg/ml;  $n$  = 4), but cannot



**FIGURE 1.** Th-polarizing ability of PDCs after 1- or 3-day culture with the different stimuli. Freshly isolated PCs were cultured with IL-3 or Sendai virus (SV) for 1 or 3 days. *A*, Expression intensities of CD40, CD80, and CD86 on the cultured cells were analyzed by flow cytometry. The results are shown as the change in mean fluorescence intensity ( $\Delta$ MFI), which is calculated by subtraction of the MFI with the isotype-matched control from that with each mAb. The data shown are representative of three independent experiments. *B* and *C*, The PDCs were cocultured with allogeneic CD4<sup>+</sup> naive T cells for 7 days, then T cells were restimulated with PMA and ionomycin. The T cell cytokine profile was analyzed intracellularly by flow cytometry (*B*) and measured in supernatants by ELISA (*C*; details in *Materials and Methods*). *B*, The percentages of the respective cytokine-producing T cells are indicated in each dot-blot profile. This figure represents the results from one of four experiments. *C*, Results are shown as the mean  $\pm$  SEM of four experiments.

produce any under culture with IL-3 ( $<3$  pg/ml;  $n = 4$ ; Fig. 2A). This result is in line with previous studies (1, 2, 34). As shown in Fig. 2B, 1d-SV-PDCs could still residually produce a substantial amount of IFN- $\alpha$  during the following 24 h, although their ability to produce IFN- $\alpha$  was not significantly increased when subsequently stimulated by sCD40L or when cocultured with allogeneic naive Th cells. In contrast, residual IFN- $\alpha$  production by 3d-SV-PDCs was detectable, but at a much lower level than that of 1d-SV-PDCs. In contrast to Sendai virus-stimulated PDCs, IL-3-treated PDCs (both 1d-IL-3-PDCs and 3d-IL-3-PDCs) can barely produce IFN- $\alpha$  even after the subsequent activation with CD40L or T cell interaction. No bioactive IL-12 p70 was detectable in any of the culture conditions described above ( $<3$  pg/ml;  $n = 4$ ; data not shown).

