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Dendritic cells from spleen, mesenteric lymph node and Peyer's patch can induce the production of both IL-4 and IFN-γ from primary cultures of naive CD4$^+$ T cells in a dose-dependent manner

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Abstract

Dendritic cells (DCs) as antigen presenting cells can stimulate naive CD4$^+$ T cells and initiate the primary immune response which controls Th1/Th2 development. It has been suggested that DCs derived from different tissues have distinct properties. We investigated whether DCs from mesenteric lymph nodes (MLN), Peyer's patches (PP) and spleen (SPL) could induce different responses of naive CD4$^+$ T cells to varying doses of antigen by using a co-culture system of DCs and T cells. DCs from each tissue induced IL-4 secretion from naive CD4$^+$T cells in the presence of low dose antigenic peptide, and induced IFN-γ production at high doses of antigen. When purified CD11c$^+$/B220$^-$ DCs were used, MLN-derived DCs induced a higher amount of IFN-γ secretion from naive CD4$^+$ T cells, compared with SPL-derived DCs. We could not detect large differences in the expressions of costimulatory molecules on the surface of these two populations of DCs. On the other hand, we found that large amounts of IL-12 were secreted from MLN DCs in an antigen dose-dependent fashion. In conclusion, DCs from SPL, MLN and PP can induce the production of both IL-4 and IFN-γ from naive CD4$^+$ T cells, depending on antigen dose. MLN-derived CD11c$^+$/B220$^-$ DCs induce higher IFN-γ production from naive CD4$^+$ T cells than SPL-derived DCs, through efficient IL-12 secretion.

Abbreviations: Ab – antibody; APC – antigen presenting cell; DC – dendritic cell; EDTA – ethylenediaminetetraacetic acid; ELISA – enzyme-linked immunosorbent assay; FITC – fluorescein isothiocyanate; GALT – gut-associated lymphoid tissue; HBSS – Hank's balanced salt solution; IFN – interferon; IL – interleukin; mAb – monoclonal antibody; MHC – major histocompatibility complex; MLN – mesenteric lymph nodes; OVA – ovalbumin; PBS – phosphate-buffered saline; PP – Peyer's patches; SPL – spleen; TCR – T cell receptor; Th – helper T cell

Introduction

Dendritic cells (DCs) are potent antigen presenting cells (APCs), that are more effective in their ability to sensitize naive CD4$^+$ T cells engaging antigens than other professional APCs (B cells or macrophages). DCs exist in gut organized lymphoid tissues, such as mesenteric lymph nodes

(MLN) and Peyer's patches (PPs), where they are specialized in the uptake and presentation of foreign antigens, including components of pathogenic microorganisms and orally absorbed soluble proteins. Previous reports have shown that DCs from PP located in the small intestine elicit different T cell responses compared with DCs from the spleen (SPL) (Everson et al. 1998; Iwasaki and Kelsall 1999; Sato et al. 2002). Such distinctive characteristics of gut-derived DCs may play an important role in the unique responses in gut-associated lymphoid tissue (GALT). However, a comparison of T cell responses induced by gut-derived DCs and those induced by DCs from other tissues have not been adequately investigated.

T cell antigen receptors (TCRs) recognize antigenic peptides bound to the major histocompatibility complex (MHC) class II molecules on the cell membrane of professional APCs. T cell responses to antigenic stimulation are controlled by many factors, such as costimulatory molecules on APCs and the cytokine environment. Regarding the role of the cytokine environment in T cell differentiation, it has been shown that interleukin 12 (IL-12) induces the development of type 1 helper T (Th1) cells which secrete interferon-gamma (IFN-γ). In contrast, interleukin (IL)-4 induces type 2 helper (Th2) T cells that possess the ability to produce high amounts of IL-4, IL-5 and IL-6. Of the factors which regulate T cell responses, it has been shown that antigen concentration affects the type and magnitude of T cell responses. However, little is known about the role of different APC populations on this phenomenon. In the present study, we focused on the T cell response to varying doses of antigen induced by DCs of GALT tissues, in particular, DCs in the MLN. Although previous reports have shown that DCs from PP in the small intestine show a tendency to induce Th2 cytokines, whereas splenic DCs induce Th1 type immune responses (Everson et al. 1998; Iwasaki and Kelsall 1999), the effect of antigen dose has not been examined. Furthermore, the characteristics of DCs from MLN have been poorly defined. Here we investigated the cytokine response of naive $CD4^+$ T cells induced by freshly isolated DCs from SPL, PP and MLN.

