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Plasmacytoid Dendritic Cells Induce Plasma Cell Differentiation through Type I Interferon and Interleukin 6

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Summary

Dendritic cells (DCs) initiate and control immune responses. Plasmacytoid DCs (pDCs) represent a unique DC subset able to promptly release large amounts of type I interferon (IFN-αβ) upon viral encounter. Here we report that depletion of pDCs from human blood mononuclear cells abrogates the secretion of specific and polyclonal IgGs in response to influenza virus. Furthermore, purified pDCs triggered with virus induce CD40-activated B cells to differentiate into plasma cells. Two pDC cytokines act sequentially, with IFN-αβ generating non-Ig-secreting plasma blasts and IL-6 inducing their differentiation into Ig-secreting plasma cells. These plasma cells display the high levels of CD38 found on tissue plasma cells. Thus, pDCs are critical for the generation of plasma cells and antibody responses.

Introduction

The immune system evolved to protect us from microbes by the coordinated actions of innate and adaptive immunity (Janeway and Medzhitov, 2002; Medzhitov and Janeway, 1998). In response to viral encounter, the cells of innate immunity, including epithelial cells, NK cells, and plasmacytoid dendritic cells (pDCs), secrete interferons, a family of cytokines with potent antiviral properties (Biron et al., 1999; Katze et al., 2002). Adaptive immunity provides T cells that secrete interferon γ and differentiate into cytotoxic T cells (Guidotti and Chisari, 2001) and B cells that generate antibody-secreting plasma cells (Bachmann and Zinkernagel, 1997). Dendritic cells (DCs), in addition to their role in innate immunity, induce and regulate adaptive immune responses (Banchereau et al., 2000; Banchereau and Steinman, 1998; Shortman and Liu, 2002; Steinman, 1991).

Distinct DC subsets exist that are endowed with different properties and lead to different types of immune responses. In the human, two major DC pathways have been identified, the myeloid DCs (O'Doherty et al., 1994) that include interstitial DCs and Langherhans cells, and plasmacytoid DCs (pDCs) (Grouard et al., 1997). pDCs display dramatically different physiology as compared to other DC subsets. For example, pDCs isolated from blood are small cells that morphologically resemble plasma cells with rich endoplasmic reticulum and readily enter into apoptosis unless provided with an appropriate signal. Perhaps the most significant feature of pDCs is their capacity to promptly produce considerable amounts of type I IFN upon exposure to viruses as well as bacterial components such as CpG oligonucleotides (Asselin-Paturel et al., 2001; Bjorck, 2001; Cella et al., 1999; Kadowaki et al., 2000; Nakano et al., 2001; Siegal et al., 1999). The release of IFN-αβ initiates a cascade of events that leads to the elimination of the virus. In particular, IFN-αβ acts directly on most cell types to turn on biochemical pathways that restrict viral replication and render host cells resistant to further viral infection (Katze et al., 2002). IFN also activates NK cells, which can lyse virally infected cells (Biron et al., 1999). Finally, virally triggered pDCs differentiate into mature DCs able to induce the differentiation of $CD4^+$ T cells into IFN-γ- and IL-10-secreting cells (Kadowaki et al., 2000). These cytokines further contribute to antiviral protection directly and indirectly by recruiting other immune effectors. Thus, IFN-γ is an antiviral agent as well as an activator of CTLs (Boehm et al., 1997). Furthermore, IL-10 (Moore et al., 2001) is a known activator of CTL precursors (MacNeil et al., 1990) and a potent stimulator of plasma cell differentiation (Fluckiger et al., 1993; Rousset et al., 1992). Activated T cells differentiate into (1) CTLs that eliminate virally infected cells and (2) memory T cells. Plasma cells produce neutralizing antibodies and, when long-lived, may provide the most efficient and life-long protection against viral infection (Manz et al., 1998, 2002; Slifka and Ahmed, 1996).

While the majority of the studies on DCs have focused on their ability to trigger T cell responses, early studies clearly indicated that they may act on other immune effectors including B cells (Dubois et al., 1997; Fayette et al., 1997; Garcia De Vinuesa et al., 1999; Sornasse et al., 1992). Indeed, myeloid DCs have been shown to trigger B cell growth and differentiation through the release of soluble factors such as IL-12 and IL-6 (Dubois et al., 1998) and/or membrane molecules such as BAFF/APRIL (Balazs et al., 2002; Litinskiy et al., 2002; MacLennan and Vinuesa, 2002). Little is known, however, about the role of pDCs in B cell differentiation. We now show that virus-triggered pDCs induce, through IFN-αβ and IL-6 release, the generation of plasma cells secreting virus-specific antibodies.

Results

pDCs Are Essential for Ig Production by Blood Mononuclear Cells

Human blood mononuclear cells can be induced to secrete Igs in vitro in response to a wide variety of agents including polyclonal stimulators (for example, PWM, SAC, PHA) as well as viruses (for example, influenza virus) (Banchereau and Rousset, 1992). Given the role of pDCs in controlling virus spread through the production of large amounts of type I IFN, we wondered whether they would also be involved in the generation of virus-specific antibodies. To this end, we exposed peripheral

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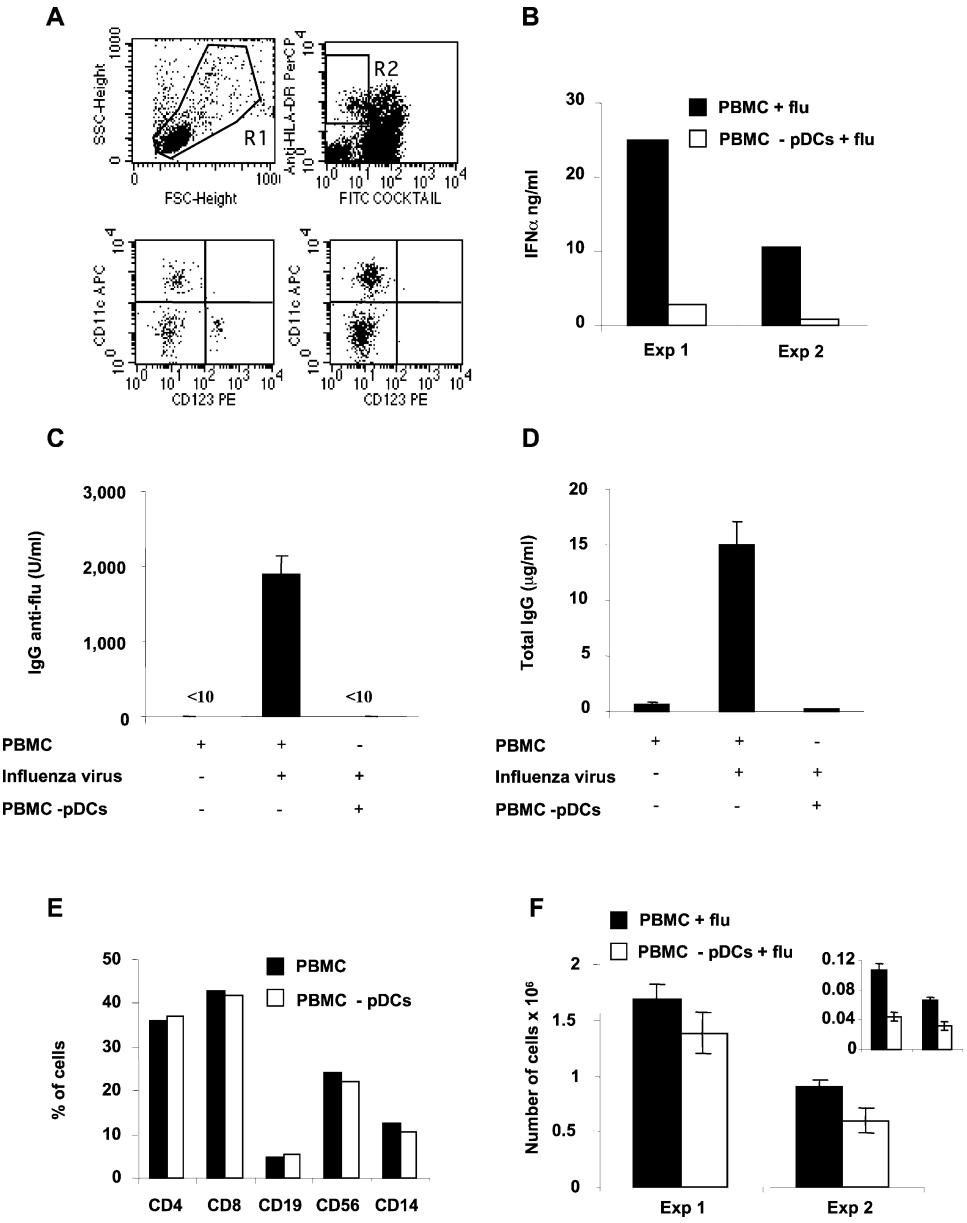