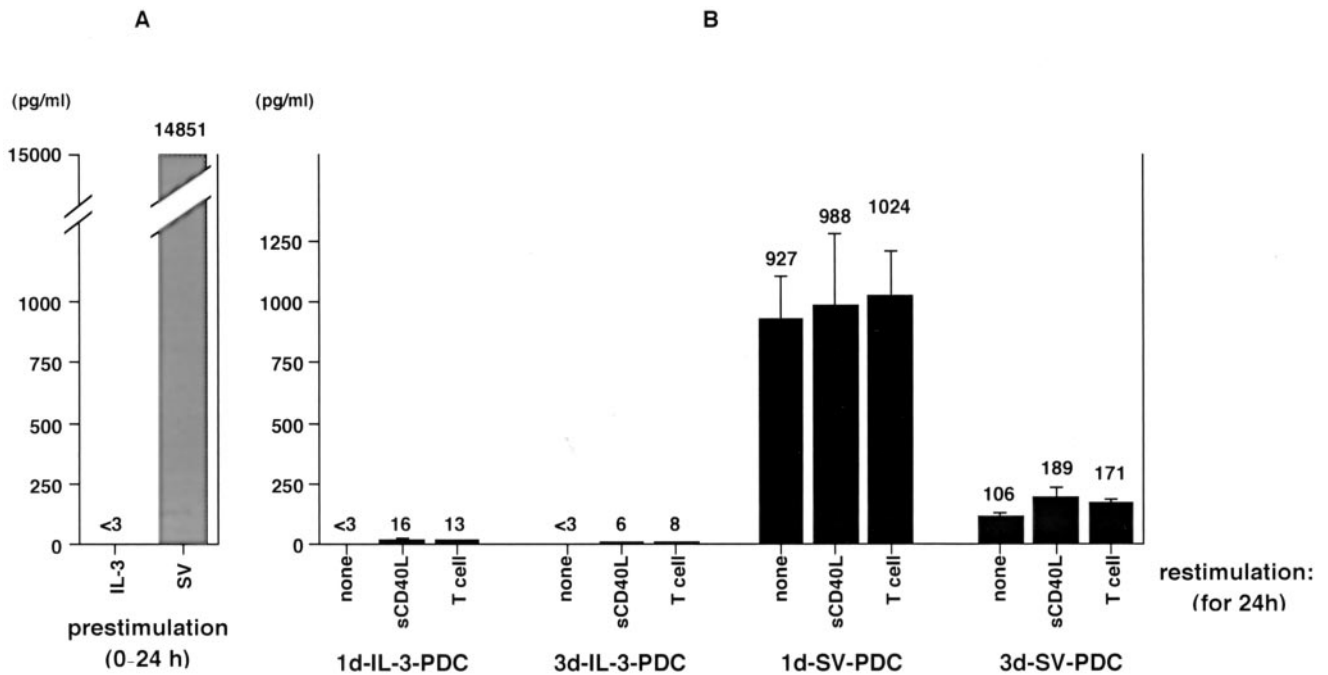
#### Expression of OX40L on PDCs

We next examined the expression of OX40L on PDCs after the culture with IL-3 or Sendai virus by RT-PCR (Fig. 3A) and flow cytometry (Fig. 3B). OX40L mRNA and its surface protein could

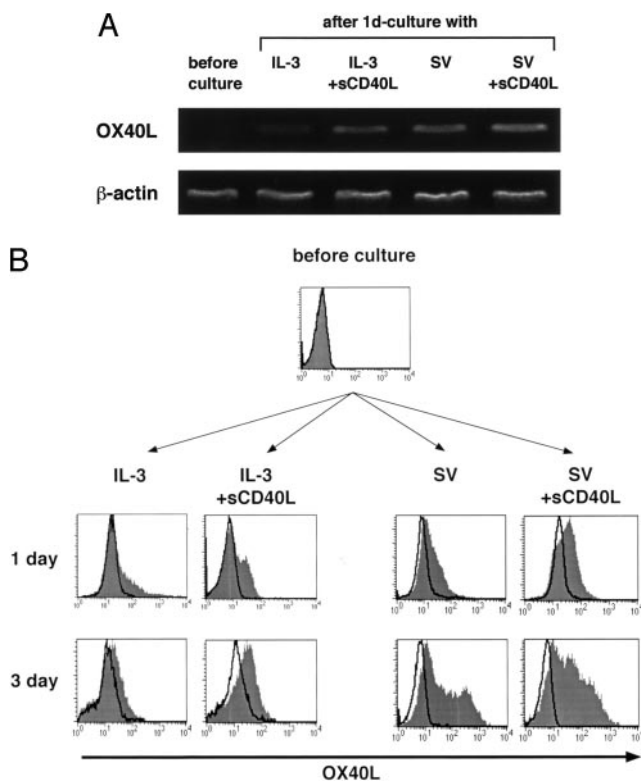
not be detected in freshly isolated PCs. After 1-day culture, IL-3 induced PCs to express OX40L mRNA at a very low level, whereas Sendai virus induced it at a definitive level (Fig. 3A). Furthermore, the OX40L expression induced by IL-3 or Sendai virus was up-regulated by the presence of sCD40L. Consistent with this expression profile of OX40L mRNA, a fraction of the cells began to express surface OX40L after 1-day culture with IL-3, and a majority of the cells became OX40L-positive at low level even after 3-day culture. The addition of sCD40L up-regulated their OX40L expression. Meanwhile, Sendai virus readily induced OX40L on the majority of the cells even after the 1-day culture and up-regulated its expression during 3-day culture; sCD40L augmented its expression.

#### Effects of PDC-derived OX40L and type 1 IFNs in Th polarization

To elucidate how PDC-derived OX40L and IFN- $\alpha\beta$  impact the quality of Th cell responses, anti-OX40L Ab and/or a mixture of



