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DAP12 Signaling Regulates Plasmacytoid Dendritic Cell Homeostasis and Down-Modulates Their Function during Viral Infection

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DAP12 is an ITAM-containing adaptor molecule conveying activating properties to surface receptors on many cell types. We show here that DAP12 paradoxically down-modulates plasmacytoid dendritic cell (pDC) cytokine production in vivo during murine CMV (MCMV) infection. Higher levels of IFN-αβ and IL-12 were detected upon MCMV infection or CpG treatment in DAP12-deficient (DAP12°) mice as compared with wild-type (WT) mice. This resulted from altered homeostasis and enhanced responsiveness of pDCs in DAP12° animals. Increased numbers of pDCs were observed in the periphery of both naïve and MCMV-infected DAP12° mice. A higher proportion of pDCs was activated in infected DAP12° mice, as demonstrated by intracellular staining using an optimized protocol for simultaneous detection of IFN-α and IFN-β. The homeostasis of WT and DAP12° pDCs did not differ in mixed bone marrow chimeric mice. In addition, a similar efficiency of pDC differentiation was observed in vitro in Fms-like tyrosine kinase receptor 3 ligand cultures of WT and DAP12° bone marrow cells. This suggests that DAP12 signaling effects on pDC homeostasis are indirect. In contrast, in response to CpG, DAP12-mediated effects on both IL-12 and IFN-αβ production were intrinsic to the pDCs. However, in response to MCMV, only IL-12 but not IFN-αβ production was affected by pDC-intrinsic DAP12 signaling. Thus, DAP12 signaling in pDCs can mediate different regulatory effects on their functions, depending on the mechanisms of pDC activation. The potential implications of the regulation of pDC functions by DAP12 for promoting health over disease are discussed. The Journal of Immunology, 2006, 177: 2908–2916.
Some receptors on DCs require association with the KARAP/DAP12/TYROBP adaptor molecule for cell surface expression and signal transduction. DAP12 carries an ITAM and mediates a multiplicity of functions in a variety of cell types (23–25). DAP12 and the associated receptor triggering receptor expressed on myeloid cells 1 amplify macrophage inflammatory responses and increase mortality in mice undergoing LPS-induced shock or septic peritonitis upon caecal ligation and puncture (26, 27). In contrast, DAP12 has also been reported to down-modulate macrophage inflammatory responses to TLR or CD16 triggering and to limit susceptibility to another model of endotoxic shock induced by the combination of LPS and β-galactosamine (28). Thus, the role of DAP12 in regulating inflammation may depend on the cell type, the stimuli, or the disease studied. Interestingly, NKp44 signals through DAP12, suggesting a possible involvement of this signal-adaptor in the inhibition of IFN-α/β production by tonsil pDCs upon engagement of the receptor (21). Recently, DAP12 has been shown to inhibit IFN-α/β production in response to CpG stimulation in vitro and in vivo in the mouse (29). Cross-linking of the DAP12 associated receptor Siglec-H on pDCs leads to inhibition of CpG induced IFN-α/β production (29). However, it is not known whether DAP12 functions regulate pDC production of other cytokines induced by TLR-9 triggering, whether DAP12 regulates DC responses in vivo during natural infections, or what the respective roles of the intrinsic vs extrinsic effects of DAP12 for the regulation of pDC functions are.

Defense against murine CMV (MCMV), an established model for studying human CMV infection, is heavily dependent on DC and NK cell responses (30). Early after MCMV infection, IFN-α/β production by tonsil pDCs is observed in uninfected animals. In infected animals, the background staining with an isotype control Ab was <0.1% (not shown). Depending on the experiments, 2.5 × 105 to 2 × 106 events were collected on a FACS Calibur or FACSCanto.

