IL-4 Confers NK Stimulatory Capacity to Murine Dendritic Cells: A Signaling Pathway Involving KARAP/DAP12-Triggering Receptor Expressed on Myeloid Cell 2 Molecules

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IL-4 Confers NK Stimulatory Capacity to Murine Dendritic Cells: A Signaling Pathway Involving KARAP/ DAP12-Triggering Receptor Expressed on Myeloid Cell 2 Molecules

Magali Terme,* Elena Tomasello, † Koji Maruyama,* Florent Crépineau,* Nathalie Chaput,* Caroline Flamant,* Jean-Pierre Marolleau, ‡ Eric Angevin,* Erwin F. Wagner, § Benoît Salomon,* François A. Lemonnier,† Hiro Wakasugi,# Marco Colonna, ** Eric Vivier, ‡ and Laurence Zitvogel*‡

Dendritic cells (DC) regulate NK cell functions, but the signals required for the DC-mediated NK cell activation, i.e., DC-activated NK cell (DAK) activity, remain poorly understood. Upon acute inflammation mimicked by LPS or TNF-α, DC undergo a maturation process allowing T and NK cell activation in vitro. Acute inflammation is controlled in part by Th2 cytokines. In this study, we show that IL-4 selectively confers to DC NK but not T cell stimulatory capacity. IL-4 is mandatory for mouse bone marrow-derived DC grown in GM-CSF (DCGM/IL-4) to promote NK cell activation in the draining lymph nodes. IL-4-mediated DAK activity depends on the KARAP/DAP12-triggering receptor expressed on myeloid cell 2 signaling pathway because: 1) gene targeting of the adaptor molecule KARAP/DAP12, a transmembrane polypeptide with an intracytoplasmic immunoreceptor tyrosine-based activation motif, suppresses the DCGM/IL-4 capacity to activate NK cells, and 2) IL-4-mediated DAK activity is significantly blocked by soluble triggering receptor expressed on myeloid cell 2Fc molecules. These data outline a novel role for Th2 cytokines in the regulation of innate immune responses through triggering receptors expressed on myeloid cells. The Journal of Immunology, 2004, 172: 5957–5966.
chemokine (MDC) and thymus- and activation-regulated chemokine, are regulated by IL-4 and IL-13 (21, 22) and play a critical role to recruit both DC and NK cells (23, 24). Adenoviral gene transfer of MDC has been reported to mediate IL-4-dependent antioxidant effects (25). IL-13 administration to SIV-infected macaques leads to monocytes (26) and NK cell infiltration of duodenal villi with consequent apoptosis of intestinal epithelial cells (27).

Therefore, we investigated the role of IL-4 or IL-13 in regulating DC-activated NK cell (DAK) activity. Both Th2 cytokines confer to mouse and human DC the selective capacity to stimulate NK cells (but not alloreactive T cells) in vitro. Moreover, IL-4 dramatically enhances the capacity of adaptively transferred DC to activate NK cells in lymph nodes. By selectively up-regulating transcription of triggering receptors expressed on myeloid cells (TREM molecules), IL-4 facilitates engagement of KARAP/DAP12 signaling pathway in DC following encountering with NK cells, ultimately leading to NK cell activation. These data outline a new pathway through which Th2 cytokines may shape immune responses.

Materials and Methods

Mice

Female C57BL/6 (H-2b) wild-type (WT) or recombinant-activating gene (RAG) 2−/− or nude mice and female BALB/c (H-2r) WT or SCID mice were obtained from the Center d’Elevage Iffa Credo (Le Genest St Isle, France), the Center d’Elevage Janvier (Le Genest St Isle, France), the Center d’Elevage Iffa Credo (Le Genest St Isle, France), and maintained in Institut Gustave Roussy animal facilities according to the Animal Ethics Committee Guidelines. Double H-2Kb−/−/H-2Db−/− knockout mice were generated, as previously described (5), bred in the animal facility of Institut Pasteur, and used at the sixth backcross generation onto C57BL/6 mice. All female mice were used at 6–25 wk of age. Female 6–8 wk of age KARAP/DAP12 homozygous knock-in mice were previously described (28). B7-1 and B7-2 loss-of-function mice were bred in the Centre d’Études et de Recherches en Virologie et Immunologie animal facility (29).