Materials and methods

Mice

$OVA_{323–339}$-specific TCR transgenic mice (OVA23-3) on a BALB/c genetic background (Sato et al. 1994) were originaly provided by Dr. Sonoko Habu. Female mice (7–12 weeks old) were used for all experiments. Sex matched BALB/c mice (6–8 weeks old) were purchased from Japan CLEA (Tokyo, Japan)

Culture medium, and antigenic peptide

Cells were cultured in RPMI 1640 (Sigma, St Louis, MO) supplemented with 5% heat-inactivated (56 °C, 30 min) FCS, 2 mM L-glutamine, 2% potassium carbonate, 5×10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin, and 100 μg/ml streptomycin. The $OVA_{323–339}$ peptide (ISQAVHAAHAEINEAGR) was purchased from Mitokor (San Diego, CA).

Preparation of naive $CD4^+$ T cells from OVA23-3 mice

Naive $CD4^+$ T cells ($CD62L^{high}/CD4^+$ T cells) were prepared from the spleens of naive OVA23-3 mice. The tissue was digested with collagenase D (1 mg/ml, Roche, Mannheim, Germany) and DNase I (0.1 mg/ml, Roche) at 37 °C for 30 min, and further dissociated in Ca^{2+}-free HBSS in the presence of 5 mM EDTA at 37 °C for 5 min. The dissociated cells were isolated by positive selection using FITC-conjugated anti-CD4 monoclonal antibody (mAb) (H129.19, BD Pharmingen, San Diego, CA) and MACS anti-FITC multi-sort kit (Miltenyi Biotec, Bergish Gladbach, Germany), and negative selection using biotin-conjugated anti-MHC class II mAb (M5/114.15.2, prepared in our laboratory), biotin-conjugated anti-CD11c mAb (N418, prepared in our laboratory) and streptavidin-conjugated beads (Miltenyi Biotec). To obtain $CD62L^{high}/CD4^+$ naive T cells we used anti-CD62L microbeads (MEL-14, Miltenyi Biotec). The purity of isolated cells was checked by FACSort (BD Biosciences) and the percentage of $CD62L^{high}/CD4^+$ T cells were more than 95%.

DC preparation

$CD11c^+$ DCs were prepared from SPL, MLN and PP of BALB/c mice. Cells were digested as described above. After blocking of Fc receptors (purified anti-mouse CD16/32 mAb 2.4G2, prepared in our laboratory), isolated single cells were positively selected by MACS using anti-CD11c microbeads (N418, Miltenyi Biotec). To purify $CD11c^+/B220^-$ DCs, cells were positively selected using FITC-conjugated anti-CD11c mAb (N418, prepared in our laboratory) and MACS anti-FITC multi-sort kit, and negatively selected using biotin-anti-CD45R/B220 mAb (RA3-6B2, Pharmingen) and streptavidin-microbeads. The DCs from BALB/c mice were treated with 50 μg/ml of mitomycin C (Sigma) at 37 °C for 30 min.

Flow cytometry

The following antibodies (Abs) were used for the identification of cell populations and phenotype analysis: allophycocyanin-conjugated anti-mouse CD11c mAb (HL3, Pharmingen), FITC-conjugated anti-mouse CD45R/B220 mAb (RA3-6B2, Pharmingen), biotin-conjugated anti-mouse CD80 mAb (16-10A1, Pharmingen), Biotin-conjugated anti-mouse CD86 mAb (GL1, Pharmingen), biotin-conjugated anti-mouse CD40 mAb (3/23, Pharmingen), and PE-conjugated streptavidin (Pharmingen). All analysis was performed on FACSort.