Figure 1. pDC Depletion from PBMC Abrogates Influenza-Specific Antibody Production

(A) Phenotypic characterization of PBMCs before (lower left) and after (lower right) depletion of BDCA4-positive cells. pDCs ($CD11c^{neg}$ $CD123^{pos}$) are analyzed within viable cells (R1) and Lin^{neg} $HLA\text{-}DR^{high}$ cells (R2) (one of five experiments).

(B) Decreased IFN-α secretion upon influenza virus stimulation by 1×10^6 PBMCs before (black bars) and after (white bars) pDC depletion.

(C) Anti-flu IgG levels in supernatants of 1×10^6 PBMCs or PBMCs depleted of pDCs and cultured for 15 days with influenza virus and IL-2 (one of four experiments).

(D) Total IgG concentration in PBMCs or PBMC-pDCs cultured with influenza virus and IL-2 (one of five experiments).

(E) Percentage of CD4-, CD8-, CD19-, CD56-, and CD14-positive cells in PBMCs before (black bars) or after pDC depletion (white bars) (one of two experiments).

(F) Numbers of PBMCs after 15 days of culture (two of three experiments). The insert shows the number of CD19 cells in these two experiments.

blood mononuclear cells (PBMCs) as well as PBMCs depleted of pDCs to live influenza virus in vitro. pDCs were depleted with BDCA4-coupled magnetic beads (Dzionek et al., 2000), which removed >95% of pDCs identified as $CD123^+$ $CD11c^-$ $HLA\text{-}DR^+$ cells lacking lineage markers (0.3% pDCs before and <0.01% pDCs after depletion, Figure 1A). The depletion of pDCs resulted in >90% inhibition of IFN-α secretion after 48 hr exposure to live influenza virus (Figure 1B). PBMCs and pDC-depleted PBMCs were cultured with live influenza virus for 2 weeks. Supernatants were then tested by ELISA for both influenza-specific antibodies and total immunoglobulins. As shown in Figure 1C, depletion of pDCs at culture onset completely abrogated secretion of

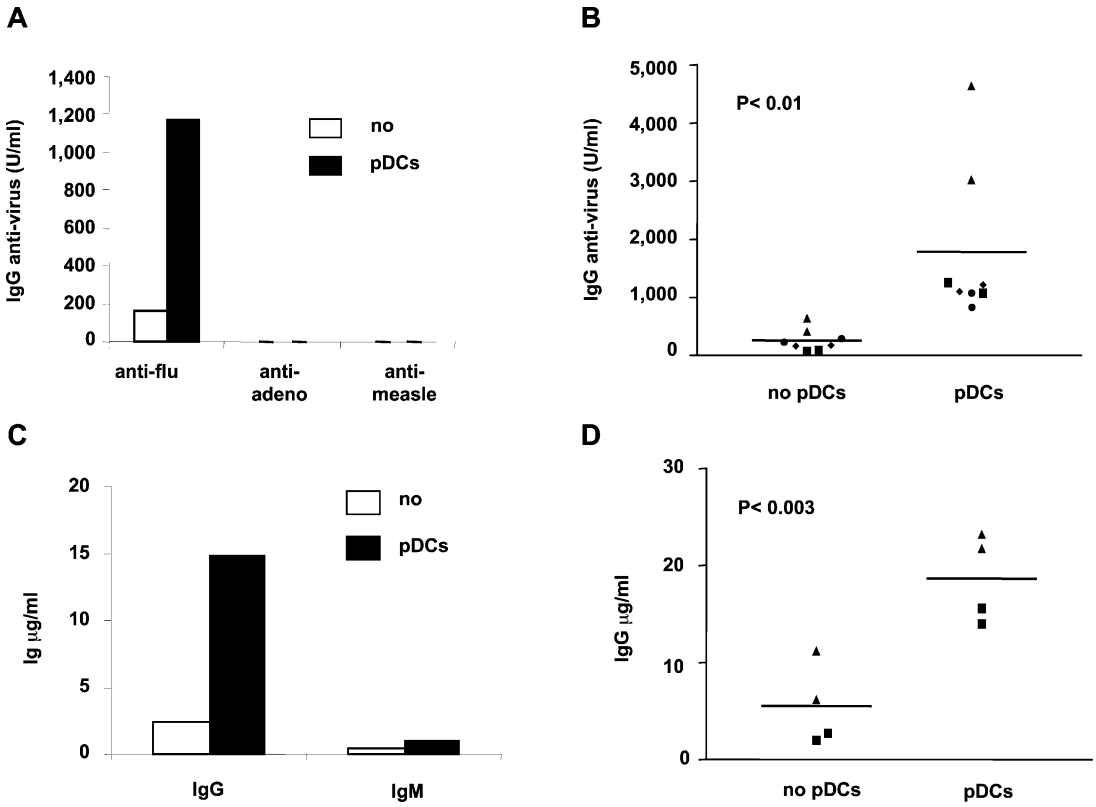

Figure 2. pDCs Enhance the Production of Influenza-Specific Antibodies by Purified B Cells Cocultured with T Cells and Virus

(A) Concentration of IgG against influenza virus, adenovirus, measles virus, in the 15 day coculture of 2×10^5 autologous B and T cells, influenza virus, and IL-2, with (black bars) or without (white bars) 5×10^3 pDCs (one of two experiments).

(B) Anti-flu IgG concentration in duplicates of four independent experiments (paired t test).

(C) Total IgG and IgM in experiment illustrated in (A) in the presence of influenza virus (one of two experiments).

(D) Total IgG from duplicates of two independent experiments in the same conditions as (A) in the presence of influenza virus (paired t test).

influenza-specific antibodies. This inhibition of specific antibody secretion reflected the inhibition of total immunoglobulin secretion, as no IgG could be detected in the supernatants of pDC-depleted PBMCs (Figure 1D). The lack of Ig secretion was not due to the death of PBMCs. Indeed, pDC depletion did not alter the frequency of various cell populations at the onset of culture (Figure 1E). Upon culturing, B cells were present in the end of the culture albeit at reduced numbers (~50%, Figure 1F).

Thus, pDCs are essential for the production of influenza virus-specific IgG by blood mononuclear cells.

Purified CD40-Activated Human B Cells Secrete Igs When Exposed to pDCs and Virus

We next analyzed whether pDCs directly interact with B cells. Human B cells, purified by positive selection using CD19-beads, were exposed to three signals: influenza virus (the antigen), pDCs (the antigen-presenting cell), and T cells. Through most of this study, pDCs were sorted from cultures of $CD34^+$ hematopoietic progenitors made in the presence of FLT-3L and TPO (Blom et al., 2000). In some cases, however, to ensure that cultured pDCs behave as those isolated from blood, experiments were carried out with pDCs isolated from blood using BDCA-4 beads. BDCA-4-positive blood pDCs were also able to induce B cell differentiation (data not shown).