**Materials and Methods**

**Mice**

C57BL/6, B10.D2, B6-DAP12.KI, B6-DAP12.KO, and B10.D2-DAP12.KI mice were bred in pathogen-free breeding facilities at Centre d’Immunologie de Marseille-Luminy (Marseille, France) and at the Microbiology and Tumorbiology Center, Karolinska Institute (Stockholm, Sweden). Bone marrow chimeric mice (BMC) were generated by irradiation of CD45.1+ C57BL/6 (B6.SJL-Ptpcr1/-) Pgp37/BoyCrl (Charles River Laboratories) at 1,000 rad and reconstitution by i.v. injection of bone marrow (BM) cells, either CD45.1 wild-type (WT) and CD45.2 WT BM cells at a 1:1 ratio or CD45.1 WT and CD45.2 DAP12-deficient (DAP12−) BM cells at a 1:1 ratio. Experiments on BMC mice were performed 12–13 wk after generation. Experiments were conducted in accordance with institutional guidelines for animal care and use. Protocols have been approved by the French Provence ethical committee (no. 04/2005), the U.S. Office of Laboratory Animal Welfare (assurance no. A5665-01), and the Committee for Animal Ethics in Stockholm, Sweden.

**In vitro treatment protocols**

Infections of MCMV were initiated at day 0 by i.p. delivery or 1 × 106 PFU of Smith strain MCMV salivary gland extracts (31). Infected mice were harvested at the peak of innate cytokine production by pDCs, which occurs at day 1.5 postinfection (31). CpG oligodeoxynucleotide (ODN) 2216 and control ODN 2243 were synthesized by Sigma-Aldrich and mixed with 1,2-dioleoyloxy-3-(trimethylammonium)propane reagent as described (4), and 10 μg of the ODN was injected i.v. CpG-treated mice were harvested at 3 h postinjection for cytokine stainings in spleen pDCs, at a time where serum cytokine titers are close to their peak, as described (4). No cytokine production was detected in mice injected with control ODN 2243 (not shown).

**Lymphocyte isolation, cell surface, and intracellular staining**

Lymphocytes were isolated from spleens, livers, and lymph nodes as previously described (31, 35). Abs against NK1.1, CD8α, TCR-β, CD11c, IL-12p40/p70, CD45.1, and CD45.2 as well as isotype controls and streptavidin were purchased from BD Pharmingen. The plasmacytoid DC Ag 1 (PDCA-1) mAb was purchased from Miltenyi Biotec, biotinylated goat anti-rat IgG was from Jackson ImmunoResearch Laboratories, and purified rat anti-mouse IFN-α (clone F18 or clone RMMMA-1) and anti-IFN-β (clone RMMMB-1) were from Tebu-bio. mAb 120G8 (36) was provided by Schering-Plough and conjugated to Alexa 488 using a labeling kit from Invitrogen Life Sciences. Protocols for the staining of cell surface markers and intracellular staining of cytokines were adapted (4, 31). No staining for IFN-γ was observed in uninfected animals. In infected animals, the background staining with an isotype control Ab was <0.1% (not shown). Depending on the experiments, 2.5 × 105 to 2 × 106 events were collected on a FACS Calibur or FACSCanto.

**Cell sorting, RNA isolation, and RT-PCR**

NK cells (NK1.1−TCRβ+, pDCs (CD11cint3−medium120G8+), CD8α−CD11c−CD45.1, CD8α+CD103−CD45.1, and CD8α−CD103−CD45.2−) were enriched from the spleen of C57BL/6 mice by positive selection using magnetic beads (anti-DX5 or a mix of anti-CD11c and anti-PCDA1, respectively, all from Miltenyi Biotec) and further purified to >98% on a FACS Aria flow cytometer. RNA were isolated using the RNeasy micro kit (Qiagen) and contamination by genomic material in accordance with the instructions of the manufacturer. The levels of expression of different genes were evaluated by RT-PCR after 28, 30, 32, or 40 amplification cycles. The data shown for each primer pair correspond to the cycle number at which it could be detected but was not yet at saturation. The primer pairs in parentheses were used for the following target genes: DAP12 (forward, 5′-GCTCTGGAGCCCTCTGGTGC-3′; reverse, 5′-CTGATGGTTCAGTGGTGG-3′; product size: 261 bp on cDNA, no genomic product); CD200R4 (forward, 5′-TCCACATGAGGGACTTAC-3′; reverse, 5′-GGGACCATCAACACAACTC-3′); product size: 636 bp on cDNA, >5000 bp on genomic); Ly49H (forward, 5′-AGGATTCATTCTTCTTACC-3′; reverse, 5′-ACTCTGGTTTCTGACTGCC-3′; product size: 482 bp on cDNA, >4000 bp on genomic), Mair-II (forward, 5′-TTCGAGGCTTAGGATGAGTA-3′, reverse, 5′-GCTCGAGAACAGGAAACCACTCTC-3′; product size: 178 bp on cDNA, >1300 bp on genomic); NKGD2 (forward, 5′-AACAAGGAGTCCTCCGATTCC-3′; reverse, 5′-TTACAGACCCCTTTTCTAAGC-3′; product size: 402 bp on cDNA, >3000 bp on genomic); SIRP-β (forward, 5′-TGTGAAGTCTTCCAGAGAGCACTGACATGGC-3′; reverse, 5′-TAGGTTCAACACACCTGACTGTGTCG-3′; product size: 299 bp on cDNA, >5000 bp on genomic); actin (forward, 5′-CATCATCATGAGGGTCTGAC-3′; reverse, 5′-CATACACTGCTCTGCTGACC-3′; product size: 247 bp on cDNA, 247 plus 370 bp on genomic).