Cytokine production assay

$CD62L^{high}/CD4^+$ T cells (1×10^6 or 1×10^5 cells) and DCs (1×10^5 or 1×10^4 cells) were co-cultured in 2 ml or 200 μl of medium in 48- or 96-well plates, respectively, with 0–5000 nM of $OVA_{323–339}$ peptide for 72 h. The cytokine concentrations in culture supernatants were measured by sandwich ELISA. For IL-4, IFN-γ, IL-12 p40 ELISA, Abs were used in the following pairs; anti-mouse IL-4 mAb (BVD4-1D11, Pharmingen) and biotinylated anti-mouse IL-4 mAb (BVD6-24G2, Pharmingen), anti-mouse IFN-γ mAb (R4-6A2, Pharmingen) and biotinylated anti-mouse IFN-γ mAb (XMG1.2, Pharmingen), anti-mouse IL-12 mAb (C15.6, Pharmingen) and biotinylated anti-mouse IL-12 mAb (C17.8, Pharmingen).

Results and discussion

We investigated the primary cytokine secretion profiles of $CD62L^{high}/CD4^+$ T cells from OVA23-3 mice on a BALB/c background, stimulated by $CD11c^+$ DCs isolated from SPL, MLN and PP of un-manipulated BALB/c mice with various concentrations of $OVA_{323–339}$ peptide. Naive $CD4^+$ T cells from OVA23-3 mice express TCR recognizing MHC class II-$OVA_{323–339}$ peptide complexes, and are suitable for examining antigen-specific cytokine responses of naive $CD4^+$ T cells in primary culture. $CD11c^+$ DCs from SPL, MLN and PP were obtained by MACS sorting (flow cytometric profiles are shown in Figure 1) and were treated with mitomycin C. In this experiment we used highly purified $CD62L^{high}/CD4^+/CD11c^-/MHC^-$ cells as naive T cells, since T cells sorted only for $CD4^+$ and $CD62L^{high}$ expression would possibly be contaminated by APC populations, such as DCs or B cells, and we would not be able to examine the exact roles of DCs from each tissue. In this experiment using $CD11c^+$ DCs, we measured cytokine secretion from naive $CD4^+$ T cells during their primary response (Figure 2). Varying doses of $OVA_{323–339}$ peptide were added to the culture medium, and concentrations of IL-4 and IFN-γ were measured by sandwich ELISA. The DC-enriched fraction induced naive $CD4^+$ T cells to secrete a maximum amount of IL-4 at 50 nM of $OVA_{323–339}$. In contrast, the secretion of IFN-γ increased in a dose-dependent fashion above 50 nM of $OVA_{323–339}$. We repeatedly observed the tendency of SPL DCs to induce a higher production of IFN-γ compared to MLN and PP DCs in the presence of high doses of antigen (500 nM and 5000 nM). It has been reported that splenic DCs induced a Th1-dominant immune reaction and mucosal DCs (such as Peyer's patch DCs) drive a Th2 type immune response (Everson et al. 1998; Iwasaki and Kelsall 1999). On the other hand, we found that PP DCs induce secretion of IFN-γ from naive $CD4^+$ T cells (Sato et al. 2002). In the present study, we demonstrated that SPL, MLN and PP DCs could induce both IL-4 and IFN-γ production from naive $CD4^+$ T cells depending on the antigen dose. A similar pattern was observed in

