Inflammatory Mediators Modulate NK Cell-stimulating Activity of Dendritic Cells by Inducing Development of Polarized Effector Function

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ABSTRACT

Background: It is well established that cross talk between natural killer (NK) cells and myeloid dendritic cells (DC) leads to NK cell activation and DC maturation. In the present study, we investigated whether type 1-polarized DC (DC1) matured in the presence of IFN-γ and type 2-polarized DC (DC2) matured in the presence of PGE2 can differentially activate NK cells. **Methods:** In order to generate DC, plastic adherent monocytes were cultured in RPMI 1640 containing GM-CSF and IL-4. At day 6, maturation was induced by culturing the cells for 2 days with cytokines or PGE2 in the presence or absence of LPS. Each population of DC was cocultured with NK cells for 24 h. The antigen expression on DC was analyzed by flow cytometry and cytokine production in culture supernatant was measured by ELISA or a bioassay for TNF-α determination. NK cell-mediated lysis was determined using a standard 4 h chromium release assay. **Results:** DC2, unlike DC1, had weak, if any, ability to induce NK cell activation as measured by IFN-γ production and cytolytic activity. DC2 were weakly stimulated by activated NK cells compared to DC1. In addition, IFN-γ-primed mature DC appeared to be most resistant to active NK cell-mediated lysis even at a high NK cell/DC ratio. On the other hand, PGE2-primed DC were less resistant to feedback regulation by NK cells than IFN-γ-primed mature DC. Finally, we showed that the differential effect of two types of DC population on NK cell activity is not due to differences in their ability to form conjugates with NK cells. **Conclusion:** These results suggest that different combinations of inflammatory mediators differentially affect the effector function of DC and, as a result, the function of NK cells, eventually leading to distinct levels of activation in adaptive immunity. **(Immune Network 2007;7(3): 133-140)**

Key Words: DC, NK cells, cytokine production, PGE2, NK cell-mediated killing

Introduction

Natural killer (NK) cells and dendritic cells (DC) represent two distinct components of the innate immune system. NK cells are effector cells of innate resistance that are able to lyse a variety of cells, including tumor and virus-infected cells, spontaneously in the absence of Ag-specific recognition (1). The cytolytic activity of NK cells is augmented by IL-2 and, to a lesser extent, by other cytokines, including IL-12 and IFN-α. Activated NK cells also acquire the capacity to lyse target cells that are resistant to lysis by resting NK cells, and to produce cytokines, particularly IFN-γ. IFN-γ-producing NK cells regulate innate resistance by activating phagocytic cells and priming APC for IL-12 production, thus shaping adaptive immunity toward a Th1 response (2).

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DC are considered sentinels of the immune system because of their role as initiators of both innate and antigen-specific immunity (3,4). Located in the periphery as an immature form, DC take up substrates such as proteins and dying cells from the surrounding environment. Upon encounter with microbial antigens or pro-inflammatory cytokines, the DC mature, process, and present the internalized antigens on MHC molecules. Mature DC then prime naive T cells, selected from the recirculating T cell pool in secondary lymphoid organs, producing active antigen-specific MHC class I-restricted CTLs (3,5,6). Although NK cells are part of innate immunity, they share many phenotypic and functional features with T cells (7-9). T cells cooperate with DC in the initiation/amplification of antigen-specific responses. Several groups have reported that the DC-NK interaction can activate each of the cells (10,11).

In addition, tissue-derived factors, such as IFN-γ and PGE2, present during the activation of human monocyte-derived sentinel DC promote the generation of type 1 effector DC (DC1), which produce high amounts of IL-12 upon subsequent engagement with naive T cells, or the generation of IL-12-deficient DC2, which drive the development of Th2 cells (12,13).

In the present study, we investigated whether DC1 and DC2 can differentially activate NK cells. Monocyte-derived DC were stimulated with TNF-α, IL-1β, and IFN-γ (DC1) or PGE2 (DC2). In order to examine the interaction between different phenotypes of DC and NK cells, DC differentiated from monocytes were cocultured with autologous NK cells. Our results indicated that DC2, unlike DC1, had a weak, if any, ability to induce NK cell activation. IFN-γ-primed mature DC were resistant to feedback regulation by NK cells. Therefore, different combinations of inflammatory mediators differentially affect the effector function of DC and, as a result, the function of NK cells, eventually leading to distinct levels of activation in adaptive immunity.