Supernatants of cocultures with influenza virus were harvested at day 15 for determination of total immunoglobulins and virus-specific antibodies. In the absence of pDCs, B cells cocultured with influenza virus, T cells, and IL-2 produce low levels of influenza-specific IgG (Figure 2A) with a mean level of 255 A.U./ml (Figure 2B). However, addition of only 5000 pDCs to the cocultures results in a 7-fold enhancement of Flu-specific IgG secretion (Figure 2A) with a mean level of 1782 A.U./ml (Figure 2B, $p < 0.01$). Figure 2B shows the replicates of four independent experiments carried out with cells from four different donors. The secretion of Flu-specific antibodies is accompanied by polyclonal activation of IgG secretion (Figures 2C and 2D, 3.4-fold increase, $p < 0.003$). Yet, neither anti-measles nor anti-adenovirus antibodies could be detected in cocultures with influenza virus (Figure 2A). Figure 2D shows the results of the replicates of two independent experiments carried out with cells from two different donors. The Ig secretion appears to be mostly IgG, as little IgM could be detected (Figure 2C, right panel).

To further understand the mechanisms through which

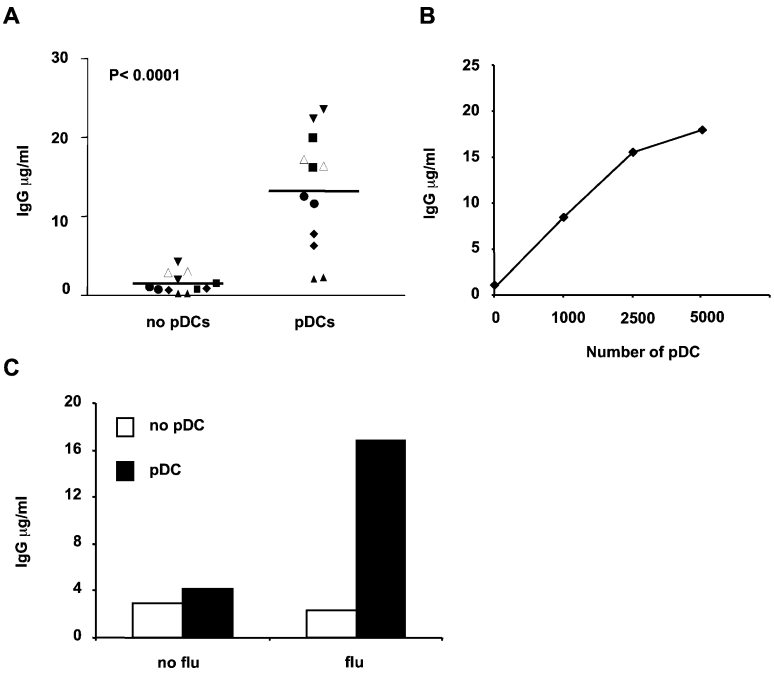

Figure 3. pDCs Enhance IgG Production by CD40-Activated B Cells

10^4 B cells were cultured over CD40L L cells with IL-2 in the absence or presence of pDCs.
(A) Total IgG secretion in the absence or presence of 5×10^3 pDCs from duplicates of six independent experiments (paired t test).
(B) Low numbers of pDCs enhance IgG secretion. Increasing numbers of pDCs were cultured with B cells during day 15 (one of four experiments).
(C) pDC stimulation of IgG secretion is dependent on the presence of the virus. B cells were cultured with or without influenza virus in the absence (white bars) or presence of 5×10^3 pDCs (black bars) (one of two experiments).

pDCs enhance antibody secretion, we replaced the T cells by CD40L transfected L cells. As shown in Figure 3A, the addition of 5000 pDCs to CD40-activated B cells cultured with Flu virus resulted in a 10-fold increase of IgG production (12 replicates of six independent experiments, $p < 0.0001$). As few as 1000 pDCs suffice for significant enhancement of IgG secretion (Figure 3B). However, in the absence of virus (Figure 3C, left panel), pDCs do not enhance antibody production, suggesting a critical role for the virus in the secretion of IgG.

Thus, pDCs can induce activated B cells to secrete Igs.

pDCs Induce CD40-Activated B Cells to Differentiate into Plasma Cells

Antibodies are produced by plasma cells, the end stage of B cell differentiation. Hence, pDC-dependent secretion of Flu-specific antibodies suggested the role of pDC in driving plasma cell differentiation. While CD40-activated B cells are $CD20^{hi}$ $CD38^{lo}$ (Figure 4A, 1), a significant fraction of these cells (43%) become $CD20^{lo}$ $CD38^{hi}$ plasma cells when cultured with IL-2+IL-10 (Arpin et al., 1995) (Figure 4A, 2). Likewise, CD40-activated B cells exposed to pDCs and virus acquire a plasma cell phenotype (43%, Figure 4A, 3). The expression of CD38 on the $CD20^{lo}$ cells in these cultures is higher than that observed in IL-10-driven cultures. Such CD38 expression intensity is comparable to that of plasma cells isolated from either blood (Figure 4A, 4) or tonsils (data not shown). Confocal microscopy using Texas red-labeled anti-HLA-DR and FITC-labeled anti-IgG further confirms the generation of plasma cells with a typical morphology and intense intracytoplasmic Ig staining (Figure 4B).

Thus, pDCs drive the differentiation of CD40-activated B cells into plasma cells.

pDCs Induce Plasma Cell Differentiation through Type I Interferon and IL-6

We demonstrated earlier that CD40-activated mDCs induce the differentiation of activated B cells through the release of three soluble factors: IL-12, IL-6, and gp80, a component of the IL-6 receptor (Dubois et al., 1998). We thus wondered whether pDCs induced B cell differentiation was also mediated by soluble factor(s). To this end, cultures were performed in Transwells where B cells are separated from pDCs by a semipermeable membrane. There, B cells are still able to secrete large amounts of IgG (Figure 4C). The differentiation required both the virus and CD40 signaling. Consequently, the supernatant of pDCs exposed to virus and activated through CD40 is able to induce CD40-activated B cells to differentiate into $CD20^{lo}$ $CD38^{hi}$ plasma cells (Figure 4D).

This prompted us to identify the pDC factor(s) involved in plasma cell differentiation and IgG secretion. pDCs have been shown to secrete a limited set of cytokines among which IFN-$\alpha\beta$ (Morikawa et al., 1987) and IL-6 are known to signal B cells (Kumanogoh et al., 1997). In Figure 5A, the addition of pDCs to CD40-activated B cells increases IgG secretion 11-fold (from 0.18 μg/ml to 1.98 μg/ml; one experiment representative of five showing a mean 12.7 ± 9.7-fold increase; range 3–26). The secretion of IgG could be prevented by the addition of neutralizing antibodies to (1) IFN-α alone ($p < 0.05$), (2) IFN-α and IFN-β together with anti-IFN-$\alpha\beta$ receptor antibody ($p < 0.0005$), or (3) IL-6 ($p < 0.0005$) (Figures 5A and 5B). Ig secretion results from a two-step differentiation process whereby activated B cells first differentiate into proliferating non-Ig-secreting plasma blasts that subsequently yield Ig-secreting plasma cells (Jego et al., 1999). As shown in Figure 5C, adding antibodies blocking IFN-$\alpha\beta$ (against both cytokines and receptor) resulted in >60% inhibition of $CD20^{lo}$ $CD38^{hi}$ plasma cell differentiation while neutralization of IL-6 gave <25% inhibition. To further understand the role of each cytokine, CD40-activated B cells were cultured with either IFN-α, IFN-β, or IL-6, and the cell phenotype was determined 7 days later. As shown in Figure 5D, adding IFN-α or IFN-β, but not IL-6, induced activated B cells to ac-