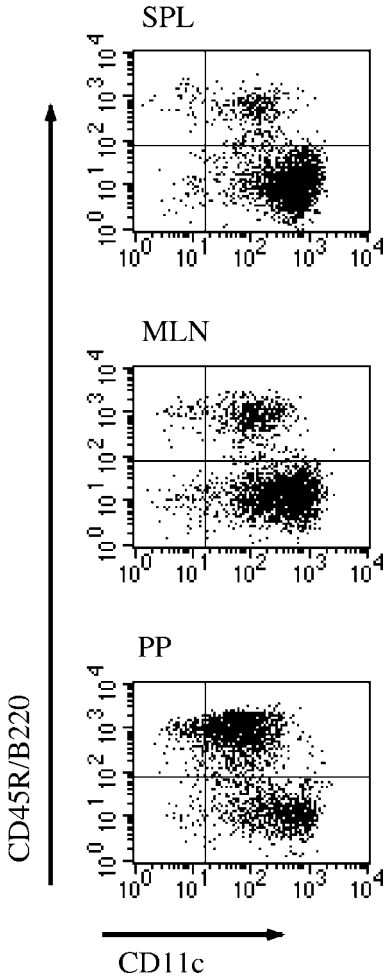

Figure 1. Cell population of CD11c$^+$ DCs sorted by MACS using anti-CD11c microbeads. DCs were prepared from the SPL, MLN, and PP of BALB/c mice, were stained with APC-conjugated anti-mouse CD11c mAb and FITC-conjugated anti-mouse CD45R/B220 mAb, and analyzed by flow cytometry.

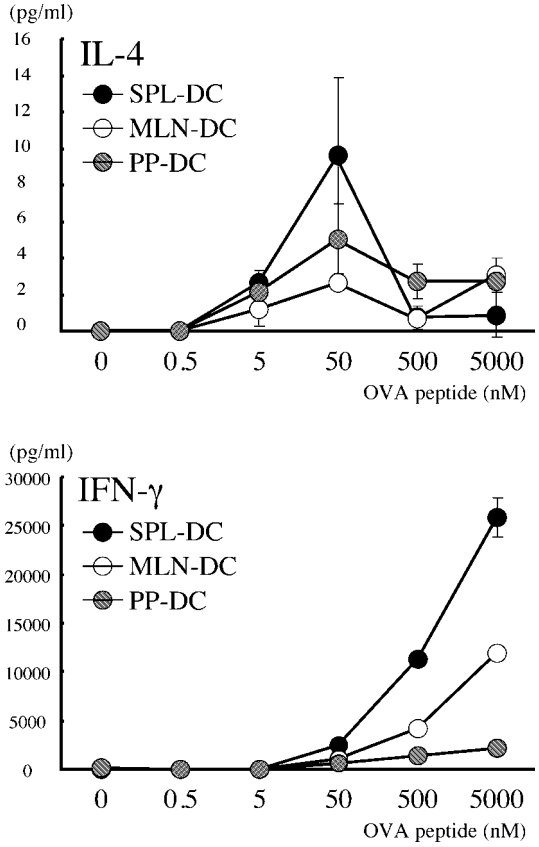

Figure 2. Cytokine production from naive CD4$^+$ T cells stimulated by CD11c$^+$ DCs from SPL, MLN or PP, with varying doses of antigen. CD11c$^+$ DCs (1×10^5 cells) from SPL, MLN, and PP were co-cultured with CD4$^+$/CD62L^{high} T cells (1×10^6 cells) with the indicated doses of $OVA_{323–339}$ peptide in 48-well plates for 72 h. IL-4 and IFN-γ in culture supernatants were measured by sandwich ELISA. The data are representative of two independent experiments.

the primary response in our previous report (Ise et al. 2002) using whole splenic APCs including DCs, B cells and macrophages: i.e., low dose antigenic peptides induced IL-4 secretion, and high dose antigens induced IFN-γ production. Recently, several groups have identified plasmacytoid DCs that are characterized by the CD11c^{mid}/B220$^+$ phenotype (Asselin-Paturel et al. 2001; Nakano et al. 2001). These cells have a deficient antigen presentation function, so it seemed necessary to exclude these cells and purify DCs that expressed high levels of CD11c to compare the T cell stimulatory function of DCs. To investigate the function of CD11c^{high}/B220$^-$ cells, DCs were obtained from SPL and MLN using a MACS multi-sort kit (Figure 3). The purity of sorted CD11c^{high}/B220$^-$ cells was >90% and we confirmed that these cells expressed high levels of CD11c and did not express CD45R/B220. We investigated the primary cytokine profiles of CD4$^+$ T cells stimulated by CD11c^{high}/B220$^-$ DCs isolated from BALB/c SPL and MLN in the presence of various doses of $OVA_{323–339}$ peptide (Figure 4). The peak of maximum IL-4 secretion shifted to 5 nM of $OVA_{323–339}$ peptide in the case of MLN CD11c^{high}/B220$^-$ DCs, which differed from MLN CD11c$^+$-enriched DC fractions. SPL DCs induced IL-4 production from naive T cells at 5 nM and 50 nM, and IL-4