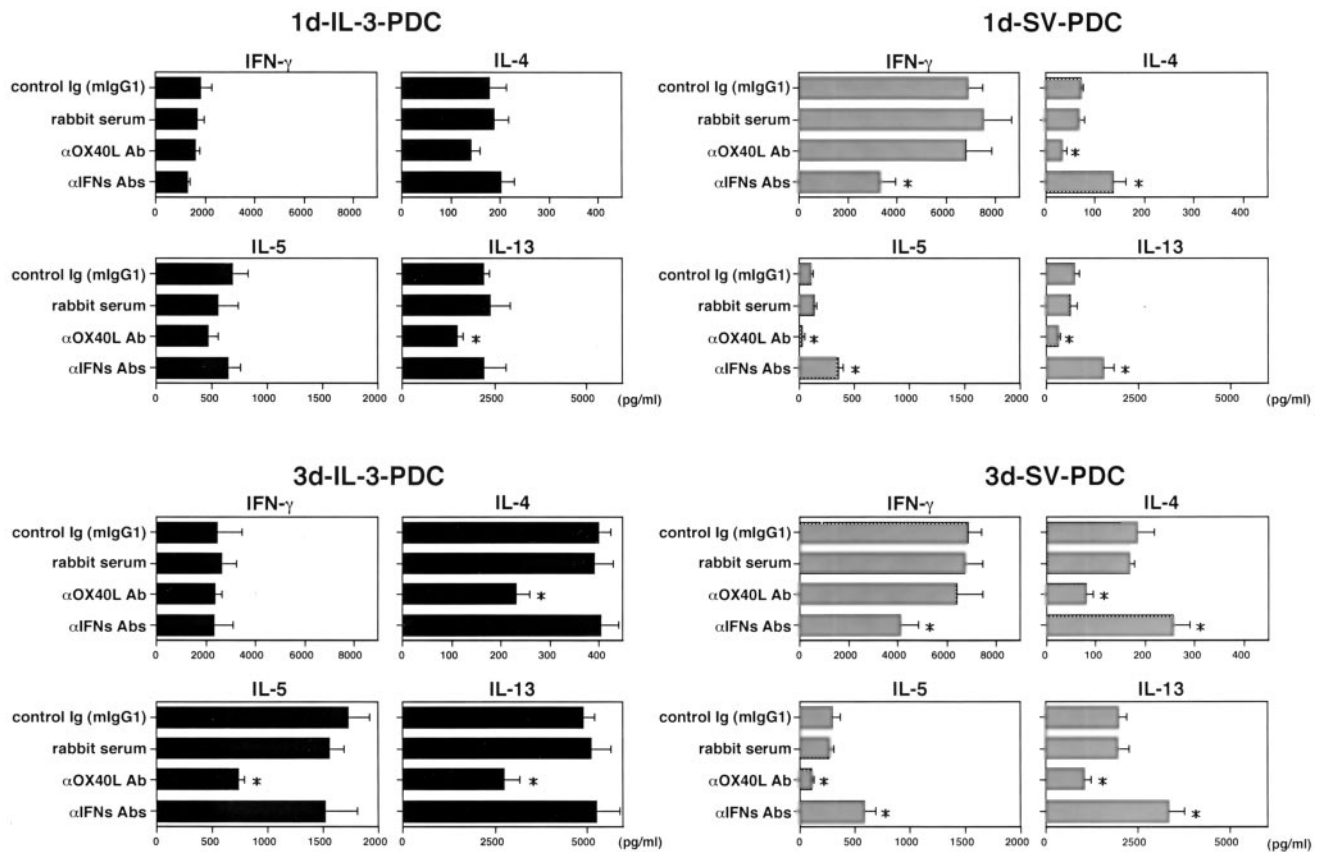
**FIGURE 2.** Capacity to residually produce IFN- $\alpha$  in PDCs. Purified PCs were cultured with IL-3 or Sendai virus (SV). **A**, After 24-h culture, the IFN- $\alpha$  concentration in the supernatant was measured by ELISA. **B**, After a 24- or 72-h culture, the supernatant was washed and completely removed, and the cells were restimulated with sCD40L or allogeneic CD4<sup>+</sup> T cells for an additional 24 h. The concentration of IFN- $\alpha$  in each supernatant was measured by ELISA. The data are shown as the mean  $\pm$  SEM of four independent experiments.



**FIGURE 3.** Expression of OX40L on PDCs. **A**, mRNA expression of OX40L was examined by RT-PCR in freshly isolated PCs or in PDCs after 24-h culture with various stimuli. **B**, During culture with IL-3 or Sendai virus (SV) in the presence or the absence of sCD40L, the surface expression of OX40L on PDCs was monitored by flow cytometry at 24 or 72 h. The staining profile of anti-OX40L mAb (ik-1) and isotype-matched control are shown by the shaded and open areas, respectively. The results shown are representative of three independent experiments.

anti-IFN- $\alpha$  Ab, anti-IFN- $\beta$  Ab, and anti-IFN- $\alpha\beta$  receptor Ab (anti-IFNs Abs) were primarily added to the PDC-T cell cocultures. As shown in Fig. 4, the addition of anti-OX40L Ab significantly decreased the production of IL-4, IL-5, and IL-13 in Th cells stimulated with 1d-SV-PDCs, 3d-SV-PDCs, and 3d-IL-3-PDCs. The addition of anti-OX40L Ab significantly decreased only IL-13 production of T cells in coculture with 1d-IL-3-PDCs. By contrast, the same Ab had no effect on IFN- $\gamma$  production from T cells under any culture conditions. In parallel with the results from ELISA, intracellular cytokine staining demonstrated that the addition of anti-OX40L Ab hampered the development, which had been induced by 3d-IL-3-PDCs or 3d-SV-PDCs, of IL-4-producing Th cells without affecting the frequency of IFN- $\gamma$ -producers (Fig. 5). These findings indicate that OX40L on the PDCs is responsible for Th cell production of IL-4, IL-5, and IL-13, but not IFN- $\gamma$ .