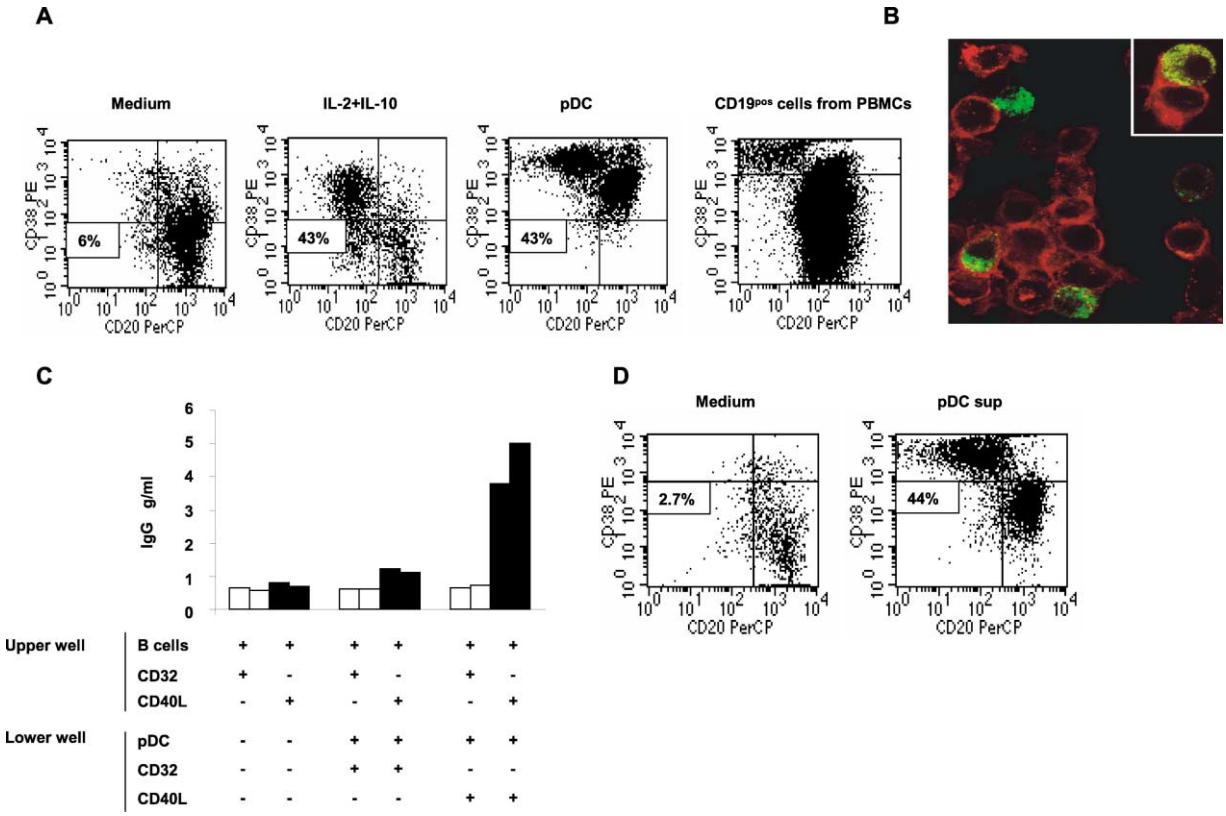

Figure 4. pDCs Induce CD40-Activated B Cells to Become Plasma Cells in a Contact-Independent Fashion

(A) Activated B cells acquire a plasma cell phenotype in coculture with pDCs and virus. Phenotype of activated B cells after 7 days of culture in medium alone, IL-2, and IL-10, or with 2×10^4 virus-activated pDCs; the boxed number is the frequency of $CD20^{lo}$ $CD38^{hi}$ plasma cells (one of five experiments with pDCs). Far right is the CD38 expression of $CD19^+$ peripheral blood cells (one of three experiments).

(B) After 8 days of coculture, plasma cells are detected by bright anti-Ig staining (green) and pDCs by bright anti-HLA-DR staining (red). Confocal microscopy, 63× magnification.

(C) The secretion of IgG is dependent on the production of soluble factor(s) by pDCs after CD40 engagement. IgG production by B cells over CD32 L cells (white bars) and over CD40L L cells (black bars) was measured in the upper well of the Transwells plate (one of two experiments). Each bar represents one well.

(D) Phenotypic differentiation of activated B cells into plasma cells is contact independent. Frequency of $CD20^{lo}$ $CD38^{hi}$ plasma cells generated in culture of 10^5 activated B cells with medium or virus-activated-pDC-supernatant after 7 days (one of three experiments).

quire a plasma cell phenotype. However, neither IFN-α nor IL-6 together with IL-2 was able to trigger Ig's secretion (Figure 5E), and only their combination resulted in the secretion of IgG, demonstrating cooperation between both cytokines (Figure 5E). Yet, Ig secretion obtained with IFN-α and IL-6 is lower than this obtained with pDCs, suggesting that other factors/cytokines produced by pDCs are involved, the nature of which is currently unknown. In those conditions, the Ig secretion was independent of the addition of the virus (data not shown). Interestingly, while virus alone was able to activate pDCs to secrete IFN-α, the levels of IL-6 were modest, thus explaining why B cells do not differentiate into Ig-secreting cells when pDCs are triggered with virus in the absence of CD40 signaling (see column 4 of Figure 4C). Indeed, pDCs required CD40 signaling for secretion of high levels of IL-6 (fold increase 31.1 ± 33, mean ± SD, range 5.6–93 [n = 6]) (Figure 5F).

Thus, pDCs induce the differentiation of activated B cells into Ig-secreting plasma cells through release of IFN-αβ and IL-6 that act in sequential fashion. First, IFN-αβ induces differentiation of activated B cells into nonsecreting plasma blasts, which in response to IL-6 become Ig-secreting plasma cells.

Discussion

In this study, we demonstrate a novel biological function of pDCs, their critical role in the generation of antibody responses. We show that depletion of pDCs from blood mononuclear cells abrogates secretion of immunoglobulins including influenza-specific ones and that pDCs control the differentiation of activated B cells into plasma cells through the secretion of IFN-αβ and IL-6. On the basis of our results we propose a model for development of virus-specific humoral immune responses (Figure 6). Accordingly, upon virus encounter pDCs promptly secrete IFN-αβ and differentiate into mature DCs presenting viral antigens to T cells (Fonteneau et al., 2003). T cells secrete IL-2 and express CD40L, which signals pDCs to secrete IL-6 and also activates B cells. pDC-derived IFN-αβ induces B cells to differenti-

