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Dendritic Cells Mediate NK Cell Help for Th1 and CTL Responses: Two-Signal Requirement for the Induction of NK Cell Helper Function

Robbie B. Mailliard,* Young-Ik Son,† Richard Redlinger,* Patrick T. Coates,* Adam Giermasz,* Penelope A. Morel,‡ Walter J. Storkus,*‡ and Pawel Kalinski§*‡

Early stages of viral infections are associated with local recruitment and activation of dendritic cells (DC) and NK cells. Although activated DC and NK cells are known to support each other’s functions, it is less clear whether their local interaction in infected tissues can modulate the subsequent ability of migrating DC to induce T cell responses in draining lymph nodes. In this study, we report that NK cells are capable of inducing stable type 1-polarized “effector/memory” DC (DC1) that act as carriers of NK cell-derived helper signals for the development of type 1 immune responses. NK cell-induced DC1 show a strongly elevated ability to produce IL-12p70 after subsequent CD40 ligand stimulation. NK-induced DC1 prime naive CD4+ T cells for high levels of IFN-γ, but low IL-4 production, and demonstrate a strongly enhanced ability to induce Ag-specific CD8+ T cell responses. Resting NK cells display stringent activation requirements to perform this novel, DC-mediated, “helper” function. Although their interaction with K562 cells results in effective target cell killing, the induction of DC1 requires a second NK cell-activating signal. Such costimulatory signal can be provided by type I IFNs, common mediators of antiviral responses. Therefore, in addition to their cytolytic function, NK cells also have immunoregulatory activity, induced under more stringent conditions. The currently demonstrated helper activity of NK cells may support the development of Th1- and CTL-dominated type 1 immunity against intracellular pathogens and may have implications for cancer immunotherapy. The Journal of Immunology, 2003, 171: 2366–2373.

Dendritic cells (DC) are carriers of pathogen-related information within the immune system. DC are crucial for the induction of primary immune responses, being the only type of APC capable of priming the lymph node-based naive T cells (1). The unique ability of DC to migrate from peripheral sites of pathogen entry to T cell areas of the lymph nodes allows them to provide naive T cells with Ag-specific “signal 1” and costimulatory “signal 2,” which inform the immune system about the molecular identity and pathogenic potential of the invader. A growing body of evidence indicates that migrating DC also carry an additional “signal 3” (polarization), driving the development of primary responses toward the Th1 or Th2 direction (2–5). Several intracellular microorganisms are known to directly induce distinct Th1- or Th2-inducing phenotypes in DC (6–8). In addition, DC can also modify their Th1/Th2-inducing functions as a result of their interaction with B lymphocytes (9) or with IFN-γ- and TNF-α-producing CD8+ T cells (10), which indicates the ability of DC to receive indirect polarizing signals from other immune cells.

Early stages of viral infection are associated with the recruitment and local activation of DC (11–13) and NK cells (14), the cell types capable of exchanging bidirectional activating signals (15–18). NK cells appear not only important for the early virus elimination, but also for the development of Ag-specific memory, since NK cell deficiencies are associated with recurrent infections both in humans and in mice (19–24). Although DC have been known to support the tumoricidal activity of NK cells (15), cytokine-precipitated NK cells have been recently demonstrated to activate DC and to induce their maturation and cytokine production (16–18).

Although the above observations suggest that NK cells and DC can mutually support each other’s functions at the site of the pathogen’s invasion, it remains unclear whether such peripheral NK-DC interaction has an impact on subsequent induction of primary responses of CD4+ and CD8+ T cells in the draining lymph nodes. Following their preactivation with IL-2, NK cells can contribute to the IL-12 induction in DC when present at the site and at the time of their CD40 ligand (CD40L)-mediated stimulation (16–18). However, in contrast to DC, the migratory ability of NK cells remains unclear. Within the lymph nodes and spleen, NK cells localize mainly in B cell follicles and in the marginal zone rather than in the T cell areas (25–27), which makes it less clear if they can directly affect naive T cells. Therefore, we tested whether and under what conditions the interaction of NK cells with DC can act as a source of the DC-transmitted Th1- and CTL-inducing signals for naive T cells.

Materials and Methods

Isolation of peripheral blood leukocyte populations

Mononuclear cells, obtained from the peripheral blood of healthy donors, were isolated by density gradient separation using Lymphocyte Separation Medium (Celgro Mediatech, Herndon, VA). Resting NK cells (>90%...
CD56+CD3- cells; <1% of the cells were positive for CD3, CD14, CD19, or HLA-DR, see the inset to Fig. 1) and naive (CD45RA+RO- CD4+ T cells (96–98% purity) were isolated using Stem-Sep-negative selection systems (StemCell Technologies, Vancouver, British Columbia, Canada). The cells were analyzed by FACScan (BD Biosciences, San Jose, CA). Fluorochrome-labeled CD3, CD4, CD8, CD11c, CD14, CD16, CD45RA CD45RO, CD56, and isotype control mAbs were purchased from BD Pharmingen (San Jose, CA). CD83 mAb was purchased from Coulter Immunotech (Miami, FL). CD1a mAb was purchased from Diacclone (Besançon, France).

Generation of DC

To obtain immature “day 6 DC” (28), monocytes were isolated from PBL using density separation, followed by plastic adherence, as described previously (10). Monocytes were cultured for 6 days in 24-well plates (Costar, Cambridge, MA) at 5 × 10^5 cells/well in IMDM with 10% FBS (both from Life Technologies, Grand Island, NY) supplemented recombinant human GM-CSF and IL-4 (both 1000 IU; gifts from Schering-Plough, Kenilworth, NJ). The typical cultures yielded 2–3 × 10^6/well of CD1a+, CD14-, CD86+, and CD83-immature DC.