**IFN-α/β bioassay was performed as described (37). IFN-α, IFN-β, and IL-12p40 or IL-12p70 ELISAs were performed using commercial kits distributed by PBL Laboratories and R&D Systems, respectively.**

**Fms-like tyrosine kinase receptor 3 ligand (FLT3-L) bone marrow cell cultures**

BM-DC were derived from bone marrow cells in cultures treated with murine recombinant FLT-3-L (25 ng/ml; R&D Systems) as previously described (38).
Statistical analyses

Statistical analyses were performed using Student’s two-tailed t tests. Unless otherwise indicated, means ± SD are shown.

Results

DAP12 and several associated receptors are expressed in pDCs at the mRNA level

Various cell types like DCs, monocytes, and NK cells express DAP12 and associated receptors (23). It has recently been shown that the lectin Siglec-H is specifically expressed on pDCs and requires DAP12 for cell surface expression (29). We studied DAP12 expression in purified NK cells and DC subsets by RT-PCR and found high levels of mRNA encoding DAP12 in all cell populations (Fig. 1). Importantly, several known DAP12-associated receptors in addition to Siglec-H were expressed in pDCs at mRNA levels comparable or higher to those observed in cDCs or in NK cells (i.e., NKG2D, CD200R4, and MAIR-II) (Fig. 1 and Table I). Thus, DAP12 and a variety of associated receptors are expressed in pDCs and could therefore directly take part in regulating pDC functions.

Increased cytokine production in DAP12° mice in response to MCMV infection or CpG treatment

To examine the impact of DAP12 functions on pDC antiviral responses, we measured titers of IFN-αβ in the sera and spleen homogenates of day 1.5 MCMV-infected DAP12° or WT animals by bioassay and ELISA. We observed a significant increase in IFN-αβ production both in the serum (Fig. 2) and spleen homogenate (not shown) of DAP12° mice compared with WT controls. We next measured IFN-αβ production in animals injected with a synthetic TLR-9 agonist, CpG ODN 2216. Again, there was a significant increase in production of IFN-αβ in the serum of DAP12°

Table I. Expression of the genes encoding DAP12 and selected associated receptors in DC subsets and NK cells as assessed by semiquantitative RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>pDC</th>
<th>CD11b DC</th>
<th>CD8α DC</th>
<th>NK</th>
</tr>
</thead>
<tbody>
<tr>
<td>dapt2</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>cd200v3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cd200v4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>ly49h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mairll</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>mlr1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nkg2d</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>pirlb</td>
<td></td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>siglec-h</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sirpβ</td>
<td></td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>trem1</td>
<td></td>
<td></td>
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<tr>
<td>trem2</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>trem3</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* +, ++, and +++ indicate increasing levels of expression; — indicates undetectable.
mice (Fig. 2). Similarly, increased levels of IL-12p40 were observed in spleen homogenates of CpG-treated or MCMV-infected DAP12° animals as compared with their WT counterparts (not shown). These data show that DAP12 signaling can lead to the down-modulation of IFN-αβ and IL-12 production by pDCs in response to MCMV infection or CpG treatment in vivo.