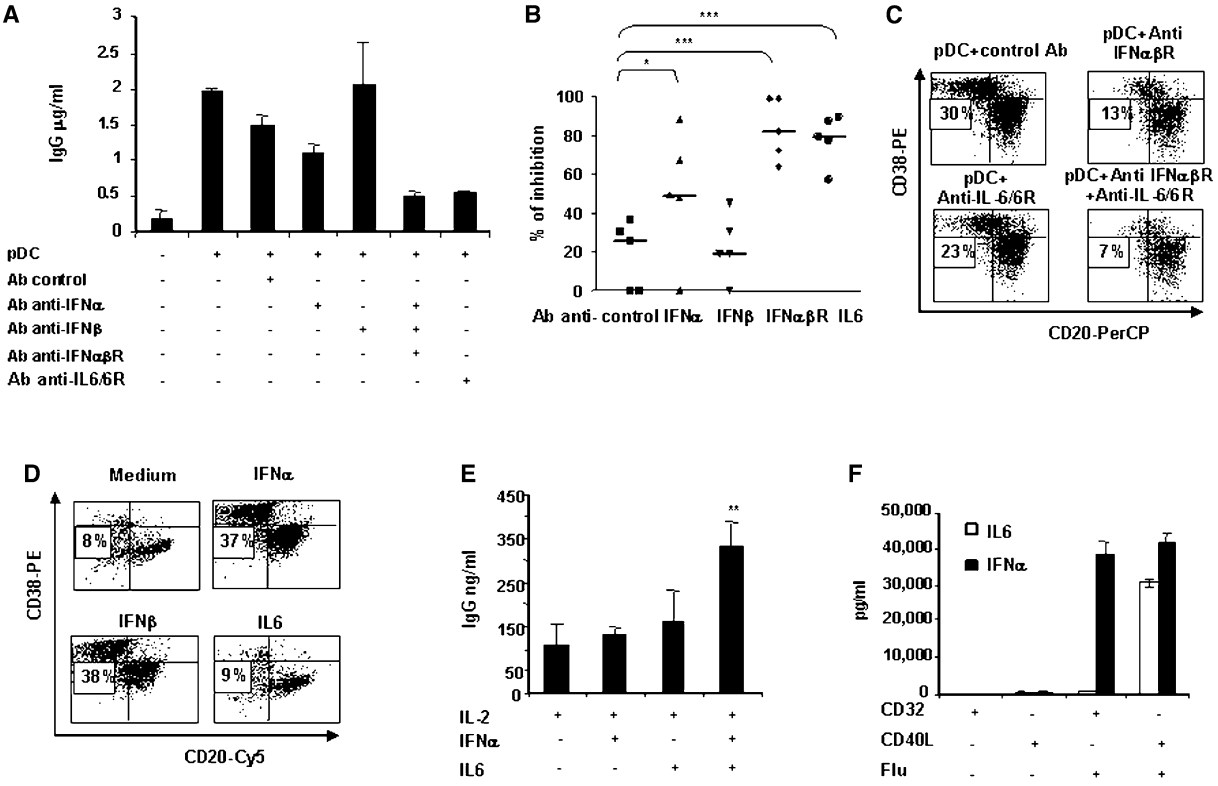

Figure 5. pDC-Induced Plasma Cell Differentiation Is Dependent on IFN-α and IL-6

(A) Neutralization of IFN-αβ and IL-6 abolishes the pDC-induced IgG secretion by B cells. Levels of IgG secreted by CD40-activated B cells in the presence of IL-2 and 5×10^3 virus-activated pDCs with or without anti-IFN-α, anti-IFN-β, anti-IFN-αβR, and anti-IL-6/6R-blocking Abs (one of five experiments).

(B) Percentage of inhibition of IgG secretion of five experiments in the same condition as in (A). Control Ab, ■; anti-IFN-α, ▲; anti-IFN-β, ▼; anti-IFN-αβ, ◆; anti-IL-6/6R, ●. *$p < 0.05$, ***$p < 0.0005$.

(C) Neutralization of IFN-αβ strongly inhibits the pDC-induced differentiation of $CD38^{hi}$ $CD20^{lo}$ B cells toward plasma cells. Frequency of plasma cells at day 7 of culture of activated B cells with medium, virus-activated-pDCs with or without anti-IFN-αβR, or/and anti-IL-6/6R-blocking Abs (one of two experiments).

(D) IFN-αβ induces the phenotypic differentiation of activated B cells into plasma cells. 10^5 activated B cells were cultured with medium, IFN-α, IFN-β, or IL-6 during 7 days before staining for $CD20^{lo}$ $CD38^{hi}$ plasma cells (one of four experiments).

(E) IgG production in cultures of CD40-activated B cells supplemented with IL-2 and either IFN-α, IL-6, or both (one of three experiments).

(F) IL-6, but not IFN-α, secretion is strongly increased after dual CD40 and virus stimulation of 4×10^4 pDCs during 48 hr (one of three experiments).

ate into plasma blasts while pDC-derived IL-6 permits plasma blasts to become antibody-secreting plasma cells.

We surmise that T cells are a necessary component in the B cell/pDC interaction for the generation and secretion of virus-specific antibodies because (1) CD40 ligand plays a critical role in the activation of B cells and in the enhancement of IL-6 secretion by pDCs, and (2) plasma cell differentiation is also supported by T cell-derived IL-2 and IL-10. However, CD40 ligand could be provided by cells other than activated T cells, including activated endothelial cells (Mach et al., 1997), mast cells (Gauchat et al., 1993), platelets (Henn et al., 1998) as well as activated B cells themselves (Grammer et al., 1995). Under these circumstances, a T cell-independent plasma cell differentiation driven by pDCs could take place. Recent studies demonstrating that myeloid DCs drive T cell-independent and even CD40-independent B cell differentiation and isotype switch, mediated by BLyS and APRIL, support this hypothesis (Balazs et al., 2002; Litinskiy et al., 2002; MacLennan and Vinuesa, 2002).

The importance of DCs in the establishment of humoral responses has been recognized for some time. However, it was demonstrated only for myeloid DCs and considered to be mediated by T cells that, once activated by DCs, would in turn activate B cells (Inaba et al., 1983; Sornasse et al., 1992). These studies, however, did not consider the possibility that DCs interact directly with B cells and present them with unprocessed antigen (Wykes et al., 1998). We have previously demonstrated that myeloid DCs, generated by culturing $CD34^+$ hematopoietic stem cells, are able to enhance B cell proliferation and isotype switching toward IgA (Fayette et al., 1997), as well as plasma cell differentiation (Fayette et al., 1998). However, at variance with pDCs, myeloid DCs, and most particularly the interstitial $CD14^+$ DCs, are able to induce the differentiation of naive B

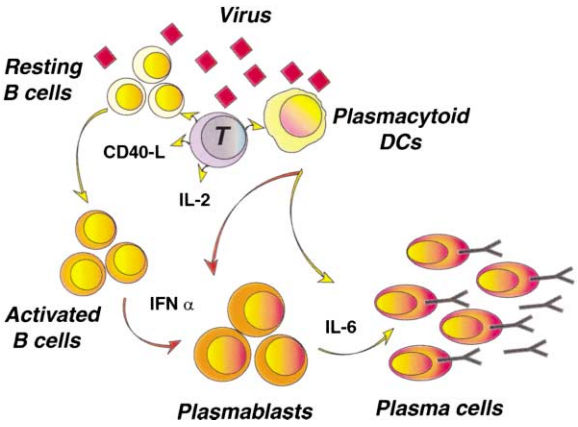

Figure 6. Plasmacytoid Dendritic Cells Induce Plasma Cell Differentiation through Interferon α and Interleukin 6

Upon virus encounter, pDCs promptly secrete IFN-αβ. The cells differentiate into DCs presenting viral antigens to T cells, which promptly secrete IL-2 and turn on CD40 L that signals pDCs to secrete IL-6 and activates B cells. Activated B cells differentiate into plasma blasts in response to the IFN-αβ. The secretion of IL-6 further induces the plasma blasts to become plasma cells. The T cells also contribute importantly through their secretion of IL-2 and IL-10, the combination of all signals leading to the generation of $CD38^{2+}$ long-lived plasma cells.

cells through the secretion of IL-12 and IL-6 (Dubois et al., 1998; Fayette et al., 1998). Our results here show that B cells activated with pDCs preferentially secrete IgG over IgM, suggesting that pDCs may specifically target memory B cells. Experiments are ongoing to establish whether the high IgG levels are due to the preferential IgG switching of naive B cells or indeed the specific activation of memory B cells. Our results show a cooperation of two pDC-derived cytokines, IFN-α and IL-6, at distinct stages of plasma cell differentiation. Yet, a combination of IL-6 and IFN-α did not fully recapitulate the effect of pDCs on Ig secretion. This suggests that other factors secreted by pDCs are involved in the process. Among others, BAFF/BLyS and APRIL are known to be involved in mDCs/B cell interactions (Balazs et al., 2002; Litinskiy et al., 2002; MacLennan and Vinuesa, 2002) and can be triggered on mDCs by IFN-α. However, our genomic analysis of resting and virus-activated pDCs did not reveal increased transcription of BAFF/Blys and APRIL (B. Piqueras and A.K. Palucka, unpublished data). Thus, distinct human DC subsets control B cell differentiation through different molecular pathways.