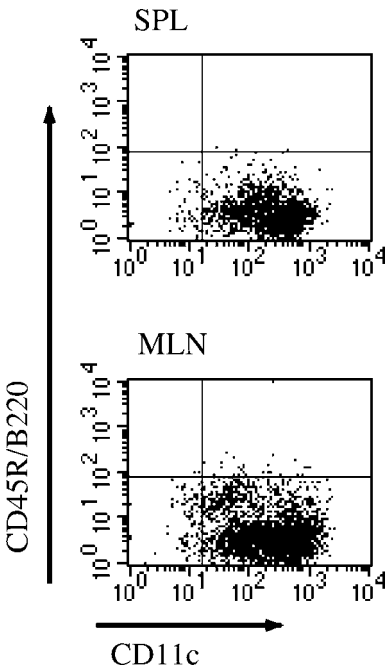

Figure 3. CD11c$^+$/B220$^-$ DCs were sorted by multi-sort MACS. DCs, prepared from the SPL and MLN of BALB/c mice, were stained with FITC-conjugated anti-CD11c mAb, biotin-conjugated anti-CD45R/B220 mAb and streptavidin-PE, and analyzed by flow cytometry.

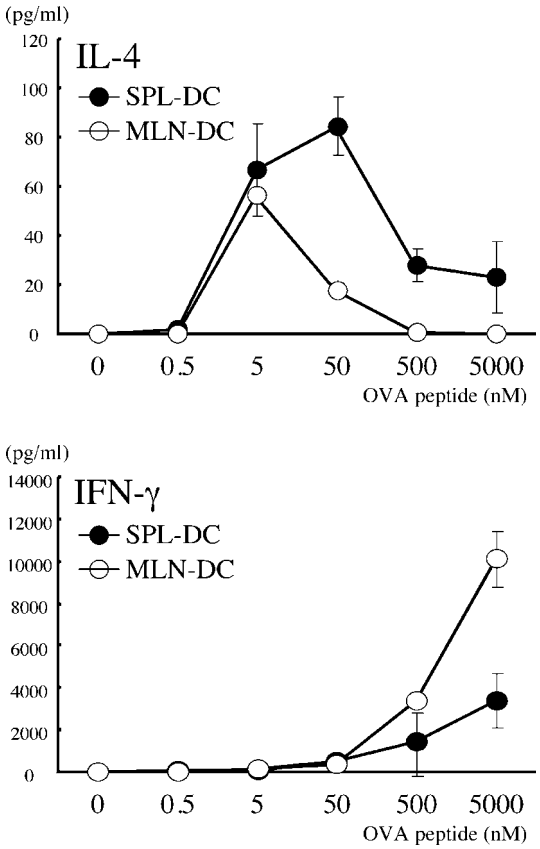

Figure 4. Cytokine production from naive CD4$^+$ T cells stimulated by CD11c$^+$/B220$^-$ DCs from SPL or MLN, with varying doses of antigen. SPL and MLN DCs (1×10^4 cells) were cultured with CD4$^+$/CD62L^{high} T cells (1×10^5 cells) with the indicated antigen in 96-well plates for 72 h. IL-4 and IFN-γ in the culture supernatants were measured by cytokine ELISA. The data are representative of three independent experiments.