Generation of DC in vitro

Bone marrow-derived DC were propagated from BM progenitor cells in culture medium supplemented with 1000 IU/ml murine rGM-CSF (R&D Systems, Minneapolis, MN) or with or without 1000 IU/ml murine rIL-4 (R&D Systems), as previously described (30). For the induction of maturation, cultures of bone marrow-derived DC (BM-DC) were supplemented with 2 μg/ml LPS (Sigma-Aldrich, St. Louis, MO) or 20 ng/ml murine rTNF-α (R&D Systems) on day 6 of culture for 24 h before use. Culture medium was renewed at days 2 and 4. Day 6 DC were harvested, spun down, and transferred into new six-well plates. For phenotypic analyses, cells were incubated with FITC-conjugated anti-I-Ak (AF6-1201); PE-conjugated anti-CD11c (HL-3); FITC-conjugated anti-CD86 (GL1), CD40 (3/23), and H-2Kb (AF6-885); and PE-conjugated anti-CD80 (16-10A1) and H-2Db (KH95). All Abs were purchased from BD Pharmingen (San Diego, CA). Cells were gated according to size and granularity with exclusion of propidium iodide-positive cells. Residual B lymphocytes (Gr1−/CD19−/CD11b−/NK1.1−) and granulocytes (Gr1+/CD11b−/CD19−/NK1.1+) were detected in the CD11c−/I-Ak− cells and constitute <20% of whole cell population. T and NK cells were not propagated in these DC culture conditions. Phenotypic profiles are shown for each individual condition in Fig. 1. BM-DC derived from gene-targeted mice were analyzed for MHC class I expression and exhibited levels of MHC class II, CD80, CD86, and CD40 expression comparable to those of WT BM-DC and a similar allostimulatory capacity in the absence or presence of LPS (data not shown).

Preparation of NK cells

Splenocytes were harvested from BL6-RAG2−/− or BALB/c SCID mice, as stated in figure legends. Splenic nonadherent cells were generated by subjecting RBC-deprived splenocytes to 3-h adherence at 37°C. Nonadherent cells were incubated overnight with 1000 U/ml human rIL-2, and analyzed in a FACSscan cytofluorometer using anti-CD3 FITC, DX5 PE (or NK1.1 PE in BL6 mice), and CD69 CyChrome (Cyc) mAb (or an isotype control Ab) before coculture with BM-DC. Up to 40% of such splenocytes were CD3−/DX5+. In some experiments, spleen-derived NK cells from SCID mice or KARAP/DAP12 knock-in mice were negatively selected using the Spin Sep kit (StemCell Technologies, Vancouver, Canada).

Preparation of DC/NK cell cocultures

Procedures have been previously described (9). NK cells (splenocytes from immunodeficient mice) were seeded at 1.5 × 10^5/well in 96-well plates for 20 h. DC/NK ratios of mouse cocultures were 0.5:1 (unless otherwise specified). In blocking experiments, mouse TREM2Fc or CTLA4Ig (Roche, Milan, Italy), both containing an IgG1, were added at increasing dosages in the mouse BM-DC/NK cell cocultures or to NK or DC alone (as described in Fig. 6 legend). Mouse TREM2 extracellular region was amplified by PCR from cloned TREM2 cDNA (31) using the following oligonucleotides: 5′-TAGTAGGAAATCGCCACATGGAACCTCCACAGCTTTCTCTGTCG-3′ and 5′-TAGTAGAACCTCTACTTACCGGAATGTTGGAAGAGGT-3′. The PCR product was digested with EcoRI and HindIII, subcloned into a vector encoding human IgG1 Fc, and expressed as TREM2-human IgG1 fusion protein in J558L mouse myeloma cells, as previously described (32).

Allogeneic MLR

Splenocytes were harvested from BALB/c (H-2b) mice. Splenic nonadherent cells were generated by subjecting RBC splenocytes to 3-h adherence at 37°C. Procedures of MLR have been detailed in figure legends.

Assessment of NK cell effector functions

Cytotoxicity assays. Cells from 20 h of coculture were collected. Viable trypan blue-excluded NK cells were counted and used as effector cells. Cytotoxicity of NK cells was measured in a standard 4-h 51Cr release assay using Na235CrO4-labeled YAC-1 targets. Experiments were conducted in triplicate at various E:T ratios.

Cytokine detection and quantification (murine IFN-γ). After 20 h of BM-DC/NK coculture, supernatants were harvested, stored at −80°C, and assayed either directly or after 2–10 times dilution using commercial ELISA kits (OptEIA ELISA kit; BD Pharmingen). The sensitivity of the murine IFN-γ kit was >31.5 pg/ml.