Our study shows IFN-αβ as an important molecule in the generation of human antibody-secreting plasma cells, thus providing a cellular mechanism for earlier studies that showed increased Ig secretion by IFN-α-treated B cells (Morikawa et al., 1987; Neubauer et al., 1985; Peters et al., 1986). This function of type I interferon in plasma blast differentiation herein described adds to the list of IFN immunomodulatory effects initially recognized 40 years ago (De Maeyer and De Maeyer-Guignard, 1980, 1988) and explains the recently observed adjuvant effect of type I IFN on humoral immune responses (Le Bon et al., 2001). Yet, mice with targeted IFN-αβR appear to mount normal antibody responses (van den Broek et al., 1995) suggesting alternative (redundant) pathways of plasma cell differentiation, possibly through T cells and myeloid DCs which induce B cell differentiation independently of type I IFN. Interestingly, however, the double IFN-$\gamma^{-/-}$ IFN-α$R^{-/-}$ mice fail to produce LCMV-specific IgG2a and IgG2b (van den Broek et al., 1995) while the single receptor IFN-α-targeted mice produce normal levels (Muller et al., 1994). This illustrates the limits of redundancy within the immune system. The further elucidation of the role of pDCs in the generation of virus-specific humoral immune responses will require methods for specific pDC depletion in vivo. The recently described lack of pDCs in $ICSBP^{-/-}$ mice (Schiavoni et al., 2002) may help us address these questions.

The present findings are also relevant to systemic lupus erythematosus (SLE), an autoimmune disease characterized by the break of tolerance to self-antigens. We had shown earlier that the interplay between DC subsets may underlie immune alterations in SLE (Bennett et al., 2003; Blanco et al., 2001). There, the pDCs release large amounts of IFN-α (Bennett et al., 2003; Blanco et al., 2001), which induces monocytes to differentiate into mDCs. These DCs phagocytose cell nuclei that circulate in these patients' blood, and their components are presented to autoreactive T cells and B cells, leading to the increased production of autoantibodies by plasma cells, the hallmark of SLE. The skewed B cell differentiation in SLE could be explained by pDC-derived IFN-α in at least two mechanisms, either through induction of BAFF/BLys on mDCs and/or direct effect on plasma blasts. The excess of IFN-αβ in SLE may also explain the actual increase of plasma blast and plasma cells found in SLE blood (Arce et al., 2001). Accordingly, two recent studies on murine SLE models showed aggravation of the disease and increased plasma cell numbers after induction of IFN-αβ (Braun et al., 2003; Santiago-Raber et al., 2003). Another unusual feature of SLE blood B cells, i.e, coexpression of IgD and CD38, could also be attributed to IFN-α as we have previously shown that IFN-α can induce the expression of CD38 on B cells in vitro (Galibert et al., 1996). In keeping with this, plasma cells generated with IFN-α express very high levels of CD38 similar to those found on circulating and tissular human plasma cells, further supporting the role of IFN-αβ in the generation of plasma cells in vivo.

In conclusion, pDCs appear critical for generation of plasma cells and anti-viral antibodies. Hence, the in vivo targeting of pDCs, as proposed for mDCs (Hawiger et al., 2001), may represent a novel approach to induce potent antiviral immune responses.

Experimental Procedures

Culture of PBMCs and Depletion of pDC

PBMCs were obtained from normal adult donors. pDCs were depleted using BDCA-4 microbeads (Miltenyi Biotec). BDCA-4 antigen is specifically expressed on all human plasmacytoid dendritic cells in blood. Isolated BDCA-4-positive cells have a typical phenotype, morphology, and cytokine secretion profile of plasmacytoid dendritic cells (Dzionek et al., 2000; Dzionek et al., 2002). 1×10^6 depleted or nondepleted PBMCs were then cultured with or without 10^4 influenza virus particles (Charles Rivers) in a 48-well plate with

50 U/ml of IL-2 (R&D). At d15, supernatants were harvested and used for indirect anti-influenza Ab and IgG ELISA.

Isolation and Culture of B Cells and pDCs

B and CD4 T cells were purified from blood or tonsils with CD19 and CD4 microbeads (Miltenyi Biotech), respectively. To remove germinal center B cells, tonsils were first depleted of $CD38^{hi}$ cells as previously described (Dubois et al., 1998). For Ig production, 4×10^3 irradiated CD40L cells (9000 rads) were seeded with 10^4 B cells with or without 2.5×10^3 influenza virus particles, and with or without pDC in a 96-well plate with 50 U/ml of IL-2. Monoclonal Abs against IL-6 and IL-6R and/or a rabbit anti-IFN-α, rabbit anti-IFN-β, and mouse anti-IFN-R mAb (PBL biomedical) were added at the beginning of the culture. To induce the Ig secretion by a cytokine combination, IL-6 (100 ng/ml; R&D) and IFN-α (100 U/ml; Schering-Plough) were added in the culture to IL-2 (50 U/ml). For Transwell (Costar) experiments, pDC (2×10^4) were cultured over overirradiated CD40L- or CD32-transfected L cells (5×10^4) in the lower compartment (0.8 ml). B cells (1.5×10^4) were cultured over irradiated CD40L- or CD32-transfected L cells (4×10^3) in the upper compartment (0.2 ml). Influenza virus was in both compartments, and IL-2 was added in the upper compartment. Supernatants were recovered at day 15 and used for indirect IgG, IgM, or IgA ELISA. For the induction of anti-influenza Ab, 2×10^5 B cells + 2×10^5 CD4 T cells and 50 U/ml of IL-2 were cultured with 5×10^3 of influenza virus particles with or without 5×10^3 pDCs.

Generation of pDCs

G-CSF mobilized $CD34^+CD45RA^-$ hematopoietic progenitor cells were cultured (5×10^4 cells/well) in Yssel's medium (Irvine Scientific) supplemented with 2% human serum, FLT3 ligand (100 ng/ml; R&D), and TPO (35 ng/ml; R&D) (Blom et al., 2000). Medium was changed every 7 days. At day 26, pDCs were sorted as lineage negative (CD3, CD14, CD16, CD19, CD20, CD56), $HLA\text{-}DR^+$ $CD123^+$, and $CD11c^-$ cells. Alternatively, pDCs were purified from buffy coats using BDCA-4 microbeads. pDC supernatants were generated by stimulating 4×10^4 pDCs with 2×10^4 influenza virus particles over CD40L L cells for 24 hr. IFN-$\alpha\beta$ and IL-6 concentrations were measured in the supernatant by ELISA (Biosource and R&D, respectively).

Quantification of Immunoglobulins and Antibodies

The ELISA was done as previously described (Jego et al., 1999). The anti-influenza Ab and anti-adenovirus Ab ELISA were set up by coating influenza or adenovirus overnight in PBS in a 96-well plates. The ELISA was run as previously, and standardization was performed with human serum. The anti-measles ELISA was from IBL GmbH.

Phenotypic Studies and Flow Cytometry

B cells (1×10^6) were first activated during day 4 as previously described (Arpin et al., 1995), then cells were harvested, washed, and recultured with either IL-2 and IL-10, 100 U/ml IFN-α or -β alone, 50 ng/ml IL-6 alone, 2×10^4 pDCs, or pDC supernatant. In all experiments, 2 μg/ml of anti-CD40L-blocking mAb was added to neutralize remaining CD40L fibroblasts and 2.5×10^3 influenza virus particles. The beginning of the second culture constituted day 0, cells were harvested at day 7 and stained for CD38-PE, CD20-PerCP, and CD19-APC. The percentage of $CD20^{lo}$ $CD38^{hi}$ plasma cells (Pcs) was analyzed on a FACS Calibur (Becton Dickinson) by gating on $CD19^+$ cells.