Increased pDC numbers in the periphery of DAP12° mice

Because the tissue distribution of cells bearing generic markers of DCs such as CD11c or MHC class II is affected in DAP12° mice (39), we investigated whether the increased production of IFN-αβ observed in DAP12° animals could be attributed to an alteration of pDC homeostasis. We therefore measured DC subset frequency and numbers in multiple organs of DAP12° and WT animals. There was a significant increase in the proportions of pDCs to total splenocytes in DAP12° mice (Fig. 3, A and B). In contrast, the percentages of CD8α cDCs and NK cells were unchanged, whereas the frequency of CD11b DCs was slightly but not significantly decreased. Overall, the percentages and absolute numbers of pDCs were significantly increased in spleen, lymph nodes, and livers of DAP12° mice (Table II). Viral infection (Fig. 3, C and D) and CpG stimulation (data not shown), preserved higher numbers of pDCs in DAP12° mice as compared with WT controls. pDC accumulation in the periphery of DAP12° mice resulted neither from increased differentiation of pDCs in BM nor from longer half-life in the periphery. BM from WT and DAP12° animals had the same frequency and numbers of pDCs (Fig. 4, A and B), and BM cells from both strains showed similar potential for pDC differentiation in vitro upon FLT3-L stimulation (Fig. 4, C and D). No major differences were observed in the half-life of WT vs DAP12° pDC in the periphery as measured by the kinetics of BrdU incorporation in vivo (data not shown).

To determine whether DAP12 regulation of pDC homeostasis was at least in part cell intrinsic, we generated mixed BMC mice from lethally irradiated CD45.1° WT recipients reconstituted with BM of CD45.2° DAP12° or CD45.2° WT donors mixed in a 1:1 ratio with the BM of WT CD45.1° donors. We analyzed the reconstitution of WT and DAP12° pDCs, cDCs, and NK cells in the spleens of those animals. There was no increase in the proportion of CD45.2° over CD45.1° pDCs in the DAP12°/WT as compared with the WT/WT BMC. Indeed, similar percentages of CD45.2° cells, whether WT or DAP12°, were observed in the three cell types studied: pDCs, cDCs, and NK cells (Fig. 5). Altogether, these results show that DAP12 regulation of pDC homeostasis is not cell intrinsic but must occur as a consequence of DAP12 modulation of the functions of other cells. Irrespective of the nature of these cells, which remains to be identified, the accumulation of

FIGURE 3. Increased numbers of pDCs in the spleen of DAP12° mice in naive mice and at day 1.5 (d1.5) post-MCMV infection. Splenic leukocytes were isolated from naive WT and DAP12° mice on day 1.5 post-infection and analyzed for pDC, CD11b DC, CD8α DC, and NK cell frequencies. A, pDC (PDCA-1°CD11cintCD8α°), CD11b DC (PDCA-1°CD11cintCD8α°), CD8α DC (PDCA-1°CD11chighCD8α°), and NK cell (TCRβ°NK1.1°) frequency is shown for one representative WT and one representative DAP12° animal from groups of three mice. Numbers in dot plots represent the percentage of gated cell population within total splenocytes. T and NK lineage positive cells (TCRβ° or NK1.1°) were electronically excluded from the DC plots. B, Graphic representation of the relative frequency of the three splenic DC subsets in comparison to one another for one representative WT and DAP12° animal. C, cDC (120G8°CD11chigh) and pDC (120G8°CD11cint) frequency is shown for one representative d1.5 CMV-infected WT animal and DAP12° animal from groups of three mice. Numbers in dot plots represent the percentage of gated cell population within total splenocytes. D, Total numbers of splenic cDCs and pDCs from naive and d1.5 MCMV-infected WT and DAP12° mice. Results are expressed as mean ± SD of three mice per group. One experiment representative of three is shown in each panel. *, p ≤ 0.01; **, p ≤ 0.05.

*The Journal of Immunology
pDCs in the periphery of DAP12° mice was true for two different genetic backgrounds, C57BL/6 and B10.D2, and for two different models of genetic inactivation (Table II), mice expressing a mutated DAP12 molecule devoid of signaling function (B6.DAP12.KI) (39) and mice knocked out for the dap12 gene (B6.DAP12.KO) (40). Thus, DAP12 functions regulate pDC homeostasis by limiting pDC numbers in the periphery. The higher numbers of pDCs in DAP12° mice could in part explain the increased production of IFN-α/β in these animals.