Statistical analyses of cytokine levels

Student’s t test was used to compare means ± SE of IFN-γ production in between various culture conditions, and significant differences at 95% confidence are depicted with * on each graph.

Lymph node analyses of NK cells

In some experiments, three washing steps in PBS, one million day 7 BM-DC, grown with various cytokine mixtures and derived from various strains of mice, were inoculated in one footpad of BL6 nude mice. Popliteal homo- and contralateral nodes were harvested at 24 or 72 h. Lymph node mononuclear cells were mechanically minced. Cells were enumerated using trypan blue exclusion before immunostaining with three-color mAb (anti-CD3 FITC, anti-DX5 PE, anti-CD69 Cy or anti-CD53 Cy, anti-NK1.1 PE, anti-CD69 FITC).

Analysis of KARAP/DAP12 and associated membrane receptors

RT-PCR studies. Total RNA was extracted from BM-DC using RNeasy Mini kit (Life Technologies, Carlsbad, CA). One microgram of RNA was then used to produce cDNA in a final reaction volume of 50 μl using SuperScript II reverse transcriptase, according to the manufacturer’s instructions. Potential contamination by genomic DNA was avoided by treatment with RNase-free DNase (Life Technologies). Serial dilutions of cDNA (5, 0.5, and 0.05 μl) were then used to perform PCR in a final volume of 50 μl. Following primers were used: myeloid DAP12-associated lectin-1 (MDL-1) forward, ATTTGGCATTCATTTCGCAGA; MDL-1 reverse, ATTTGGCATTCATTTCGCAGA; TREM forward, GCTCTGGAGCCCTCCTGGTGC; TREM reverse, ATTTGGCATTCATTTCGCAGA; TREM forward, GCTCTGGAGCCCTCCTGGTGC; KARAP reverse, CTCAGTCTCAGCAATGTTGTTG; actin forward, ATGGAACGGCATCAGTCTTGTCA; actin reverse, GATGGACCTTCCTCACAGTTT; TREM reverse, CTCAGTCTCAGCAATGTTGTTG; actin forward, ACTCTCCCTGTCGTTGCTCC; actin reverse, CCTCTCCCTGTCGTTGCTCC. Reaction was performed with the following program: 94°C for 2 min, followed by 40 cycles of 94°C for 50 s, 55°C for 50 s, 72°C for 1 min, and finally 72°C for 7 min.

Results

DAK activity upon acute inflammation

The dynamics of the human DC/NK cell cross talk using monocyte-derived DC and peripheral blood CD3−/CD56+ purified NK

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cells have been reported recently (12–14). In resting conditions, when immature DC encounter resting NK cells, NK cell effector functions remain inert. In contrast, in the presence of inflammatory stimuli such as LPS, DC readily promote NK cell activation (12). Similarly, resting NK cells encountering mature DC become activated (14). We investigated the dynamics of the mouse BM-DC/NK cell interaction using DC stimulated with the acute inflammatory cytokine TNF-α or the microbial product LPS. The vast majority of day 7 BM-DC propagated in GM-CSF alone (BM-DCGM) express low levels of MHC class II (I-A^b), MHC class I (K^b, D^b), and costimulatory CD86, CD40 molecules (Fig. 1) compared with their counterparts stimulated with TNF-α or LPS. Indeed, BM-DCGM/TNF and BM-DCGM/LPS homogeneously display a mature phenotype with surface expression of MHC class II molecules (Fig. 1) and a strong allostimulatory capacity (Fig. 2A). In contrast to BM-DCGM, such mature BM-DC enhanced NK cell lytic activity against YAC-1 cells (Fig. 2B) and IFN-γ secretion (inset, Fig. 2B). At various DC/NK cell ratios, BM-DCGM remain poorly effective. We conclude from these experiments that mouse DC, like human DC, become potent T/NK cell stimulators when exposed to danger signals such as LPS or TNF-α.