Materials and Methods

Generation of immature DC and maturation induction by different stimuli. For DC generation, the method used by Sallusto *et al*. (14) was slightly modified. Briefly, PBMC from healthy donors (Red Cross Blood Center, Daejeon, South Korea and volunteers) were isolated by density centrifugation on Histopaque 1077 (Sigma, St. Louis, MO). After lysis of erythrocytes, the populations were resuspended in RPMI 1640 (Sigma) medium supplemented with 10% heat-inactivated fetal bovine serum (FBS; HyClone, Logan, UT). Plastic adherent cells (2 h, 37°C) that are usually CD14-positive monocytes (data not shown) were cultured in RPMI 1640 containing GM-CSF (provided by LG Biotech, Iksan, South Korea) and IL-4 (Endogen Pierce, Woburn, MA). At day 6, maturation was induced by culturing the cells for 2 days with the following factors alone or with in combinations as indicated in the text: IL-1β (10 ng/ml; Endogen Pierce), TNF-α (50 ng/ml; PeproTec Inc., Rocky Hill, NJ), IFN-γ (1000 u/ml; LG Biotech), PGE2 (10^{-6} M; Sigma), or LPS (100 ng/ml or 1 μg/ml; Sigma). All subsequent tests were performed after harvesting and extensive washing of the cells to remove all factors.

Determination of expression level of cell surface molecules. At day 8, the effector DC obtained were analyzed for the expression of cell surface molecules with a FACSCalibur (Becton Dickinson, Mountain View, CA). Mouse anti-human mAbs were used against the following molecules: CD80, CD86, CD83, and HLA-DR (Pharmingen, San Diego, CA). FITC/PE-conjugated IgG (Pharmingen) was used as an isotype control.

NK-DC coculture. PBMC were allowed to adhere to plastic, and nonadherent lymphocytes were then extensively washed, then subjected to NK Cell Isolation Kit (Miltenyi Biotec., Bergisch Gladbach, Germany). The percentage of NK cells in the population was evaluated by using PE-αCD56 mAb (Pharmingen) staining followed by flow cytometric analysis. In general, the various numbers of purified NK cells were added to 1×10^5 autologous DCs (DC1 or DC2) at ratios indicated in the text. After coculture of DC and NK cells, the supernatants were saved for cytokine determination, and the cells were harvested and used as effector cells against target cells (K562 cells).

Cytokine assay. To detect the production of IL-12 and IFN-γ by NK/DC cocultures, ELISA were carried out with commercially available kits (Endogen Pierce) for detecting the cytokines according to the manufacturer's instruction. TNF-α production was measured by bioassay based on a cell lysis assay where WEHI-164 cells are lysed by TNF-α treatment.

Cell-mediated cytotoxic assay. NK cell killing of K562 cells or DC was determined using a standard 4 h chromium release assay (15). Briefly, K562 cells or DC were labeled with 100 μCi (NEN-Dupont) of sodium chromate for 1.5 h at 37°C in a CO_2 incubator. A total 1×10^4 cells in 100 μl complete culture medium were distributed into each well of U-bottomed microtiter plates in triplicate. After incubation for either 4 h or 24 h in the case of DC at 37°C, 100 μl of supernatant was collected and the ^{51}Cr release was counted in a gamma counter (Wallac, Gaithersburg, MD). The percentage of specific lysis was calculated as follows: 100×[(cpm of test sample − cpm of spontaneous lysis)/(cpm of maximum control − cpm of spontaneous lysis)], where cpm denotes counts per minute.

Conjugate formation assay. NK cell-DC conjugates were detected by flow cytometry after activated NK cells and DC were labeled with PKH26 red or PKH67 green fluorescent dyes (Sigma), respectively. After labeling, the cells were washed and incubated together (NK-DC) at a 1 : 1 ratio for 1 h at 37°C in the presence or absence of EGTA. The percentage of conjugates formed was calculated with a FACSCalibur.

Results

Activating interaction between NK cells and DC is dependent on cell-cell contact. We began our study by investigating the interaction between autologous DC and resting NK cells. These initial experiments demonstrated that both cell types become activated after coculture for 24 h. The immature DC before coculture with NK cells showed evidence of maturation as determined by their increased expression of both HLA-DR and CD83 antigens (Fig. 1A), and cytokine production (Fig. 1B), which were augmented by both increasing the number of NK cells and the addition of LPS. However, IFN-γ production by NK cells was only detected in the group of DC-NK cells cocultured in the presence of LPS (Fig. 1C). Of note, this production was dependent on cell-cell contact, because NK cells did not produce IFN-γ in a transwell plate system where DC and NK cells were located in upper and lower wells, respectively (Fig. 1D).