secretion induced by SPL DCs was constantly greater compared to MLN DCs. On the other hand, the production of IFN-γ provoked by MLN CD11c^{high}/B220$^-$ DCs was larger than that induced by SPL DCs which increased in a dose-dependent manner above 50 nM. In high-dose antigen presenting conditions CD11c^{high} DCs from MLN were markedly more effective in IFN-γ induction than SPL CD11c^{high} cells. Since we could only collect small numbers of highly purified CD11c^{high}/B220$^-$ DCs, this experiment was performed in a 96-well plate. The experiments using DC enriched population (CD11c$^+$ cells) were performed in a 48-well plate, so the levels of IFN-γ induced by the two populations could not be compared. We cultured the highly purified CD11c^{high}/B220$^-$ DCs in a 48-well plate, albeit only at high antigen concentration (5000 nM OVA peptide). The levels of IFN-γ in the cultures were 39,839 $\pm$ 4134.6 pg/ml for SPL-DCs, and 75,676 $\pm$ 20,148.5 pg/ml for MLN-DCs. The well size seemed to affect the absolute levels of IFN-γ secretion in the cultures, which was higher in the 48-well plate cultures. The results of the 48-well plate cultures suggest there was a difference in IFN-γ induction between the DC-enriched population (CD11c$^+$ cells) and the highly purified CD11c^{high}/B220$^-$ DCs (CD11c$^+$ and B220$^-$ cells); the highly purified CD11c^{high}/B220$^-$ DCs appeared to induce higher levels of IFN-γ compared to the CD11c$^+$ cells, especially in the case of MLN. McRae et al. reported in the human system that type I IFNs (IFN-α and -β) prevented Th1 development through direct inhibition of IL-12 production from DCs (McRae et al. 1998). The ratio of plasmacytoid DCs in MLN, which produce enormous amounts of IFN-α, may be larger than in SPL DCs. Therefore, the possibility exists that plasmacytoid DCs in DC-enriched populations from MLN cells released high levels of IFN-α, and as a result, inhibited IFN-γ production.

To elucidate the mechanism of strong IFN-γ induction by MLN DCs, we determined the expression of CD80, CD86, CD40 molecules on freshly isolated DCs from SPL and MLN (Figure 5). Many reports have referenced the importance of interactions between T cells and APCs mediated by the B7 family or CD40 molecules and their ligands. CD40L (Ruedl et al. 2000), CD28 and OX-40 (Rogers and Croft 2000) on the T cell surface, or CD80, CD86 (Ranger et al. 1996) and CD40 on APCs contribute to T cell differentiation and cytokine production. We found that CD80, CD86 and CD40 molecules were constitutively expressed on SPL and MLN DCs, and similar levels of expression were observed for both SPL DCs and MLN DCs (Figure 5). Thus it appeared that the difference in IFN-γ induction between SPL and MLN DCs was not attributed to the differences in expression of costimulatory molecules such as CD80, CD86 and CD40.

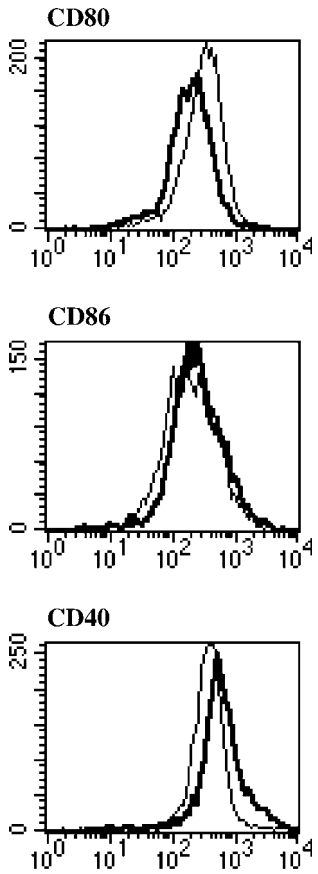

Figure 5. Costimulatory molecule expression on SPL-derived DCs (thin line) and MLN-derived DCs (bold line). Freshly isolated SPL and MLN DCs were gated on CD11c$^+$/B220$^-$ cells and were analyzed for cell surface expression of CD80, CD86 and CD40. Results are representative of two experiments.