**IL-4 confers NK stimulatory capacity to DC**

When cultured in the presence of IL-4 or IL-13, BM-DC exhibit low MHC class II surface expression (Fig. 1, and data not shown) and poor allostimulatory capacity, yet manifesting the capacity to enhance NK cell effector functions (Fig. 3, A and B). The phenotypic comparison between BM-DCGM vs BM-DCGM/IL-4 does not reveal major differences in the expression of MHC class I, II, CD80, CD86, and CD40 molecules (Fig. 1) as well as in the allogeneic MLR (Fig. 3A). Although BM-DCGM barely trigger NK cell activation in coculture at various DC:NK cell ratios, BM-DCGM/IL-4 are apparently endowed with elective NK cell stimulatory capacity in vitro at a DC:NK cell ratio of 1:2 and 1:5 (Fig. 3B, and data not shown). As previously reported (9), cell-to-cell contact was required for this effect and BM-DCGM/IL-4 were not lysed by NK cells. Moreover, BM-DCGM/IL-4 also trigger the secretion of IFN-γ by NK cells (Fig. 3B, inset). IL-13 (≥ 2 ng/ml) was as potent as IL-4 (between 500 and 1000 IU/ml) to endow DC with the capacity to activate NK cells (Fig. 3B). However, neither of these Th2 cytokines does directly modulate NK cell functions in the absence of DC in vitro (data not shown). Importantly, the IL-4-mediated BM-DC capacity to activate NK cells was comparable whether NK cells were syngeneic or allogeneic (Fig. 3C). A kinetic study for the introduction of IL-4 in the BM-DC culture underscores a requirement for IL-4 in the last 4 days of in vitro cultures (Fig. 3D).

Altogether, these data indicate that while IL-4 or IL-13 do not promote overt DC maturation, IL-4 and IL-13 do confer a NK cell stimulatory capacity i.e., DAK activity to mouse DC in vitro.

**IL-4-propagated DC promote NK activation in vivo**

To test whether IL-4 modulates DAK activity in vivo, we examined NK cell activation in the draining lymph nodes of BL6 Nu/Nu mice inoculated with syngeneic BM-DC in the footpad at 24 and 72 h. NK cells represented ~5% of total lymph node mononuclear cells in nude BL6 mice and could be stained either with anti-DX5 mAb or anti-NK1.1 mAb. The proportion of CD69^+ cells among gated NK1.1^+ or DX5^+/CD3^- NK cells in these nodes is shown in Fig. 4A, and enumeration is depicted at 24 h in Fig. 4B. Absolute numbers of homolateral popliteal lymph node resident DX5^-/CD3^- NK cells were calculated 72 h following injection of

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**FIGURE 1.** Phenotypic analyses of ex vivo propagated BM-DC. BM-DC propagated either in GM-CSF alone or a combination of GM-CSF + IL-4 for 7 days were analyzed in flow cytometry. BM-DC propagated in GM-CSF alone were subjected to 24-h stimulation at day 6 with either TNF-α or LPS. A two-color staining (anti-CD11c PE and anti-I-A^b FITC mAb) or one-color staining (anti-CD40, anti-CD86, anti-H-2K^b FITC mAb, anti-CD80, anti-H-2D^b PE mAb) was performed at day 7 before analysis. Hatched lines represent staining with a control isotype-matched mAb.
IL-4-mediated DAK activity does not involve B7 or MHC class I molecules

Because CD28 is expressed on resting NK cells and has been claimed to be costimulatory for NK cell activation (33, 34), we investigated the role of B7-1 and B7-2 in BM-DC\textsuperscript{GM,IL-4}–mediated NK cell activation in vivo and in vitro. B7 loss-of-function BM-DC\textsuperscript{GM,IL-4} remain fully competent to recruit and activate a subset of DX5\textsuperscript{+}/CD3\textsuperscript{−} NK cells in the draining lymph node of Nu/Nu mice (Fig. 4D). Such B7-deficient BM-DC\textsuperscript{GM,IL-4} also maintain their capacity to activate NK cells in vitro, as determined by YAC-1 killing and IFN-γ secretion (Fig. 5A). NK cell activation results from a balance between inhibitory and activating signaling regulated by specific ligands (35). Abrogation of inhibitory signals could account for the BM-DC\textsuperscript{GM,IL-4}–mediated NK cell activation. However, the surface expression of MHC class I molecules, i.e., H-2D\textsuperscript{b} and H-2K\textsuperscript{b}, is not down-modulated on DC propagated in the presence of IL-4 (Fig. 1). Moreover, we performed DC/NK cell cocultures using H-2D\textsuperscript{b}/K\textsuperscript{b} double-knockout BM-DC propagated in GM-CSF + IL-4 in H-2-matched model systems. The loss of inhibitory ligands significantly augmented the capacity of DC\textsuperscript{GM,IL-4} to promote NK cell effector functions, i.e., cytolytic activity against YAC-1 (Fig. 5B) and IFN-γ secretion (Fig. 5B, inset). Thus, a down-regulation of the inhibitory pathway is unlikely to account for the IL-4–mediated DAK activity.