In contrast to the above findings, anti-IFN Abs significantly suppressed IFN- $\gamma$  production and increased IL-4, IL-5, and IL-13 production by Th cells when primed with 1d-SV-PDCs or 3d-SV-PDCs (Fig. 4). The intracellular cytokine staining also showed an impairment of IFN- $\gamma$ -producing cell development and a concomitant increase in the number of IL-4-producing cells when anti-IFN Abs were added into the coculture of 1d-SV-PDCs and 3d-SV-PDCs (Fig. 5, *third panels*). The reduction of IFN- $\gamma$  production (and also IFN- $\gamma$ -producing cells) by the neutralization of IFN- $\alpha\beta$  was more potently observed in the coculture of 1d-SV-PDCs than in that of 3d-SV-PDCs. A further addition of anti-OX40L Ab to anti-IFNs Abs counteracted the enhanced IL-4-producing cell development by IFN- $\alpha\beta$  neutralization (Fig. 5, *bottom panels*). However, in the coculture with 1d-IL-3-PDCs and 3d-IL-3-PDCs, the addition of anti-IFNs Abs barely altered the T cell production of cytokines (Figs. 4 and 5). These findings suggest that PDCs, in response to either IL-3 or Sendai virus, acquire some potential to induce OX40L-dependent Th2 responses, and that PDC-derived IFN- $\alpha\beta$ , in response to Sendai virus, interferes with the



**FIGURE 4.** Neutralizing effects of PDC-derived OX40L and IFN- $\alpha\beta$  on T cell cytokine production. PDCs preincubated with IL-3 or Sendai virus (SV) for 1 or 3 days were cocultured with allogeneic CD4<sup>+</sup> naive T cells for 7 days in the presence of anti-OX40L ( $\alpha$ OX40L) mAb (ik-1) or a mixture of neutralizing anti-IFN- $\alpha$  Ab, neutralizing anti-IFN- $\beta$  Ab, and anti-IFN- $\alpha\beta$  receptor mAb ( $\alpha$ -IFNs Abs). Mouse IgG1 and heat-inactivated rabbit serum were used as a control. The procedure for measuring the cytokine production from T cells in supernatants by ELISA is described in *Materials and Methods*. Statistical significance was determined using paired Student's *t* test (\*,  $p < 0.05$ ). Results are shown as the mean  $\pm$  SEM of four independent experiments.

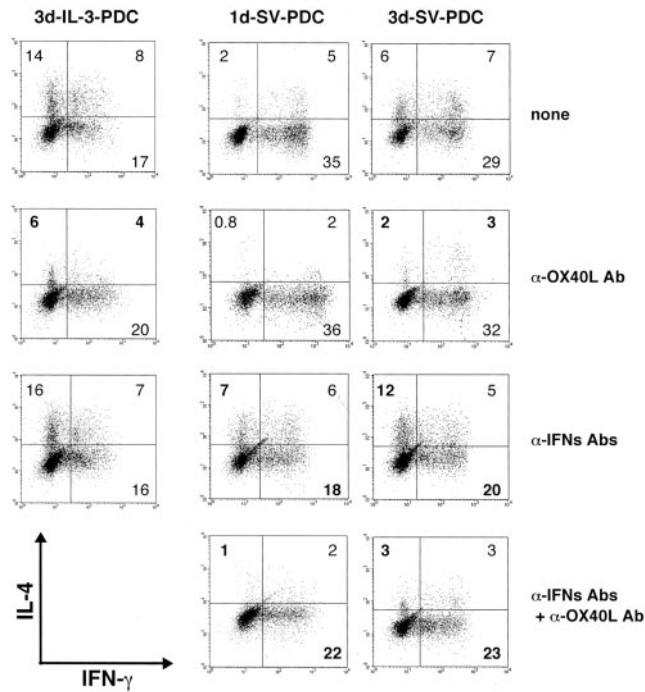
OX40L-dependent responses and consequently favors Th1-type responses.