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References

Arce, E., Jackson, D.G., Gill, M.A., Bennett, L.B., Banchereau, J., and Pascual, V. (2001). Increased frequency of pre-germinal center B cells and plasma cell precursors in the blood of children with systemic lupus erythematosus. J. Immunol. *167*, 2361–2369.

Arpin, C., Dechanet, J., Van Kooten, C., Merville, P., Grouard, G., Briere, F., Banchereau, J., and Liu, Y.J. (1995). Generation of memory B cells and plasma cells in vitro. Science *268*, 720–722.

Asselin-Paturel, C., Boonstra, A., Dalod, M., Durand, I., Yessaad, N., Dezutter-Dambuyant, C., Vicari, A., O'Garra, A., Biron, C., Briere, F., and Trinchieri, G. (2001). Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. Nat. Immunol. *2*, 1144–1150.

Bachmann, M.F., and Zinkernagel, R.M. (1997). Neutralizing antiviral B cell responses. Annu. Rev. Immunol. *15*, 235–270.

Balazs, M., Martin, F., Zhou, T., and Kearney, J. (2002). Blood dendritic cells interact with splenic marginal zone B cells to initiate T-independent immune responses. Immunity *17*, 341–352.

Banchereau, J., and Rousset, F. (1992). Human B lymphocytes: phenotype, proliferation, and differentiation. Adv. Immunol. *52*, 125–262.

Banchereau, J., and Steinman, R.M. (1998). Dendritic cells and the control of immunity. Nature *392*, 245–252.

Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y., Pulendran, B., and Palucka, K. (2000). Immunobiology of dendritic cells. Annu. Rev. Immunol. *18*, 767–812.

Bennett, L., Palucka, A.K., Arce, E., Cantrell, V., Borvak, J., Banchereau, J., and Pascual, V. (2003). Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. J. Exp. Med. *197*, 711–723.

Biron, C.A., Nguyen, K.B., Pien, G.C., Cousens, L.P., and Salazar-Mather, T.P. (1999). Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu. Rev. Immunol. *17*, 189–220.

Bjorck, P. (2001). Isolation and characterization of plasmacytoid dendritic cells from Flt3 ligand and granulocyte-macrophage colony-stimulating factor-treated mice. Blood *98*, 3520–3526.

Blanco, P., Palucka, A.K., Gill, M., Pascual, V., and Banchereau, J. (2001). Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus. Science *294*, 1540–1543.

Blom, B., Ho, S., Antonenko, S., and Liu, Y.J. (2000). Generation of interferon alpha-producing predendritic cell (Pre-DC)2 from human CD34(+) hematopoietic stem cells. J. Exp. Med. *192*, 1785–1796.

Boehm, U., Klamp, T., Groot, M., and Howard, J.C. (1997). Cellular responses to interferon-gamma. Annu. Rev. Immunol. *15*, 749–795.

Braun, D., Geraldes, P., and Demengeot, J. (2003). Type I interferon controls the onset and severity of autoimmune manifestations in lpr mice. J. Autoimmun. *20*, 15–25.

Cella, M., Jarrossay, D., Facchetti, F., Alebardi, O., Nakajima, H., Lanzavecchia, A., and Colonna, M. (1999). Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. Nat. Med. *5*, 919–923.

De Maeyer, E., and De Maeyer-Guignard, J. (1980). Host genotype influences immunomodulation by interferon. Nature *284*, 173–175.

De Maeyer, E., and DeMaeyer-Guignard, J. (1988). Interferons and other regulatory cytokines. (New York: Wiley), pp. 91–113.

Dubois, B., Vanbervliet, B., Fayette, J., Massacrier, C., Van Kooten, C., Briere, F., Banchereau, J., and Caux, C. (1997). Dendritic cells enhance growth and differentiation of CD40-activated B lymphocytes. J. Exp. Med. *185*, 941–951.

Dubois, B., Massacrier, C., Vanbervliet, B., Fayette, J., Briere, F., Banchereau, J., and Caux, C. (1998). Critical role of IL-12 in dendritic cell-induced differentiation of naive B lymphocytes. J. Immunol. *161*, 2223–2231.

Dzionek, A., Fuchs, A., Schmidt, P., Cremer, S., Zysk, M., Miltenyi, S., Buck, D.W., and Schmitz, J. (2000). BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. J. Immunol. *165*, 6037–6046.

Dzionek, A., Inagaki, Y., Okawa, K., Nagafune, J., Rock, J.J., Sohma, Y., Winkels, G., Zysk, M., Yamaguchi, Y., and Schmitz, J.J. (2002). Plasmacytoid dendritic cells: from specific surface markers to specific cellular functions(1). Hum. Immunol. *63*, 1133–1148.

Fayette, J., Dubois, B., Vandenabeele, S., Bridon, J.M., Vanbervliet, B., Durand, I., Banchereau, J., Caux, C., and Briere, F. (1997). Human dendritic cells skew isotype switching of CD40-activated naive B cells towards IgA1 and IgA2. J. Exp. Med. *185*, 1909–1918.

Fayette, J., Durand, I., Bridon, J.M., Arpin, C., Dubois, B., Caux, C., Liu, Y.J., Banchereau, J., and Briere, F. (1998). Dendritic cells enhance the differentiation of naive B cells into plasma cells in vitro. Scand. J. Immunol. *48*, 563–570.

Fluckiger, A.C., Garrone, P., Durand, I., Galizzi, J.P., and Banchereau, J. (1993). Interleukin 10 (IL-10) upregulates functional high affinity IL-2 receptors on normal and leukemic B lymphocytes. J. Exp. Med. *178*, 1473–1481.

Fonteneau, J.F., Gilliet, M., Larsson, M., Dasilva, I., Munz, C., Liu, Y.J., and Bhardwaj, N. (2003). Activation of influenza virus-specific CD4+ and CD8+ T cells: a new role for plasmacytoid dendritic cells in adaptive immunity. Blood, *101*, 3520–3526.

Galibert, L., Burdin, N., de Saint-Vis, B., Garrone, P., Van Kooten, C., Banchereau, J., and Rousset, F. (1996). CD40 and B cell antigen receptor dual triggering of resting B lymphocytes turns on a partial germinal center phenotype. J. Exp. Med. *183*, 77–85.

Garcia De Vinuesa, C., Gulbranson-Judge, A., Khan, M., O'Leary, P., Cascalho, M., Wabl, M., Klaus, G.G., Owen, M.J., and MacLennan, I.C. (1999). Dendritic cells associated with plasmablast survival. Eur. J. Immunol. *29*, 3712–3721.

Gauchat, J.F., Henchoz, S., Mazzei, G., Aubry, J.P., Brunner, T., Blasey, H., Life, P., Talabot, D., Flores-Romo, L., Thompson, J., et al. (1993). Induction of human IgE synthesis in B cells by mast cells and basophils. Nature *365*, 340–343.

Grammer, A.C., Bergman, M.C., Miura, Y., Fujita, K., Davis, L.S., and Lipsky, P.E. (1995). The CD40 ligand expressed by human B cells costimulates B cell responses. J. Immunol. *154*, 4996–5010.

Grouard, G., Rissoan, M.C., Filgueira, L., Durand, I., Banchereau, J., and Liu, Y.J. (1997). The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. J. Exp. Med. *185*, 1101–1111.

Guidotti, L.G., and Chisari, F.V. (2001). Noncytolytic control of viral infections by the innate and adaptive immune response. Annu. Rev. Immunol. *19*, 65–91.