**KARAP/DAP12-TREM2 is involved in the IL-4-mediated DAK activity**

Knock-in mice bearing a nonfunctional KARAP/DAP12 immunoreceptor tyrosine-based activation motif (ITAM) exhibit altered innate immune responses associated with an accumulation of DC in cutaneous and mucosal epithelia, suggesting that KARAP/DAP12 acts as an endogenous modulator of DC functions (28, 36). We therefore investigated the role of this transmembrane protein in the DAK activity mediated by BM-DC\textsuperscript{GM,IL-4}. Homozygous KARAP/DAP12 loss-of-function mutant BM-DC\textsuperscript{GM,IL-4} were cocultured with WT NK cells. Homozygous knock-in DC completely failed to activate NK cells at the level of cytolytic activity and IFN-γ secretion (Fig. 5C). Interestingly, KARAP/DAP12 knock-in NK cells do respond to WT BM-DC (Fig. 5D), strengthening that BM-DC and not NK cells require KARAP-DAP12 signaling. We next addressed the possibility that IL-4 might up-regulate the mRNA expression of KARAP/DAP12 adaptor and/or its membrane-associated receptors expressed on DC, i.e., triggering receptors expressed on myeloid cells (32, 37) and the myeloid DAP12-associated lectin-1 (38). Although KARAP/DAP12 mRNA or protein expression levels were not modulated by incubation of BM-DC with IL-4, TREM, but not MDL-1 cDNA were significantly more transcribed in BM-DC\textsuperscript{GM,IL-4} as compared with BM-DC\textsuperscript{GM} (Fig. 6A). TREM mRNA levels were also augmented in KARAP/DAP12 knock-in DC (data not shown). Bouchon et al. (31) reported a strong down-regulation of MDL-1 and TREM-1 during monocyte differentiation toward the dendritic lineage in the presence of IL-4 associated with a concomitant up-regulation of TREM2. TREM2 is a member of the Ig superfamily characterized by a single V-type extracellular domain, a transmembrane region with a charged residue of lysine, and a short cytoplasmic tail with no signaling motifs. TREM2 is associated with KARAP/DAP12 in human monocyte-derived DC (32). To further demonstrate that TREM2 is involved in the IL-4–mediated DAK activity, soluble mouse TREM2Fc molecules were used to block the BM-DC\textsuperscript{GM,IL-4}–mediated NK cell activation in vitro. Indeed, while CTLA4Ig had no effect, confirming the results achieved with B7 loss-of-function

![FIGURE 2. Activated BM-DC promote both T and NK cell stimulation.](https://example.com/figure2.png)

**FIGURE 2.** Activated BM-DC promote both T and NK cell stimulation. A, Day 6 H-2\textsuperscript{b} BM-DC incubated for 24 h in GM-CSF alone or a combination of GM-CSF + LPS, or GM-CSF + TNF-α were used to stimulate the proliferation of 10\textsuperscript{5} allogeneic H-2\textsuperscript{b} splenocytes for 5 days at various DC:T ratios in an MLR assay. Incorporation of [\textsuperscript{3}H]Tdr is measured at day 6. The DC:T ratio of 1:25 is shown. Means of proliferation counts with SE of triplicate wells are depicted. B, \textsuperscript{51}Cr release cytosis assay against YAC-1 target cells of cocultures containing BM-DC and BALB/c SCID-derived NK cells at a 1 NK:0.5 DC ratio for 20 h. In the inset are depicted the levels of IFN-γ secretion (measured in ELISA) of NK cells alone or cocultured in the presence of BM-DC. A typical experiment is shown of three performed and resulting in similar data.

BM-DC in the homolateral footpad and compared with those residing in contralateral popliteal nodes. Although BM-DC\textsuperscript{GM} neither activated (Fig. 4, A and B) nor recruited (Fig. 4C) resident DX5\textsuperscript{+}/CD3\textsuperscript{−} cells, BM-DC\textsuperscript{GM,IL-4} significantly promoted NK cell recruitment and/or proliferation in situ (63,140 ± 7,153 NK cells in homolateral vs 47,470 ± 7,365 on contralateral node, p = 0.006) at 72 h as well as activation of a subset of resident NK cells (10,650 ± 1,364 CD69\textsuperscript{+} NK cells in homolateral vs 5,958 ± 933 on contralateral node, p = 0.002) at 24 h. Similarly, splenic NK cell activation was achieved using i.v. injection of BM-DC\textsuperscript{GM,IL-4}, but not BM-DC\textsuperscript{GM} (data not shown).