A combination of inflammatory stimuli induces either effector DC1 or DC2. To study the direct effect of different stimuli (IFN-γ or PGE2) on the maturation of senti-

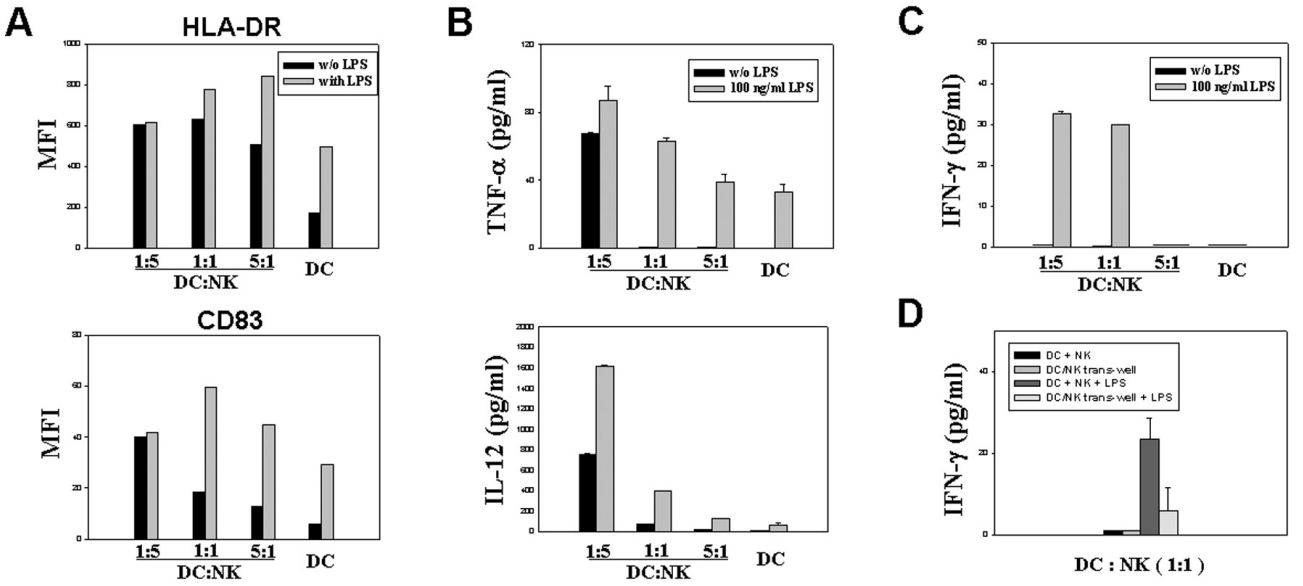

Figure 1. Reciprocal and cell contact-dependent activating interaction between NK cells and DC. (A) DC were cultured for 24 h in the absence or presence of LPS (100 ng/ml). After cells (1×10^5) were washed and cocultured for 24 h with purified autologous NK cells at different ratios of DC to NK cells, the expression of HLA-DR and CD83 antigen on DC was analyzed by flow cytometry. (B, C, D) TNF-α production in culture supernatant was measured with a bioassay based on WEHI-164 lysis (B, upper). IL-12 (B, low) and IFN-γ (C) were measured by ELISA. Cell-cell contact-dependent IFN-γ production (D) was tested in a transwell plate that allows separate cultures of each population.

nel DC into effector DC, uncommitted monocyte-derived DC were cultured with the combination of IL-1β and TNF-α (maturation factor: MF) and/or IFN-γ or PGE2. All stimuli induced final maturation within 48h, as evident from the induction of HLA-DR, CD80, CD86, and CD83 expression (Fig. 2). The level of surface antigens was not significantly different between the DC1 (MF+IFN-γ) and DC2 (MF+PGE2) groups. In these conditions, DC1 could produce bioactive IL-12p70, whereas DC2 produced IL-10 (Fig. 3A). We used these cells as activators of NK cells after extensive washing to remove soluble factors. After extensive washing, IL-12 and IL-10 were not detectable (data not shown).

DC1 function as a potent activator of NK cells. After final maturation of DC, we investigated which type of DC,