**Increased frequency of cytokine-producing pDCs in DAP12° mice upon MCMV infection or CpG injection**

In addition to increased pDC numbers, enhanced reactivity of pDCs for response to viral infection could also contribute to the overall increase in cytokine production observed in DAP12° mice. To address this issue, we established an optimized intracellular staining protocol for simultaneous detection of both IFN-α and IFN-β. We measured IFN-α/β and IL-12 production ex vivo in individual cells of infected animals by intracellular staining. There was a clear staining for IFN-α/β in pDCs (Fig. 6A) without any detectable signals in cDCs from the same sample (not shown). These data confirm our previous demonstrations that pDCs are the major producers of these cytokines early after MCMV infection (3, 31, 32). The proportions of IFN-α/β- or IL-12-expressing pDCs were significantly higher in the DAP12° mice as compared with WT animals (Fig. 6, A and B). These data were further confirmed by measuring ex vivo cytokine production in conditioned medium from pDCs purified from MCMV-infected mice. These experiments showed a >3-fold increase in the production of IL-12p70 and IFN-α/β by the pDCs from DAP12° mice over that from the WT animals (5335 vs 1420 pg/ml and 233 vs 67 U/ml, respectively, for one representative experiment of two). Increases in the overall cytokine production observed in DAP12° mice.

**Contrasting role of DAP12 signaling in pDCs for regulation of IL-12 vs IFN-α/β production in response to MCMV infection**

To determine whether DAP12 regulation of pDC cytokine production in vivo was at least in part cell intrinsic, we generated mixed BMC mice from DAP12° and WT donors as described above. We then analyzed, in each BMC mouse, pDC cytokine production separately for the two types of donor cells as identified by CD45.1 or CD45.2 allotypic markers. CpG stimulation of the mixed BMC mice gave rise to a strong and significant increase in the percentages of both IFN-α/β and IL-12 producing cells within the DAP12° pDCs as compared with the WT pDCs (Fig. 7). Thus, DAP12 is functional in pDCs and limits their production of IL-12 and IFN-α/β in vivo in response to TLR-9 triggering.

NK cell-mediated control of viral replication is heavily reduced in the DAP12° mice (35) because of the loss of NK cell ability to kill infected cells through Ly49H engagement and subsequent DAP12-dependent triggering of cytotoxic effector functions. Thus, the increased cytokine production in DAP12° animals could have resulted at least in part from increased amounts of viral products able to stimulate pDCs. For the purpose of comparing cytokine production by WT vs DAP12° pDCs in response to viral infection within the exact same pathophysiological context, including the efficiency of NK cell control of viral replication, we next infected mixed BMC mice with MCMV (Fig. 7). In MCMV-infected mixed BMC mice a higher proportion of DAP12° pDCs produced IL-12 as compared with their WT counterparts, consistent with what was observed in response to CpG treatment. However, within the same mixed BMC mouse infected with MCMV there were no differences in the percentages of IFN-α/β-producing DAP12° pDCs as compared with the WT pDCs. Thus, intrinsic DAP12 signaling in pDCs specifically inhibited production of IL-12, but not IFN-α/β, during MCMV infection.

Collectively, these data demonstrated that DAP12 is functional in pDCs and differentially limits their production of IL-12 vs IFN-α/β in vivo in response to infectious or noninfectious challenges.

**Discussion**

We demonstrate here for the first time that DAP12 signaling regulates pDC homeostasis and down-modulates pDC cytokine production in response to viral infection in vivo. Compared with WT controls, DAP12° mice produced more IL-12 and IFN-α/β in response to viral infection or to CpG stimulation. This result was due both to increased total numbers of pDCs and to higher frequencies of cytokine-producing pDCs in DAP12° animals. The development of WT and DAP12° pDCs did not differ in mixed BMC mice or in BM cell cultures. This finding shows that DAP12 regulation of pDC homeostasis is not cell intrinsic. Thus, it must occur as a consequence of DAP12 modulation of the functions of other cells. The outcome of intrinsic DAP12 signaling in pDCs for cytokine production upon CpG challenge or viral infection was evaluated in mixed BMC mice. DAP12 signaling in pDCs reduced both IL-12 and IFN-α/β production in response to CpG. In contrast, MCMV infection was able to override the intrinsic inhibitory signals mediated by DAP12 in pDCs specifically for IFN-α/β production, but not for that of IL-12.