In conclusion, adoptively transferred DC propagated in IL-4 are capable of eliciting NK cell activation in lymph nodes.
DC, soluble TREM2Fc molecules significantly abrogated BM-DC(GM/IL-4)-mediated NK cell lytic activity against YAC-1 cells (Fig. 6, B and C). However, soluble TREM2Fc molecules were not sufficient to directly trigger NK cell lytic activity in vitro (Fig. 6B), even when TREM2Fc molecules were coated on Maxisorb wells to allow cross-linking of NK cell counterreceptors. Interestingly, incubation of NK cells with IL-2 and TREM2Fc in the absence of BM-DC could trigger NK cell IFN-γ production, while neither agent alone could trigger it, and while CTLA4Ig could not, emphasizing that TREM2 molecules do interact with counterreceptors on mouse NK cells (data not shown). However, we ruled out a role for IL-2 secreted by BM-DC(GM/IL-4) in the IL-4-mediated DAK activity (data not shown). Therefore, one possibility suggested by these results is that NK cell binding to DC TREM2 molecules: 1) triggers TREM2/KARAP signaling in DC, 2) promotes KARAP/DAP12-dependent up-regulation of a membrane surface molecule that will engage activating receptors on NK cells, 3) thereby counterbalancing DC MHC class I inhibition of NK cells. To exemplify that NK cell binding to DC TREM2 molecules signals through KARAP/DAP12 pathway, we investigated the levels of I-Ab and costimulatory molecules expressed on CD11c+/H11001 BM-DC WT vs KARAP/DAP12+/H11002 knock-in BM-DC following incubation with negatively selected NK cells from BALB/c SCID mice. Although I-Ab and CD40 did not significantly translocate to cell surface in WT nor in KARAP knock-in BM-DC, CD80 molecules were up-regulated, with no significant difference between WT and KARAP/DAP12 knock-in BM-DC (Table I). However, and in accordance with the reported TREM2 signaling in human MD-DC (32), CD86 was significantly up-regulated in WT BM-DC encountering WT NK cells, but not in KARAP knock-in BM-DC, suggesting that an alternate pathway of activation via KARAP/DAP12 ITAM signaling in DC is triggered.
Discussion

Our data point to a critical role of IL-4 in regulating: 1) NK cell recruitment or in situ proliferation and activation through DC in lymph nodes; 2) DAK activity through KARAP-dependent triggering receptors expressed on myeloid cells.

The effects of IL-4 on DC functions have been mostly studied in vitro. In contrast to GM-CSF, which plays a primary role in precursor survival, proliferation, and early differentiation, IL-4 promotes the final differentiation of DC. IL-4 has been reported to induce the loss of macrophage features, increase MHC expression, up-regulate costimulatory and DC-SIGN molecules, enhance Ag uptake and processing, and synergize with other factors to increase IL-12 production (39–43). However, when added to DC precursors differentiated from CD34+ progenitors, IL-4 delays maturation of Langerhans cells and reduces the allostimulatory function of DC (44, 45). We confirmed these findings and showed that mouse BM-DCGM/IL4 behave like BM-DCGM in the sense that they acquired a fully mature phenotype only after LPS stimulation (M. Terme, data not shown). Similarly, addition of human rIL-4 to CD34+ human DC progenitors incubated for 5 days in stem cell factor, GM-CSF, and TNF-α did not enhance their allostimulatory capacities, but significantly promoted the lytic activity against K562 of CD3+/CD56+ NK cells purified from...
IL-4 facilitates NK cell stimulation at the level of DC precursors and not at that of differentiated DC, because addition of IL-4 the last 24 h of a day 6 BM-DC culture did not empower DC for NK cell stimulation (Fig. 3D). It is also conceivable that IL-4Rα could be expressed at various kinetics during in vitro bone marrow cultures in GM-CSF + IL-4, rendering DC precursors optimal for the responsiveness to IL-4. Interestingly, Roth et al. (46, 47) reported increased DC numbers and functions in both mice and cancer patients following continuous administration of GM-CSF and IL-4. As compared with GM-CSF alone, the combination of GM-CSF plus IL-4 promoted antitumor effects associated with signs of DC differentiation. Despite these changes, freshly isolated DC from GM or GM/IL-4 groups exhibited a functionally immature phenotype in MLRs. Moreover, earlier studies reported that a mixture of tumor cell lines secreting rGM-CSF + rIL-4 generated more effective antitumor responses than did the same number of tumor cells secreting only rGM-CSF (48, 49). Although NK cell functions were not assessed in these reports, it is likely that the enhanced antitumor effects observed in the elective presence of IL-4 be mediated through NK cells. Indeed, enhanced antitumor CTL responses were reported following NK cell-mediated tumor regression (50). IL-4 does not directly trigger NK cell activation, as indicated by the fact that addition of IL-4 to human or mouse NK cells (in the absence of DC) fails to enhance NK cell functions (our data not shown). According to published reports, IL-4 negatively regulates adhesion and transendothelial migration of activated NK cells (51). Our findings unravel a novel regulatory role of IL-4 on DC, conferring to DC NK cell stimulatory capacity in vitro and in vivo.