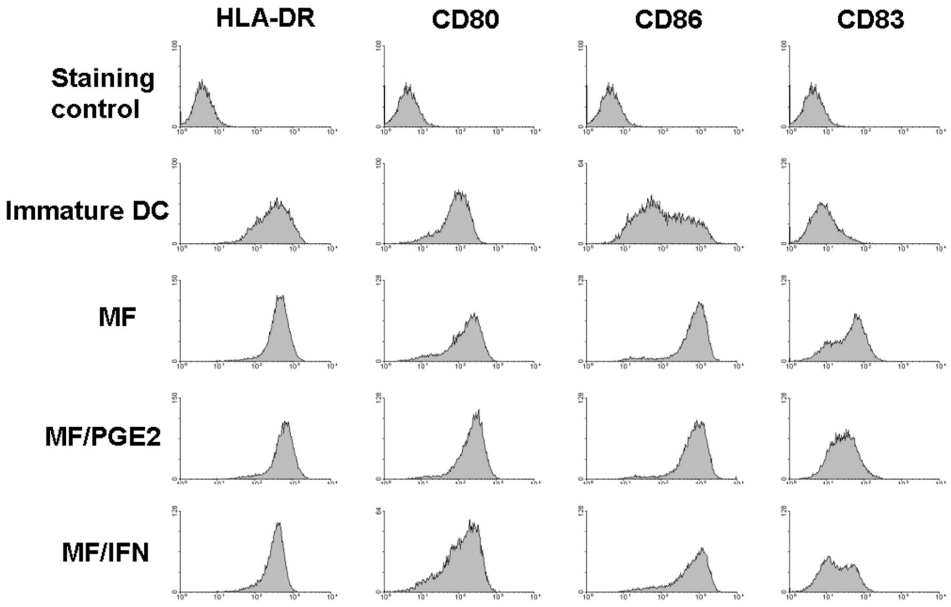

Figure 2. Induction of mature DC phenotype by a combination of inflammatory cytokines. Immature monocyte-derived dendritic cells at day 6 were cultured for 48 h with MF (TNF-α and IL-1β) or MF in combination with IFN-γ or PGE2. Each population of DC was analyzed for the expression of HLA-DR, CD80 (B7.1), CD86 (B7.2), and CD83 antigens by flow cytometry.

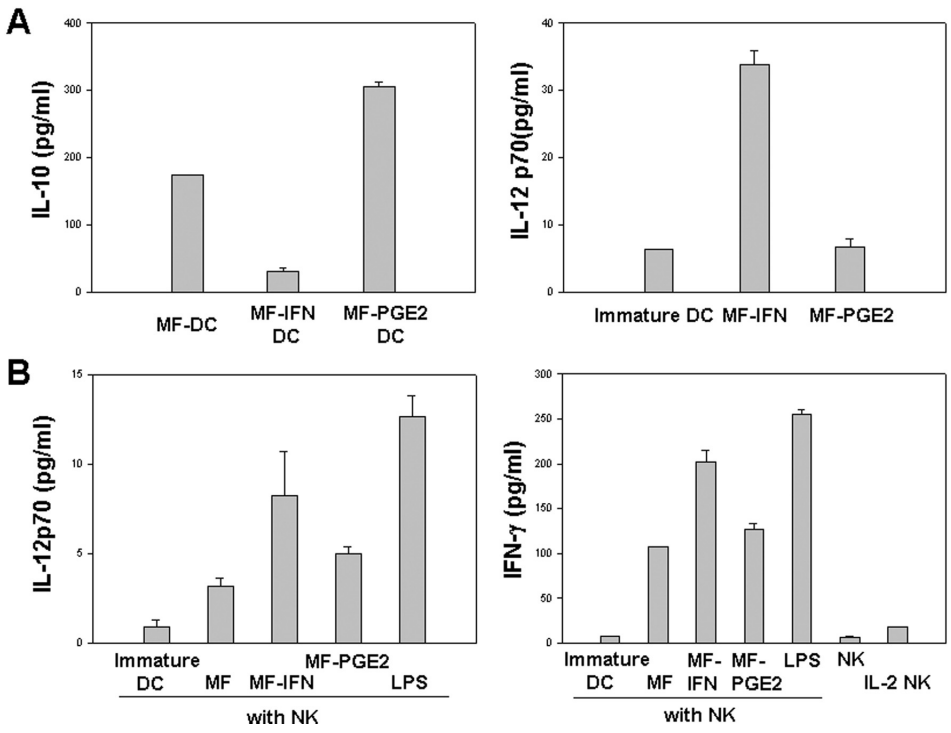

Figure 3. Cytokine production of dendritic cells matured by MF and IFN-γ or PGE2. (A) Immature dendritic cells at day 6 were cultured for 48 h with MF (TNF-α and IL-1β) or MF in combination with IFN-γ or PGE2. The culture supernatants from each population of DC were harvested and cytokines were detected with ELISA kit specific for IL-10 or IL-12p70. (B) Immature DC or mature DC stimulated with LPS (1 μg/ml), MF, or MF plus IFN-α or PGE2 were harvested and washed with PBS three times. Each population of DC was cocultured with 1×10^5 NK cells for 24 h. After coculture, the supernatant was harvested and cytokine levels were measured with ELISA kits specific for IL- 12p70 or IFN-γ.