IL-12 secreted by DCs is a strong initiator of IFN-γ production from T cells and induces Th1 development *in vivo* and *in vitro*. We investigated the quantity of IL-12, which is mainly secreted by CD11c^{high} DCs, in the same co-culture system (Figure 6). We found that cultures containing CD11c^{high} DCs from MLN and naive CD4$^+$ T cells secreted a higher amount of IL-12 compared to cultures containing SPL DCs with $OVA_{323–339}$ concentrations above 50 nM. Lymphoid DCs (CD8$^+$/CD11c$^+$ DCs) have been shown to be the main IL-12 contributor to skew the response into Th1 dominant immunity (Maldonado-Lopez et al. 1999). This comparison of IL-12 production in co-cultures of T cells and SPL DCs or MLN DCs suggested that MLN DCs induced increasing levels of IFN-γ through the secretion of IL-12. In our experiment, to assess the concentration of IL-12, we measured the level of the p40 subunit of IL-12, which is generally proportional to the level of the bioactive IL-12 p35/40 heterodimer. However, recently it has been shown that the p40 subunit of IL-12 has other biological functions. p19 is a recently identified protein which is structurally related to IL-6 and the p35 subunit of IL-12, and forms a complex with the p40 subunit of IL-12 designated as IL-23 (Oppmann et al. 2000). IL-12

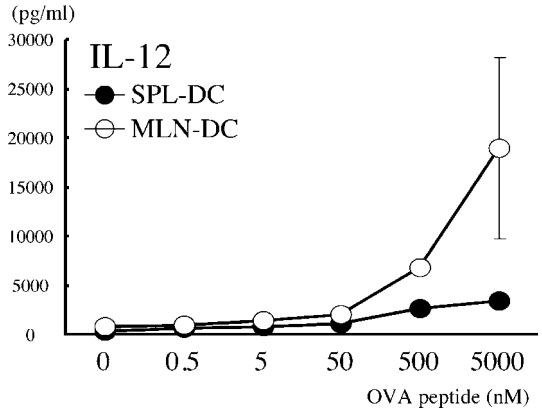

Figure 6. MLN DCs produce higher levels of IL-12 p40 when co-cultured with naive CD4$^+$ T cells in the presence of antigenic peptide. DCs (1×10^4 cells) and CD4$^+$/CD62L^{high} T cells (1×10^5 cells) were cultured with the indicated antigen in 96-well plates for 72 h. The levels of IL-12 in the culture supernatants were measured by cytokine ELISA. This data is representative of three independent experiments.

(p35/p40 complex) and IL-23 (p19/p40 complex) are expressed by human and murine DCs affecting the T cell response. In our experimental system, there remained the possibility that higher IFN-γ production was induced by functional IL-23 secreted from MLN DCs.

In our experiment, we could not find any differences in the expression of costimulatory molecules on DCs, but there remains the possibility that other B7 family molecules such as B7-H1, -H2 and B7-DC contributed to IFN-γ production. Of particular importance is B7-DC which controls both IFN-γ production by T cells (Tseng et al. 2001) and IL-12 production by DCs (Nguyen et al. 2002). In the case of MLN DC cultures, it is possible that higher levels of IFN-γ and IL-12 production originated from these costimulatory molecules.

In summary, we have demonstrated that DCs from SPL, MLN and PP can induce IL-4 production from naive $CD4^+$ T cells at low antigen dose and IFN-γ production in the presence of high antigen dose. Moreover, MLN $CD11c^+/B220^-$ DCs were found to induce higher levels of IFN-γ secretion through the production of high levels of IL-12. These results extend the findings of our previous study using unfractionated cells as APCs (Ise et al. 2002; Yoshida et al. 2002), and indicate that the local concentration of antigen is an important factor in determining the outcome of immune responses in each lymphoid tissue such as the GALT.

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