## Discussion

In this study we have demonstrated that an OX40L-dependent mechanism is functional in PDC-mediated Th responses. In dictating the quality of Th cell responses, OX40L selectively induced Th2-type immune responses by promoting CD4<sup>+</sup> T cells to secrete IL-4, IL-5, and IL-13 (Figs. 4 and 5) (14–21, 27). Meanwhile, IFN- $\alpha\beta$  not only induced IFN- $\gamma$  production, but also inhibited Th2 cytokine production (36, 37), resulting in the preferential development of Th1-type immunity. We have also demonstrated that IL-3-treated PDCs, which are widely noted as Th2-inducible DCs (so-called DC2) (4), expressed OX40L (Fig. 3), but barely produced any IFN- $\alpha$  (Fig. 2B). Indeed, the costimulatory function of OX40L on 3d-IL-3-PDCs was responsible for the preferential priming of Th2 cells (Figs. 4 and 5). However, even the immature 1d-IL-3-PDCs, which partially and weakly expressed OX40L, could induce Th2 cells to some extent, and the addition of anti-OX40L Ab could not completely block Th2 cytokine production in Th cells when stimulated by 3d-IL-3-PDCs. These findings suggest that mechanisms other than OX40L may also be involved in the PDC-mediated Th2 responses. In this regard, it should be noted that human monocyte-derived DCs can actively induce Th2 cell development independently of OX40L in response to cAMP up-regulators such as PGE<sub>2</sub> or cholera toxin (11). In addition, there is evidence that Th2 development induced by monocyte-derived DCs is dependent not only on OX40-OX40L interaction, but also on the

B7-CD28 costimulation pathway (27). Furthermore, a series of studies has suggested that the ICOS-ICOS ligand pathway plays an important role in Th2 development and function (38–40). Thus, multiple signals, including the OX40-OX40L pathway, may possibly cooperate to dictate Th2 development in the DC-mediated immune responses.

Like IL-3-treated PDCs, Sendai virus-stimulated PDCs substantially expressed OX40L (Fig. 3). However, they did not favor preferential Th2 responses, but actively promoted Th1-type immune responses according to their high potency to produce IFN- $\alpha$  (Fig. 1, B and C, and Fig. 2), in accord with previous studies (6, 7). Considering the findings that the neutralization of IFN- $\alpha\beta$  inhibited IFN- $\gamma$  production and supported OX40L-dependent Th2 responses when primed with 1d-SV-PDCs or 3d-SV-PDCs (Figs. 4 and 5), it might well be that the effect of PDC-derived IFN- $\alpha\beta$  predominates over that of OX40L in PDC-mediated immune responses upon viral infection. Thus, PDCs have the distinct means to control Th responses: IFN- $\alpha\beta$  as endogenous cytokines and OX40L as a costimulatory molecule, which differentially regulate T cell production of IFN- $\gamma$  and Th2 cytokines, including IL-4, IL-5, and IL-13. Recent progress in DC biology has led to the concept that a given DC subset can alter its functional property depending on the microenvironment. The myeloid DC subset basically has the ability to induce immunogenic Th1 immune responses against microbial invasion in an IL-12-dependent manner. By contrast, the same myeloid DCs favor Th2 cell development in response to stimuli associated with parasites or allergens. For example, soluble egg Ags of helminth inhibit IL-12 production from



**FIGURE 5.** PDC-derived IFN- $\alpha\beta$  interferes with OX40L-dependent Th responses. 3d-IL-3-PDCs, 1d-SV-PDCs, and 3d-SV-PDCs were cocultured with allogeneic CD4<sup>+</sup> naive T cells for 7 days in the presence or the absence of anti-OX40L ( $\alpha$ -OX40L) Ab and/or anti-IFNs ( $\alpha$ -IFNs) Abs. Intracellular cytokine profiles in the T cells were analyzed by flow cytometry. The percentages of the respective cytokine-producing T cells are indicated. This figure represents the results from one of five experiments.

monocyte-derived DCs and then induce DC-mediated Th2 responses through an OX40L-dependent mechanism (11). Furthermore, human thymic stromal lymphopoietin, which is highly expressed in epithelial tissues of atopic dermatitis, activates the blood myeloid DC subset to produce the Th2-attracting chemokines thymus- and activation-regulated chemokine and macrophage-derived chemokine, but not IL-12, and thymic stromal lymphopoietin-activated DCs instruct naive CD4<sup>+</sup> T cells to differentiate into Th2 effector cells (41, 42). In parallel with the functional plasticity of myeloid DCs, the current study findings indicate that PDCs could induce Th1-type immune responses against microbial invasion (i.e., in response to virus or CpG DNA) and induce Th2 responses in an IL-3 milieu (which is generally formed under some allergic conditions) depending on PDC-derived IFN- $\alpha\beta$  and OX40L, respectively.