DC1 or DC2, could activate NK cells through cell-cell interaction. Mature DC were cocultured with purified peripheral NK cells at a 1:1 ratio for 24 h. The supernatants were harvested and used for the determination of cytokine levels by ELISA, and the cells were used as effector cells for the determination of cytolytic activity against K562 cells. Interestingly, NK cells (1×10^5) cocultured with DC showed a marked enhancement of IL-12 and IFN-γ production (Fig. 3B) as well as cytolytic activity (Fig. 4) in the DC1 group only. We used LPS-stimulated DC or IL-2 stimulated NK cells as positive controls. Although DC are known as effector cells capable of inducing NK cell activation, *in vitro* interactions of NK cells with DC2, which can induce a Th2 immune response, could not activate NK cells, and DC2 were not activated by NK cells. On the contrary, we observed that DC2 inhibit NK cytolytic activity and do not induce increased levels of IL-12 and IFN-γ after interaction with NK cells (Fig. 4 and 3B).

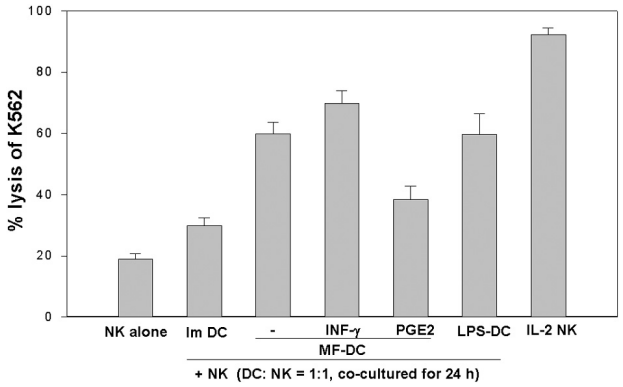

Figure 4. Enhancement of cytolytic activity by NK cells cocultured with mature DC. The NK cells were cocultured with each population of DC for 24 h as described in Fig. 3B. K562 cells used as NK target cells were labeled with ^{51}Cr. The labeled K562 cells were co-incubated with stimulated NK cells in a 96 well U-bottom plate for 4 h. The supernatants were harvested and the ^{51}Cr release into the supernatant was counted in a gamma counter.

Mature DC escape from feedback regulation by NK cells. It was reported that activated NK cells could inhibit DC development through NK-mediated killing (Piccioli *et al.*, 2002). Thus, we tested whether mature DC such as DC1 and DC2 could escape NK-mediated killing. After stimulation of NK cells with or without IL-2, the effector NK cells were cocultured with ^{51}Cr-labeled immature DC, DC1, or DC2 at different NK : DC ratios (3 : 1 or 10 : 1) for 4 h (Fig. 5A) or 24 h (Fig. 5B). Although DC1 were most resistant to NK cytolytic activity, mature DC generally became much more resistant to NK cell cytolytic activity than immature DC.

Conjugate formation with NK cells is increased for both DC 1 and DC2. As mentioned earlier, cell-cell contact is

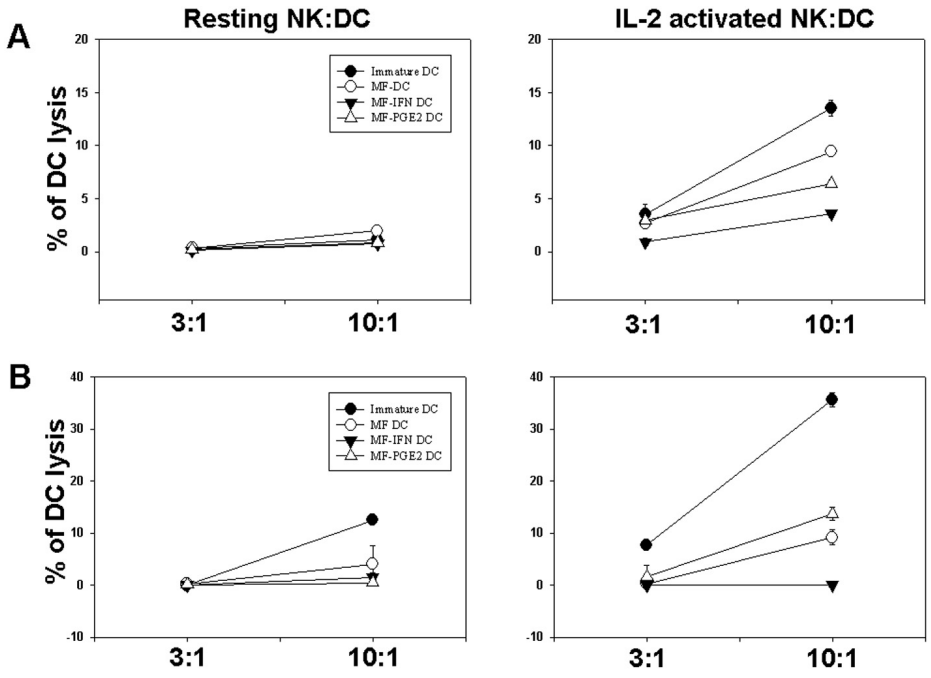

Figure 5. Mature DC, especially DC1, were remarkably resistant to NK cell-mediated lysis. DC were labeled with ^{51}Cr and then co-incubated with freshly isolated resting or IL-2 stimulated autologous NK cells at two ratios. After incubation for 4 h (A) or 24 h (B), the culture supernatants were harvested and the ^{51}Cr release into the supernatant was counted in a gamma counter.