Why were IFN-α/β serum levels elevated in the DAP12° mice after MCMV infection, when the experiments based on mixed

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**Table II. Frequencies of pDCs and NK cells in different organs of WT or DAP12° mice**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Spleen (×10⁶)</th>
<th>Lymph Node (×10⁶)</th>
<th>Liver (×10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pDC</td>
<td>NK</td>
<td>pDC</td>
</tr>
<tr>
<td>B10.D2</td>
<td>1.46 ± 0.04</td>
<td>1.1 ± 0.1</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>B10.DAP12.KI</td>
<td>1.48 ± 0.03</td>
<td>3.0 ± 0.3</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>1.44 ± 0.06</td>
<td>0.6 ± 0.1</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>B6.DAP12.KI</td>
<td>1.43 ± 0.07</td>
<td>1.8 ± 0.4</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>1.40 ± 0.03</td>
<td>0.8 ± 0.2</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>B6.DAP12.KO</td>
<td>1.44 ± 0.02</td>
<td>1.9 ± 0.1</td>
<td>3.5 ± 1.5</td>
</tr>
</tbody>
</table>

*Bold, p ≤ 0.01.*

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BMC mice demonstrated that intrinsic DAP12 signaling in pDCs did not modulate significantly IFN-γ production. Our interpretation is that in DAP12° animals the DAP12-mediated regulation of pDC IFN-γ production upon MCMV infection is indirect, occurring through altered functions of other cell types, probably NK cells. Indeed, antiviral NK cell responses are decreased in DAP12° animals because of the loss of triggering of cytotoxic effector functions upon the recognition of infected cells by the DAP12-associated receptor Ly49H (30, 35). This deficit in NK cell functions leads primarily to increased viral replication, which could in turn enhance triggering of pattern recognition receptors on pDCs and thus increase IFN-γ production.

During the course of this study, DAP12 deficiency was shown to increase IFN-γ titers in serum after CpG challenge in mice and in pDC cultures in vitro (29). Our data confirm and extend this finding. Together with our studies during MCMV infection, the data support a role of DAP12 signaling in pDCs for regulation of their responses to TLR triggering. Intracellular signaling cascades originating from DAP12 could interfere with the TLR signaling pathways in different ways. Lack of endogenous DAP12 signaling during pDC development or maturation might alter expression levels of molecules involved in TLR signaling and prime pDCs for enhanced reactivity to pathogen-associated molecular patterns. However, because it was recently shown that triggering of the DAP12-associated receptor Siglec-H on mature WT pDCs limits IFN-γ production upon CpG challenge (22, 29), there clearly exists direct cross-talk between TLR-mediated and DAP12-mediated signaling pathways in mature pDCs. The cross-linking of DAP12 associated receptors may directly interplay with molecules otherwise involved in TLR signaling pathways, either in a competition for shared molecules as was recently suggested (29) or by directly recruiting inhibitory intracellular factors that dampen TLR signaling or expression of IFN-α/β genes (41).