It is of note that PDCs are speculated to directly enter lymphoid tissues or inflammatory lesions, such as allergic mucosa from peripheral blood, in an L-selectin-dependent manner (2, 3, 43). IL-3 stimulation alone only weakly evoked OX40L expression on PCs. However, CD40L, which is delivered from T cells, substantially up-regulated its expression (Fig. 3). Therefore, it is suggested that in *in vivo* situations such as inflammations against allergens or parasites, PDCs may optimize their Th2-inducing ability through interaction with T cells in an IL-3 milieu. During the maturation in response to Sendai virus, the ability of PDCs to residually produce IFN- $\alpha$  was down-regulated (Fig. 2), whereas the expression level of OX40L on PDCs was up-regulated (Fig. 3B). In accordance with these findings, PDCs with prolonged stimulation of Sendai virus (3d-SV-PDCs) could instruct naive Th cells to more actively produce Th2 cytokines than 1d-SV-PDCs (Fig. 1). This may lead to a scenario in which viral infection causes the PDC-mediated Th1-type responses in the early phase of immune responses, and

thereafter induces some potential to mount Th2 responses, which may contribute to eliciting an immunological memory and/or counteracting the overload of Th1 responses. Furthermore, the Th2 responses may also induce the humoral immunity against viruses. Immunity against viruses involves Ab production, induction of cellular effectors such as CTL, and cytokines including IFN- $\alpha\beta$  and IFN- $\gamma$ . The findings obtained in this study suggest that PCs/PDCs are closely related to the mechanisms of antiviral immunity described above. Thus, PDCs could dictate the quality of Th responses depending on the balance of IFN- $\alpha\beta$  and OX40L expressions.

In conclusion, the current study delineated a biological significance of OX40L and type I IFNs in PDCs in regulating Th responses. Therefore, the findings obtained in this study may not only offer a new insight into the essential nature of PDCs, but also be helpful in understanding the etiological importance of PDCs in various diseases and thus in establishing a new therapeutic strategy for the diseases.

## References

- Siegal, F. P., N. Kadowaki, M. Shodell, P. A. Fitzgerald-Bocarsky, K. Shah, S. Ho, S. Antonenko, and Y.-J. Liu. 1999. The nature of the principal type I interferon-producing cells in human blood. *Science* 284:1835.
- Ito, T., R. Amakawa, and S. Fukuhara. 2002. Roles of toll-like receptors in natural interferon-producing cells as sensors in immune surveillance. *Hum. Immunol.* 63:1120.
- Grouard, G., M.-C. Rissoan, L. Filgueira, I. Durand, J. Banchereau, and Y.-J. Liu. 1997. The enigmatic plasmacytoid T cells develop into dendritic cells with Interleukin (IL)-3 and CD40-ligand. *J. Exp. Med.* 185:1101.
- Rissoan, M.-C., V. Soumelis, N. Kadowaki, G. Grouard, F. Briere, R. W. Malefyt, and Y.-J. Liu. 1999. Reciprocal control of T helper cell and dendritic cell differentiation. *Science* 283:1183.
- Liu, Y.-J. 2001. Dendritic cell subsets and lineages, and their functions in innate and adaptive immunity. *Cell* 106:259.
- Kadowaki, N., S. Antonenko, J. Y.-N. Lau, and Y.-J. Liu. 2000. Natural interferon  $\alpha/\beta$ -producing cells link innate and adaptive immunity. *J. Exp. Med.* 192:219.
- Cella, M., F. Facchetti, A. Lanzavecchia, and M. Colonna. 2000. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. *Nat. Immunol.* 1:305.
- Lanzavecchia, A., and F. Sallusto. 2001. Regulation of T cell immunity by dendritic cells. *Cell* 106:263.
- Liu, Y.-J., H. Kanzler, V. Soumelis, and M. Gillet. 2001. Dendritic cell lineage, plasticity and cross-regulation. *Nat. Immunol.* 2:585.
- Langenkamp, A., M. Messi, A. Lanzavecchia, and F. Sallusto. 2000. Kinetics of dendritic cell activation: impact on priming of Th1, Th2 and nonpolarized T cells. *Nat. Immunol.* 1: 311.
- de Jong, E. C., P. L. Vieira, P. Kalinski, J. H. N. Schuitemaker, Y. Tanaka, E. A. Wierenga, M. Yazdanbakhsh, and M. L. Kapsenberg. 2002. Microbial compounds selectively induce Th1 cell-promoting or Th2 cell-promoting dendritic cells *in vitro* with diverse Th cell-polarizing signals. *J. Immunol.* 168:1704.
- Vieira, P. L., E. C. de Jong, E. A. Wierenga, M. L. Kapsenberg, and P. Kalinski. 2000. Development of Th1-inducing capacity in myeloid dendritic cells requires environmental instruction. *J. Immunol.* 164:4507.
- Ito, T., R. Amakawa, M. Inaba, S. Ikehara, K. Inaba, and S. Fukuhara. 2001. Differential regulation of human blood dendritic cell subsets by IFNs. *J. Immunol.* 166:2961.
- Akiba, H., Y. Miyahira, M. Atsuta, K. Takeda, C. Nohara, T. Futagawa, H. Matsuda, T. Aoki, H. Yagita, and K. Okumura. 2000. Critical contribution of OX40 ligand to T helper cell type 2 differentiation in experimental leishmaniasis. *J. Exp. Med.* 191:375.
- Flynn, S., K. M. Toellner, C. Raykundalia, M. Goodall, and P. Lane. 1998. CD4 T cell cytokine differentiation: the B cell activation molecule, OX40 ligand, instructs CD4 T cells to express interleukin 4 and upregulates expression of the chemokine receptor, Bln-1. *J. Exp. Med.* 188:297.
- Jember, A. G., R. Zuberi, F. T. Liu, and M. Croft. 2001. Development of allergic inflammation in a murine model of asthma is dependent on the costimulatory receptor OX40. *J. Exp. Med.* 193:387.
- Salek-Ardakani, S., J. Song, B. S. Halteman, A. G. Jember, H. Akiba, H. Yagita, and M. Croft. 2003. OX40 (CD134) controls memory T helper 2 cells that drive lung inflammation. *J. Exp. Med.* 198:315.
- Ishii, N., L. C. Ndhlovu, K. Murata, T. Sato, M. Kamanaka, and K. Sugamura. 2003. OX40 (CD134) and OX40 ligand interaction plays an adjuvant role during *in vivo* Th2 responses. *Eur. J. Immunol.* 33:2372.
- Hoshino, A., Y. Tanaka, H. Akiba, Y. Asakura, Y. Mita, T. Sakurai, A. Takaoka, S. Nakaie, N. Ishii, K. Sugamura, et al. 2003. Critical role for OX40 ligand in the development of pathogenic Th2 cells in a murine model of asthma. *Eur. J. Immunol.* 33:861.