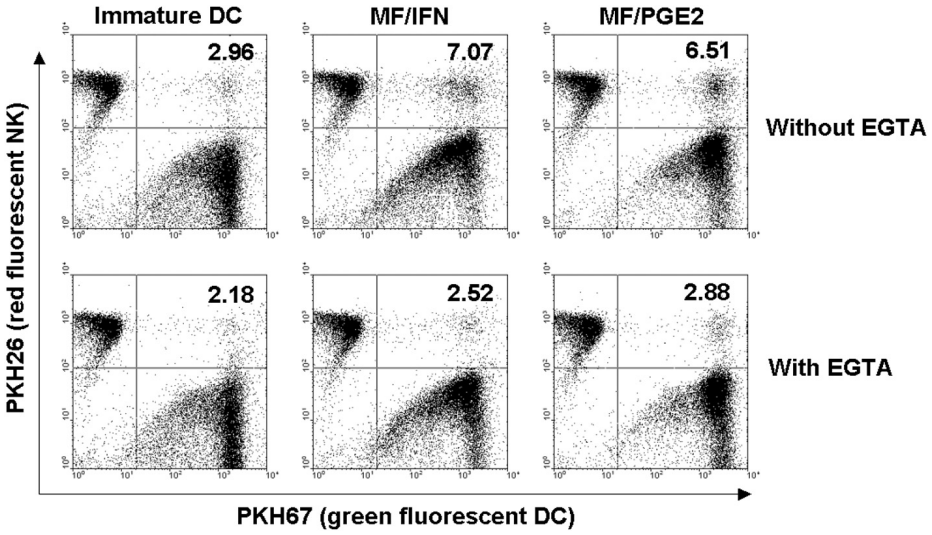

Figure 6. Conjugate formation of mature DC with NK cells. DC were stained with PKH67 green fluorescent dye, whereas IL-2-activated NK cells were stained with PKH26 red fluorescent dye. DC were co-incubated with NK cells in the absence or presence of 10 mM EGTA at a 1 : 1 ratio for 1 h. Whole cells, including DC and NK cells, were analyzed by flow cytometry. Numbers represent the relative ratio of the double positive population, indicating a conjugate formation of DC and NK cells.

required for efficient communication between DC and NK cells (Fig. 1D). Therefore, we tested whether DC2 have a poor conjugate-forming capacity with NK cells. After differentiation of DC, the cells were labeled with PKH67 green fluorescent dye and co-incubated with IL-2-treated NK cells, which were labeled with PKH26 red fluorescent dye, at 37°C for 1 h. The conjugation between DC and NK cells was enhanced after the induction of DC maturation (Fig. 6). However, contrary to our expectation, we observed that conjugate formations by DC1 and DC2 were not significantly different from each other. This conjugate formation was inhibited by treatment with a calcium chelator, EGTA, indicating that this interaction is Ca^{2+} dependent.

Discussion

Cognate interactions are a common feature of the antigen-specific immune response, where cell-cell contact regulates such critical events as antigen presentation and the delivery of T cell help to CTLs and B cells (16-18). In contrast, coordinated control of innate immunity is thought to be mediated almost exclusively by cytokine feedback loops (19-21). Recently, one exception to this general rule was proposed for the innate immune system and, importantly, it involves contact-dependent activation of murine or human NK cells by immature DC or mature DC (11,22).

A role for DC in the process of NK cell activation has been clearly demonstrated in many different experimental systems (23,24). In most instances, DC-mediated NK cell activation is dependent on IL-12 and other DC-derived cytokines/molecules. IL-12 appears to be a crucial cytokine for the induction of IFN-γ by NK cells (25,26). In our study, although the IL-12 necessary for NK cell activation was released even after non-stimulated DC and NK cell contact, LPS-activated DC that produce large amount of IL-12 most efficiently induced NK cells to produce IFN-γ (Fig. 1B, C), suggesting the presence of other LPS-inducible soluble factors in the activation process of NK cells. NK cell activation through NK cell-DC interaction also required cell-cell contact for effective cross talk between the two cell types (Fig. 1D). We investigated whether the cytokine environment and the mode of activation affect maturation of DC derived from monocytes and corresponding NK cell-DC interactions. DC were matured with a set of pro-inflammatory mediators or in combination with IFN-γ or PGE2. PGE2, which was used for activation of DC in our short-term protocol, has also been shown to prevent secretion of bioactive IL-12 (27). These findings were also confirmed by our results showing that PGE2 has a suppressive effect on IL-12 production by DC matured with inflammatory mediators. In contrast, an elevated level of IL-10 expression by mature DC was induced by the addition of PGE2, suggesting suppressive activity of PGE2 upon Th1-type immune responses. It has been shown previously that PGE2 has different and sometimes opposite effects on DC function depending on the nature of the maturation signals (28-30). It was noteworthy that IFN-γ-pri-