The DC/NK cell interaction has been formally described in the setting of acute inflammation mimicked by TNF-α, IFN-α, LPS, and Mycobacterium tuberculosis (12, 14). However, numerous clinical acute inflammatory settings such as asthma, autoimmune skin or bowel diseases, and arteriosclerosis convert into chronic inflammatory disorders. Th2 cytokines have been found in such chronic inflammatory lesions, and their role in the pathogenesis has been documented. Limited studies have investigated the role of infiltrating NK cells in chronic inflammatory processes. Chronic inflammatory diseases of the lungs, such as asthma, are frequently associated with
mixed Th1 and Th2 T cell responses. Trying to recapitulate the disease, Ford et al. (52) have coinstilled IL-13 along with IFN-H9253 into mouse airways. IFN-H9253 and IL-13 synergized to provoke recruitment of NK cells and CD11c/H11001/MHC class II/H11001/CD86/APCs, resulting in goblet cell hyperplasia, airway eosinophilia, and hyperreactivity. Evidence for enhanced NK cell activity after bronchial allergen challenge in asthmatic subjects was brought up (53) and later on, Korsgren et al. (54) demonstrated the role of NK cells during the immunization phase of allergen-induced eosinophilic airway inflammation in mice.

Our data point to a role for Th2 cytokines in providing NK cell-derived local IFN-H9253 production that might enhance ongoing Th1 inflammatory processes.

The role of the IL-4-mediated DAK activity in the Th2 differentiation pathway remains to be clarified. IL-4-treated DC were shown to enhance IL-4 production in a paracrine/autocrine positive feedback loop (55). MacDonald and Pearce (56) recently reported that Th2 polarization following adoptively transferred IL-4-producing DC only depends on IL-4 production by recipient cells. These data suggest a role for IL-4-producing DC at the site of Ag encounter rather than during DC/T cell interaction. The source of the recipient-derived IL-4 remains unknown, but it is conceivable that the DC/NK cell cross talk could result in the secretion of recipient NK cell-derived regulatory cytokines such as IL-5, IL-13, or IFN-H9253 (57–59).

![Table I](image)

Table I. WT, but not KARAP/DAP12−/− BM-DC up-regulate CD86 molecules in coculture with WT NK cells

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<td>CD40</td>
<td>87 ± 2</td>
<td>87 ± 1</td>
</tr>
<tr>
<td></td>
<td>81 ± 3</td>
<td>83 ± 3</td>
</tr>
<tr>
<td>CD40</td>
<td>169 ± 24</td>
<td>168 ± 27</td>
</tr>
<tr>
<td></td>
<td>139 ± 13</td>
<td>152 ± 22</td>
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</table>

* Spleen-derived NK cells of SCID mice were negatively selected using the Spin Sep kit (StemCell Technologies), then stimulated overnight in 1000 IU/ml IL-2 and incubated (or not) with BM-DC (GM/IL-4) WT vs KARAP/DAP12 knockin for 24 h at a ratio of 1 DC to 2 NK cells. After coculture or incubation alone, FACS analyses gating on CD11c− cells were performed using anti-I-Ab, CD40, CD80, and CD86 mAbs. Mean ± SD of three independent experiments are shown for each individual molecular pattern. Student’s t test was used to compare expression of molecules on BM-DC alone vs BM-DC after coculture with NK cells. *p < 0.05.