20. Ohshima, Y., L.-P. Yang, T. Uchiyama, Y. Tanaka, P. Baum, M. Sergerie, P. Hermann, and G. Delespesse. 1998. OX40 costimulation enhances interleukin-4 (IL-4) expression at priming and promotes the differentiation of naive human CD4<sup>+</sup> T cells into high IL-4-producing effectors. *Blood* 92:3338.
21. Delespesse, G., Y. Ohshima, L.-P. Yang, C. Demeure, and M. Sarfati. 1999. OX40-mediated cosignal enhances the maturation of naive human CD4<sup>+</sup> T cells into high IL-4-producing effectors. *Int. Arch. Allergy Immunol.* 118:384.
22. Croft, M. 2003. Costimulation of T cells by OX40, 4-1BB, and CD27. *Cytokine Growth Factor Rev.* 14:265.
23. Ukyo, N., T. Hori, S. Yanagita, T. Ishikawa, and T. Uchiyama. 2003. Costimulation through OX40 is crucial for induction of an alloreactive human T-cell response. *Immunology* 109:226.
24. Murata, K., N. Ishii, H. Takano, S. Miura, L. C. Ndhlovu, M. Nose, T. Noda, and K. Sugamura. 2000. Impairment of antigen-presenting cell function in mice lacking expression of OX40 ligand. *J. Exp. Med.* 191:365.
25. Chen, A. I., A. J. McAdam, J. E. Buhlmann, S. Scott, M. L. Lupper, Jr., E. A. Greenfield, P. R. Baum, W. C. Fanslow, D. M. Calderhead, G. J. Freeman, et al. 1999. OX40-ligand has a critical costimulatory role in dendritic cell:T cell interactions. *Immunity* 11:689.
26. Ohshima, Y., Y. Tanaka, H. Tozawa, Y. Takahashi, C. Maliszewski, and G. Delespesse. 1997. Expression and function of OX40 ligand on human dendritic cells. *J. Immunol.* 159:3838.
27. Tanaka, H., C. E. Demeure, M. Rubio, G. Delespesse, and M. Sarfati. 2000. Human monocyte-derived dendritic cells induce naive T cell differentiation into T helper cell type 2 (Th2) or Th1/Th2 effectors: role of stimulator/responder ratio. *J. Exp. Med.* 192:405.
28. Krug, A., S. Rothenfusser, V. Hornung, B. Jahrsdörfer, S. Blackwell, Z. K. Ballas, S. Endres, A. M. Krieg, and G. Hartmann. 2001. Identification of CpG oligonucleotide sequences with high induction of IFN- $\alpha/\beta$  in plasmacytoid dendritic cells. *Eur. J. Immunol.* 31:2154.
29. Krug, A., A. Towarowski, S. Britsch, S. Rothenfusser, V. Hornung, R. Bals, T. Giese, H. Engelmann, S. Endres, A. M. Krieg, et al. 2001. Toll-like receptor expression reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. *Eur. J. Immunol.* 31:3026.
30. Granelli-Piperno, A., V. Finkel, E. Delgado, and R. M. Steinman. 1999. Virus replication begins in dendritic cells during the transmission of HIV-1 from mature dendritic cells to T cells. *Curr. Biol.* 9:21.
31. Inaba, K., S. Turley, T. Iyoda, F. Yamaide, S. Shimoyama, C. Reis e Sousa, R. N. Germain, I. Mellman, and R. M. Steinman. 2000. The formation of immunogenic major histocompatibility complex class II-peptide ligands in lysosomal compartments of dendritic cells is regulated by inflammatory stimuli. *J. Exp. Med.* 191:927.