med mature DC in NK cell-DC cocultures were fully capable of inducing IL-12 as well as NK cell activation, characterized by increased production of IFN-γ and cytolytic activities of NK cells. However, PGE2 did not elevate IFN-γ production by NK cells and exerted a suppressive effect on NK cell cytotoxicity.

The relative contributions of different states of DC maturation to the NK cell-mediated killing process of DC appeared to correlate with the ability to induce NK cell activity. IFN-γ-primed mature DC were most resistant to killing by IL-2-activated NK cells. In contrast, PGE2 did not provide mature DC with any significant protection from NK cell-mediated killing, although the percentage of conjugate formation between the two cell types was not different for IFN-γ-primed and PGE2-treated mature DC. These results suggest that the differential effects of two types of DC population on NK cell activity are not due to a difference in their ability to form conjugates with NK cells. In our experimental setting, NK cells were less efficient in killing mature DC capable of inducing Th1 type immunity, irrespective of conjugate formation between DC and NK cells. It has been shown previously that whereas low NK cell/DC ratios lead to NK cell-mediated DC activation, higher ratios result in NK cell-mediated DC killing (11,24). In our experiments, IFN-γ-primed mature DC showed strong resistance to active NK cell-mediated lysis even at high NK cell/DC ratios. It has been proposed that one of the signals required for NK cell-mediated DC killing may be mediated by the NKp30 receptor that, by recognizing its ligands on immature DC, appears to be crucial for this killing process *in vitro* (24). Subsequent studies showed that monoclonal antibody-mediated blocking of NKp30 strongly inhibited NK cell-dependent DC maturation mediated by TNF-α and IFN-γ (31). Thus, it is necessary to further examine whether the NK cell subsets expressing specific receptors, like the NKp30 receptor, are differentially modulated by mature DC stimulated with different combinations of inflammatory cytokines and soluble factors.

In conclusion, we show evidence of NK cell activation that is dependent on DC maturation condition, although we have not investigated the key molecules of this NK cell activation in detail. The process of NK cell activation by NK cell/DC interaction might be important for not only upregulating antitumor NK cell activity, but also complementing the NK cell-mediated editing of DC imposed by the positive selection of mature DC, favoring an enrichment of fully activated DC. Therefore, it is necessary to fully understand DC-mediated mechanisms of NK cell priming to elicit their potent function against infected and tumor cells.

References

1. Trinchieri G: Biology of natural killer cells. Adv Immunol 47;187-376, 1989
2. Trinchieri G: Natural killer cells wear different hats: effector cells of innate resistance and regulatory cells of adaptive immunity and of hematopoiesis. Semin Immunol 7;83-88, 1995
3. Banchereau J, Steinman RM: Dendritic cells and the control of immunity. Nature 392;245-252, 1998
4. Cella M, Sallusto F, Lanzavecchia A: Origin, maturation and antigen presenting function of dendritic cells. Curr Opin Immunol 9;10-16, 1997
5. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K: Immunobiology of dendritic cells. Annu Rev Immunol 18;767-811, 2000
6. Thery C, Amigorena S: The cell biology of antigen presentation in dendritic cells. Curr Opin Immunol 13;45-51, 2001
7. Lanier LL, Spits H, Phillips JH: The developmental relationship between NK cells and T cells. Immunol Today 13; 392-395, 1992
8. Sanchez MJ, Muench MO, Roncarolo MG, Lanier LL, Phillips JH: Identification of a common T/natural killer cell progenitor in human fetal thymus. J Exp Med 180;569-576, 1994
9. Valiante NM, Parham P: NK cells and CTL: opposite sides of the same coin. Chem Immunol 64;146-163, 1996
10. Gerosa F, Baldani-Guerra B, Nisii C, Marchesini V, Carra G, Trinchieri G: Reciprocal activating interaction between natural killer cells and dendritic cells. J Exp Med 195;327-333, 2002
11. Piccioli D, Sbrana S, Melandri E, Valiante NM: Contact-dependent stimulation and inhibition of dendritic cells by natural killer cells. J Exp Med 195;335-341, 2002
12. Kalinski P, Vieira PL, Schuitemaker JH, de Jong EC, Kapsenberg ML: Prostaglandin E(2) is a selective inducer of interleukin-12 p40 (IL-12p40) production and an inhibitor of bioactive IL-12p70 heterodimer. Blood 97;3466-3469, 2001
13. Vieira PL, de Jong EC, Wierenga EA, Kapsenberg ML, Kalinski P: Development of Th1-inducing capacity in myeloid dendritic cells requires environmental instruction. J Immunol 164;4507-4512, 2000
14. Sallusto F, Lanzavecchia A: Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. J Exp Med 179;1109-1118, 1994
15. Kim KD, Choi SC, Noh YW, Kim JW, Paik SG, Yang Y, Kim KI, Lim JS: Impaired responses of leukemic dendritic cells derived from a human myeloid cell line to LPS stimulation. Exp Mol Med 38;72-84, 2006
16. Davis MM, Bjorkman PJ: T-cell antigen receptor genes and