b MFI, mean fluorescence intensity.
We showed that DC differentiated in IL-4 are able to recruit or enhance in situ proliferation and activation of NK cells in the draining lymph nodes following s.c. injection. This is the first observation demonstrating NK cell recruitment or in situ NK cell proliferation driven by DC in secondary lymphoid organs. It is tempting to speculate that NK cells are recruited from peripheral blood through high endothelial venules and that MDC or thymus-and activation-regulated chemokine might be key Th2 chemokines (21, 60) to account for NK cell attraction. It is also conceivable that peripheral DC might secrete such chemokines that might leak to lymph nodes and chemotraact NK cells from the circulating blood. Therefore, the BM-DCGM/IL-4/NK cell cross talk could be considered in the design of CTL peptide-based cancer vaccines. However, whether the DC/NK cell cross talk is critical to promote the Ag-specific cognate T cell responses mediated by DC remains to be determined.

Our data show that KARAP/DAP12 are critical ITAM-bearing activating receptors involved in the DC capacitation to trigger NK cells in vitro. KARAP/DAP12 has been shown to be involved at the level of Ag presentation for T cell activation during the development of experimental autoimmune encephalomyelitis Th1 experimental model (36), and for hapten sensitization (28). KARAP/DAP12 has also been involved in NK cell-dependent protection against murine CMV replication (61). The relevance of the DC/NK cell cross talk in mediating murine CMV eradication has been recently reported (62). Indeed, the authors showed that DC promote NK cell proliferation in a cytokine-independent manner, while DAP12-dependent Ly-49H NK cells appear to mediate DC survival through cell-to-cell contact. We showed in Fig. 5D that KARAP/DAP12 knockin NK can be activated for IFN-γ secretion following contact with WT BM-DC, suggesting that KARAP/DAP12 is not required for the NK cell ability to respond to DC.

We showed in Fig. 5C and Table I that KARAP/DAP12 is a critical pathway at the DC level for DAK activity. These conclusions hold only when IL-4 mediates DAK activity, because we were not able to show that KARAP/DAP12 was critical in the DAK activity mediated through acute inflammatory signals (i.e., LPS; M. Terme and E. Tomassolo, unpublished data). Importantly, IL-4 up-regulates KARAP/DAP12-associated TREM mRNA levels in BM-DC, and soluble TREM2Fc molecules abrogate IL-4-mediated DAK activity. KARAP/DAP12-TREM2 triggering on human DC promotes incomplete maturation process, i.e., DC up-regulation of CCR7, CD40, CD86, and MHC class II molecules, but not TNF-α nor IL-12 production (32). Accordingly, in our experimental mouse setting, whereby DC are triggered through TREM2 molecules by counterreceptors of unknown origin on NK cells, we did not observe TNF-α nor IL-12 production by DC in the cocultures (our data not shown). However, we did observe a significant KARAP-dependent up-regulation of CD86. We ruled out the role for B7 molecules (Figs. 4D and 5A) and CD40 molecules (data not shown) in the DAK activity mediated by IL-4 in vitro. Lymphocyte activation gene 3, an alternate ligand for MHC class II, and activation-regulated chemokine might be key Th2 chemokines (124). It is tempting to speculate that NK cells are recruited from peripheral blood. Therefore, the BM-DCGM/IL-4/NK cell cross talk could be considered in the design of CTL-peptide-based cancer vaccines. However, whether the DC/NK cell cross talk is critical to promote the Ag-specific cognate T cell responses mediated by DC remains to be determined.

Our preliminary data using TREM2Fc molecules for NK cell cross-linking showed that IL-2 was required to trigger NK cell IFN-γ production in the absence of DC. Therefore, because BM-DCGM/IL-4 do not produce IL-2, the hypothesis is that NK cells might allow signal transduction in DC through KARAP/DAP12, thereby triggering surface expression of an alternate activating ligand for NK cell receptors. In this setting, IL-4 would contribute to DC recognition by NK cells through a TREM2/KARAP/DAP12 signaling pathway.


Canque, B., S. Camus, M. Yagello, and J. C. Gluckman. 1998. IL-4 and CD40 ligation affect differently the differentiation, maturation, and function of human CD34+ cell-derived CD1a+CD14+ and CD1a−CD14+ dendritic cell precursors. J. Leukocyte Biol. 64:235.