Why was only IL-12 but not IFN-γ production reduced by endogenous DAP12 signaling in pDCs upon MCMV infection, when upon CpG challenge both types of cytokines were affected? DC responses to CpG stimulation are completely dependent on TLR-9 (42). During MCMV infection IL-12 production is strictly TLR-9 dependent, whereas other pathways significantly contribute...
to pDC IFN-αβ responses in vivo (32). Indeed, in addition to TLR-9 at least one other MyD88-dependent receptor promotes IFN-αβ production by pDCs in vivo in response to MCMV infection, although its precise nature has not been elucidated. Potential candidates include TLR-7 or the IL1-R as discussed elsewhere (32). Thus, viral infection triggers IFN-αβ production in pDCs via multiple pathways, and the main part of the production is not affected by endogenous DAP12 signaling. In contrast, both virally induced and CpG-induced pDC IL-12 production result only from TLR-9 engagement and are down-modulated by DAP12. We thus show that MCMV infection overrides DAP12-mediated inhibition of IFN-αβ but not IL-12 production in pDCs, and we speculate that this occurs either because the TLR-9-independent signals may be less sensitive to DAP12 inhibition or because the sum of the different activation pathways inducing IFN-αβ generate a signal stronger than the one for IL-12 (Fig. 8). What could be the advantage, if any, of this differential regulation of IFN-αβ vs IL-12 production by DAP12 in pDCs during viral infection? Systemic delivery of high levels of IFN-αβ is observed during most viral but not other infections (43). It is critical for direct induction of protective antiviral defenses in potentially all of the tissue and cell types of the host (44) as well as for broad regulation of innate and adaptive immune responses (13). Lack of IFN-αβ responses in humans and mice is lethal upon cytopathogenic viral infections (44, 45). In contrast, systemic IL-12 production is observed only during certain viral infections and is not believed to play direct antiviral effects (43). IL-12 acts on discrete subsets of immune cells to promote their activation, mostly IFN-γ production (43). Activation of NK cells by DCs for IFN-γ production requires formation of an immunological synapse with local release of IL-12 (46). Thus, systemic IL-12 responses may not be required for protective antiviral functions but lead to severe immunopathology when too high (14, 47). Therefore, during viral infection DAP12 functions may limit the production of IL-12 but allow secretion of high systemic levels of IFN-αβ by pDCs to promote optimal antiviral defense by preserving strong, direct IFN-αβ antiviral functions while dampening potentially dangerous inflammatory conditions (43). This outcome could be achieved because molecular pathways promoting IFN-αβ production by pDCs in response to viral infection are multiple and, thus, stronger and more flexible than those for IL-12 production, and they are globally able to override DAP12-mediated inhibition. In this context, it is noteworthy that several mechanisms exist that cross-regulate IFN-αβ and
proinflammatory cytokine production during infections or autoimmunity, such as the fact that IFN-α/β production is enhanced through a positive feedback loop but inhibits IL-12 production, whereas TNF-α prevents pDC generation from hematopoietic progenitors and IFN-α/β release from mature pDCs (10, 13).

One could further speculate that the cross-talk between pattern recognition receptors and DAP12-associated receptors expressed in pDCs promotes health over disease under noninfectious conditions as well as during viral challenges. Integration of activation signals delivered by various pattern recognition receptors and modulation signals delivered by DAP12-associated receptors could allow pDCs to sense their environment, measure the threat of viral infection, and balance the benefits of activation of antiviral defenses against the risk of development of immunopathology. This would be consistent with the recent observation that the simultaneous triggering of selected TLR pairs has strong synergistic effects for cytokine production and induction of Th1 responses by DCs (48). In this context, it is interesting to note that low constitutive production of IFN-α/β has been proposed to occur in the absence of overt viral infection, be critical for priming of antiviral responses upon productive infection, and require attenuation to avoid development of immunopathology (49). It would be interesting to determine whether pDCs contribute to the low spontaneous IFN-α/β production and whether alterations in the cross-talk between TLRs and DAP12-associated receptors play a role in the enhanced pDC activation or increased IFN-α/β levels observed in patients suffering from autoimmune diseases such as systemic lupus erythematosus, psoriasis, or insulin-dependent diabetes mellitus (1, 9–12, 50). Clarifying the pathways for pDC regulation in vivo could contribute to the development of new therapeutic strategies to modulate pDC functions for fighting viral infections or control autoimmune disease.

In summary, we identify here a crucial role of DAP12 in the regulation of pDC biology. DAP12 signaling has been demonstrated to exert indirect effects on pDC homeostasis, probably through the modulation of the functions of other cells, but also direct pDC-intrinsic effects on pDC cytokine production. We further show that endogenous DAP12 signaling in pDCs modulates both IFN-α/β and IL-12 production upon CpG challenge in vivo, whereas it reduces only IL-12 but not IFN-α/β production during viral infection. This finding led us to propose that the impact of DAP12 signaling on the regulation of IL-12 and IFN-α/β production by pDCs depends on the physiopathological context and, more specifically, on the nature of the number of the pattern recognition receptors engaged for pDC activation.

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Disclosures

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References