T-cell recognition. Nature 334;395-402, 1988
17. Krowka JF, Singh B, Fotedar A, Mosmann T, Giedlin MA, Pilarski LM: A requirement for physical linkage between determinants recognized by helper molecules and cytotoxic T cell precursors in the induction of cytotoxic T cell responses. J Immunol 136;3561-3566, 1986
18. Scherle PA, Gerhard W: Functional analysis of influenza-specific helper T cell clones in vivo. T cells specific for internal viral proteins provide cognate help for B cell responses to hemagglutinin. J Exp Med 164;1114-1128, 1986
19. Scott P, Trinchieri G: The role of natural killer cells in host-parasite interactions. Curr Opin Immunol 7;34-40, 1995
20. Trinchieri G: Cytokines acting on or secreted by macrophages during intracellular infection (IL-10, IL-12, IFN-gamma). Curr Opin Immunol 9;17-23, 1997
21. Unanue ER: Inter-relationship among macrophages, natural killer cells and neutrophils in early stages of Listeria resistance. Curr Opin Immunol 9;35-43, 1997
22. Fernandez NC, Lozier A, Flament C, Ricciardi-Castagnoli P, Bellet D, Suter M, Perricaudet M, Tursz T, Maraskovsky E, Zitvogel L: Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses in vivo. Nat Med 5;405-411, 1999
23. Andrews DM, Scalzo AA, Yokoyama WM, Smyth MJ, Degli-Esposti MA: Functional interactions between dendritic cells and NK cells during viral infection. Nat Immunol 4;175-181, 2003
24. Ferlazzo G, Tsang ML, Moretta L, Melioli G, Steinman RM, Munz C: Human dendritic cells activate resting natural killer (NK) cells and are recognized via the NKp30 receptor by activated NK cells. J Exp Med 195;343-351, 2002
25. Moretta L, Ferlazzo G, Bottino C, Vitale M, Pende D, Mingari MC, Moretta A: Effector and regulatory events during natural killer-dendritic cell interactions. Immunol Rev 214;219-228, 2006
26. Zitvogel L, Terme M, Borg C, Trinchieri G: Dendritic cell-NK cell cross-talk: regulation and physiopathology. Curr Top Microbiol Immunol 298;157-174, 2006
27. Kalinski P, Schuitemaker JH, Hilkens CM, Kapsenberg ML: Prostaglandin E2 induces the final maturation of IL-12-deficient CD1a+CD83+ dendritic cells: the levels of IL-12 are determined during the final dendritic cell maturation and are resistant to further modulation. J Immunol 161;2804-2809, 1998
28. Dauer M, Schad K, Junkmann J, Bauer C, Herten J, Kiefl R, Schnurr M, Endres S, Eigler, A: IFN-alpha promotes definitive maturation of dendritic cells generated by short-term culture of monocytes with GM-CSF and IL-4. J Leukoc Biol 80;278-286, 2006
29. Jing H, Vassiliou E, Ganea D: Prostaglandin E2 inhibits production of the inflammatory chemokines CCL3 and CCL4 in dendritic cells. J Leukoc Biol 74;868-879, 2003
30. Vassiliou E, Sharma V, Jing H, Sheibanie F, Ganea D: Prostaglandin E2 promotes the survival of bone marrow-derived dendritic cells. J Immunol 173;6955-6964, 2004
31. Vitale M, Della Chiesa M, Carlomagno S, Pende D, Arico M, Moretta L, Moretta A: NK-dependent DC maturation is mediated by TNFalpha and IFNgamma released upon engagement of the NKp30 triggering receptor. Blood 106; 566-571, 2005