Hawiger, D., Inaba, K., Dorsett, Y., Guo, M., Mahnke, K., Rivera, M., Ravetch, J.V., Steinman, R.M., and Nussenzweig, M.C. (2001). Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. J. Exp. Med. *194*, 769–779.

Henn, V., Slupsky, J.R., Grafe, M., Anagnostopoulos, I., Forster, R., Muller-Berghaus, G., and Kroczek, R.A. (1998). CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. Nature *391*, 591–594.

Inaba, K., Steinman, R.M., Van Voorhis, W.C., and Muramatsu, S. (1983). Dendritic cells are critical accessory cells for thymus-dependent antibody responses in mouse and in man. Proc. Natl. Acad. Sci. USA *80*, 6041–6045.

Janeway, C.A., Jr., and Medzhitov, R. (2002). Innate immune recognition. Annu. Rev. Immunol. *20*, 197–216.

Jego, G., Robillard, N., Puthier, D., Amiot, M., Accard, F., Pineau, D., Harousseau, J.L., Bataille, R., and Pellat-Deceunynck, C. (1999). Reactive plasmacytoses are expansions of plasmablasts retaining the capacity to differentiate into plasma cells. Blood *94*, 701–712.

Kadowaki, N., Antonenko, S., Lau, J.Y., and Liu, Y.J. (2000). Natural interferon alpha/beta-producing cells link innate and adaptive immunity. J. Exp. Med. *192*, 219–226.

Katze, M.G., He, Y., and Gale, M. (2002). Viruses and interferon: a fight for supremacy. Nat. Rev. Immunol. *2*, 675–687.

Kumanogoh, A., Marukawa, S., Kumanogoh, T., Hirota, H., Yoshida, K., Lee, I.S., Yasui, T., Taga, T., and Kishimoto, T. (1997). Impairment of antigen-specific antibody production in transgenic mice expressing a dominant-negative form of gp130. Proc. Natl. Acad. Sci. USA *94*, 2478–2482.

Le Bon, A., Schiavoni, G., D'Agostino, G., Gresser, I., Belardelli, F., and Tough, D.F. (2001). Type I interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. Immunity *14*, 461–470.

Litinskiy, M.B., Nardelli, B., Hilbert, D.M., He, B., Schaffer, A., Casali, P., and Cerutti, A. (2002). DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. Nat. Immunol. *3*, 822–829.

Mach, F., Schonbeck, U., Sukhova, G.K., Bourcier, T., Bonnefoy, J.Y., Pober, J.S., and Libby, P. (1997). Functional CD40 ligand is expressed on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for CD40-CD40 ligand signaling in atherosclerosis. Proc. Natl. Acad. Sci. USA *94*, 1931–1936.

MacLennan, I., and Vinuesa, C. (2002). Dendritic cells, BAFF, and APRIL. Innate players in adaptive antibody responses. Immunity *17*, 235–238.

MacNeil, I.A., Suda, T., Moore, K.W., Mosmann, T.R., and Zlotnik, A. (1990). IL-10, a novel growth cofactor for mature and immature T cells. J. Immunol. *145*, 4167–4173.

Manz, R.A., Lohning, M., Cassese, G., Thiel, A., and Radbruch, A. (1998). Survival of long-lived plasma cells is independent of antigen. Int. Immunol. *10*, 1703–1711.

Manz, R.A., Arce, S., Cassese, G., Hauser, A.E., Hiepe, F., and Radbruch, A. (2002). Humoral immunity and long-lived plasma cells. Curr. Opin. Immunol. *14*, 517–521.

Medzhitov, R., and Janeway, C.A., Jr. (1998). An ancient system of host defense. Curr. Opin. Immunol. *10*, 12–15.

Moore, K.W., de Waal Malefyt, R., Coffman, R.L., and O'Garra, A. (2001). Interleukin-10 and the interleukin-10 receptor. Annu. Rev. Immunol. *19*, 683–765.

Morikawa, K., Kubagawa, H., Suzuki, T., and Cooper, M.D. (1987). Recombinant interferon-alpha, -beta, and -gamma enhance the proliferative response of human B cells. J. Immunol. *139*, 761–766.

Muller, U., Steinhoff, U., Reis, L.F., Hemmi, S., Pavlovic, J., Zinkernagel, R.M., and Aguet, M. (1994). Functional role of type I and type II interferons in antiviral defense. Science *264*, 1918–1921.

Nakano, H., Yanagita, M., and Gunn, M.D. (2001). CD11c(+) B220(+)Gr-1(+) cells in mouse lymph nodes and spleen display characteristics of plasmacytoid dendritic cells. J. Exp. Med. *194*, 1171–1178.

Neubauer, R.H., Goldstein, L., Rabin, H., and Stebbing, N. (1985). Stimulation of in vitro immunoglobulin production by interferon-alpha. J. Immunol. *134*, 299–304.

O'Doherty, U., Peng, M., Gezelter, S., Swiggard, W.J., Betjes, M., Bhardwaj, N., and Steinman, R.M. (1994). Human blood contains two subsets of dendritic cells, one immunologically mature and the other immature. Immunology *82*, 487–493.

Peters, M., Ambrus, J.L., Zheleznyak, A., Walling, D., and Hoofnagle, J.H. (1986). Effect of interferon-alpha on immunoglobulin synthesis by human B cells. J. Immunol. *137*, 3153–3157.

Rousset, F., Garcia, E., Defrance, T., Peronne, C., Vezzio, N., Hsu, D.H., Kastelein, R., Moore, K.W., and Banchereau, J. (1992). Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc. Natl. Acad. Sci. USA *89*, 1890–1893.

Santiago-Raber, M.L., Baccala, R., Haraldsson, K.M., Choubey, D., Stewart, T.A., Kono, D.H., and Theofilopoulos, A.N. (2003). Type-I interferon receptor deficiency reduces lupus-like disease in NZB mice. J. Exp. Med. *197*, 777–788.

Schiavoni, G., Mattei, F., Sestili, P., Borghi, P., Venditti, M., Morse, H.C., III, Belardelli, F., and Gabriele, L. (2002). ICSBP is essential

for the development of mouse type I interferon-producing cells and for the generation and activation of CD8alpha(+) dendritic cells. J. Exp. Med. *196*, 1415–1425.

Shortman, K., and Liu, Y.J. (2002). Mouse and human dendritic cell subtypes. Nat. Rev. Immunol. *2*, 151–161.

Siegal, F.P., Kadowaki, N., Shodell, M., Fitzgerald-Bocarsly, P.A., Shah, K., Ho, S., Antonenko, S., and Liu, Y.J. (1999). The nature of the principal type 1 interferon-producing cells in human blood. Science *284*, 1835–1837.

Slifka, M.K., and Ahmed, R. (1996). Long-term humoral immunity against viruses: revisiting the issue of plasma cell longevity. Trends Microbiol. *4*, 394–400.

Sornasse, T., Flamand, V., De Becker, G., Bazin, H., Tielemans, F., Thielemans, K., Urbain, J., Leo, O., and Moser, M. (1992). Antigen-pulsed dendritic cells can efficiently induce an antibody response in vivo. J. Exp. Med. *175*, 15–21.

Steinman, R.M. (1991). The dendritic cell system and its role in immunogenicity. Annu. Rev. Immunol. *9*, 271–296.

van den Broek, M.F., Muller, U., Huang, S., Aguet, M., and Zinkernagel, R.M. (1995). Antiviral defense in mice lacking both alpha/beta and gamma interferon receptors. J. Virol. *69*, 4792–4796.

Wykes, M., Pombo, A., Jenkins, C., and MacPherson, G.G. (1998). Dendritic cells interact directly with naive B lymphocytes to transfer antigen and initiate class switching in a primary T-dependent response. J. Immunol. *161*, 1313–1319.