32. Matumura, Y., T. Hori, S. Kawamata, A. Imura, and T. Uchiyama. 1999. Intracellular signaling of gp34, the OX40 ligand: induction of c-jun and c-fos mRNA expression through gp34 upon binding of its receptor, OX40. *J. Immunol.* 163:3007.
33. Ito, T., M. Inaba, K. Inaba, J. Toki, S. Sogo, T. Iguchi, Y. Adachi, K. Yamaguchi, R. Amakawa, J. Valladeau, et al. 1999. A CD1a<sup>+</sup>/CD11c<sup>+</sup> subset of human blood dendritic cells is a direct precursor of Langerhans cells. *J. Immunol.* 163:1409.
34. Ito, T., R. Amakawa, T. Kaisho, H. Hemmi, K. Tajima, K. Uehira, Y. Ozaki, H. Tomizawa, S. Akira, and S. Fukuhara. 2002. Interferon- $\alpha$  and interleukin-12 are induced differentially by Toll-like receptor 7 ligands in human blood dendritic cell subsets. *J. Exp. Med.* 195:1507.
35. Bogdan, C. 2000. The function of type I interferons in antimicrobial immunity. *Curr. Opin. Immunol.* 12:419.
36. Demeure, C. E., C. Y. Wu, U. Shu, P. V. Schneider, C. Heusser, H. Yssel, and G. Delespesse. 1994. In vitro maturation of human neonatal CD4 T lymphocytes. *J. Immunol.* 152:4775.
37. Brinkmann, V., T. Geiger, S. Alkan, and C. H. Heusser. 1993. Interferon  $\alpha$  increases the frequency of interferon  $\gamma$ -producing human CD4<sup>+</sup> T cells. *J. Exp. Med.* 178:1655.
38. McAdam, A. J., T. T. Chang, A. E. Lumelsky, E. A. Greenfield, V. A. Boussiotis, J. S. Duke-Cohan, T. Chernova, N. Malenkovich, C. Jabs, V. K. Kuchroo, et al. 2000. Mouse inducible costimulatory molecule (ICOS) expression is enhanced by CD28 costimulation and regulates differentiation of CD4<sup>+</sup> T cells. *J. Immunol.* 165:5035.
39. Dong, C., A. E. Juedes, U. A. Temann, S. Shresta, J. P. Allison, N. H. Ruddle, and R. A. Flavell. 2001. ICOS co-stimulatory receptor is essential for T-cell activation and function. *Nature* 409:97.
40. Coyle, A. J., S. Lehar, C. Lloyd, J. Tian, T. Delaney, S. Manning, T. Nguyen, T. Burwell, H. Schneider, J. A. Gonzalo, et al. 2000. The CD28-related molecule ICOS is required for effective T cell-dependent immune responses. *Immunity* 13:95.
41. Reche, P. A., V. Soumelis, D. M. Gorman, T. Clifford, M. Liu, M. Travis, S. M. Zurawski, J. Johnston, Y.-J. Liu, H. Spits, et al. 2001. Human thymic stromal lymphoprotein preferentially stimulates myeloid cells. *J. Immunol.* 167:336.
42. Soumelis, V., P. A. Reche, H. Kanzler, W. Yuan, G. Edward, B. Homey, M. Gilliet, S. Ho, S. Antonenko, A. Lauerma, et al. 2002. Human epithelial cells trigger dendritic cell-mediated allergic inflammation by producing TSLP. *Nat. Immunol.* 3:673.
43. Jahnsen, F. L., F. Lund-Johansen, J. F. Dunne, L. Farkas, R. Haye, and P. Brandtzaeg. 2000. Experimentally induced recruitment of plasmacytoid (CD123<sup>high</sup>) dendritic cells in human nasal allergy. *J. Immunol.* 165:4062.