Induction of cytokine production in cocultures of NK cells with target cells and DC

Resting NK cells (1 × 10^5 cells/well) were plated in 96-well round-bottom plates and stimulated with K562 cells (2 × 10^5 cells/well; obtained from American Type Culture Collection (Manassas, VA), IFN-α (Intron A-IFN-α-2b; Schering-Plough) or IFN-β (Avonex; Biogen, Cambridge, MA), IL-2 (a gift from Chiron, Emeryville, CA), IL-18 (a gift from GlaxoSmithKline, King of Prussia, PA), or their combination. Supernatants were collected after 24 h and tested for the presence of cytokines.

NK cytolytic activity

Cytolytic activity was determined by performing standard 51Cr release assays with results calculated and reported in either lytic units or the percentage of target lysis at individual E:T ratios as described elsewhere (29). When indicated, “cold” DC or K562 cells were added at the same numbers as the 51Cr-labeled target cells.

Activation and polarization of DCs by NK cells

Freshly isolated autologous NK cells (2 × 10^5 cells/well) were added to day 6 DC cultures (containing 2–3 × 10^5 cells/well) in the presence or absence of the NK-sensitive K562 or T2 (provided by Dr. P. Cresswell, Yale University, New Haven, CT) target cells (2 × 10^5 cells/well). NK cells and NK-sensitive target cells were added either directly to the DC cultures or added to a Transwell culture insert with a pore size of 0.4 μm (Costar, separated from DC). When indicated, recombinant human IFN-α or recombinant human IFN-β (both at 1000 IU/ml, unless indicated otherwise), were added to the cultures. To neutralize the biological activity of TNF-α and IFN-γ in NK cell-DC cocultures, human soluble (s) TNFR1 (sTNFR1, 1 μg/ml; R&D Systems, Minneapolis, MN) or IFN-γ-receptor 1 (10 μg/ml; R&D Systems) were added at the beginning of cocultures (10). In 96-well plates, after 3 days, the cells were harvested and analyzed for the expression of maturation-associated surface markers and the ability to produce IL-12p70.

Determination of the IL-12p70-producing capacity of DC

To test the IL12p70-producing capacity of DC, they were harvested, washed, and plated in flat-bottom 96-well plates at 2 × 10^4 cells/well. To mimic the interaction with CD40L-expressing Th cells, CD40L-transfected J558 cells (a gift from Dr. P. Lane, University of Birmingham, Birmingham, U.K.) were added at 5 × 10^5 cells/well (29). Supernatants were collected after 24 h and tested for the presence of IL-12p70 by ELISPOT.

Differential priming of Th cells

Using the Transwell model described before (10), DC cultured in the bottom chamber were exposed for 48 h to IFN-α (1000 IU/ml); K562 cells with or without freshly isolated resting NK cells were all added to the upper chamber. DC were harvested and pulsed with Staphylococcal enterotoxin B (1 ng/ml; Sigma-Aldrich, St. Louis, MO) for 1 h, washed, and placed into culture (2 × 10^5 cells/well) with naive (CD45RA+RO- CD4+ T cells) or CD4+ Th cells (10^5 cells/well). On day 3, recombinant human IL-2 (50 U/ml; a gift from the Chiron) was added to the cultures. At day 10, the expanded CD4+ Th cells (100-300-fold expansion) were washed, counted, plated in 96-well plates (10^4 cells/well), and stimulated with CD3 and CD28 mAbs (respectively, CLB-T3/4.E, 1 μg/ml, and CLB-CD28/8.1, 2 μg/ml; both purchased from Accurate Chemicals, Westbury, NY). The supernatants from the restimulated CD4+ Th cells were collected after 24 h and analyzed by ELISA for the presence of IL-4 and IFN-γ.

Induction of Ag-specific CD8+ T cells

Negatively isolated CD8+ T cells from HLA-A2-2 donors (5 × 10^5 cells) were sensitized by peptide-pulsed HLA-A2-restricted EBV BMLF1 280-288 peptide (GLCTLVAML; 2 μg/ml) autologous DC (5 × 10^5 cells) that had been matured in a Transwell system under the influence of the IFN-α and K562-activated NK cells or under the influence of IFN-α alone (no NK cells in the upper chamber). To avoid the requirement for double-expressing HLA-A2/DR4+ donors, we used 3000-rad irradiated CD40L-transfected J558 cells (5 × 10^4 cells) as a surrogate of CD40L-expressing activated DCs. Th cells (30). Recombinant human IL-2 (50 U/ml) was added at day 4, and the differentially sensitized CD8+ T cell cultures were expanded by an additional round of stimulation at day 10 using peptide-pulsed autologous PBMC (2 × 10^6 cells). At day 20, the differentially induced CD8+ T cell lines were stimulated with peptide-pulsed HLA-A2+ T2 cells to monitor the frequency of the EBV-specific CD8+ T cells by IFN-γ ELISPOT.

Cytokine detection

Concentrations of IL-12p70 and IFN-γ in cell supernatants were determined by specific ELISAs performed with matched Ab pairs, standards, and reagents from Endogen (Woburn, MA). IL-4 ELISA was performed using the OPT-EIA Human IL-4 Set from BD Pharmingen. IFN-γ ELISPOT reagents were purchased from Mabtech (Cincinnati, OH).

Results

Differential activation requirements for NK cell cytotoxicity and cytokine production

Peripheral blood-isolated resting NK cells effectively recognized and killed K562 targets cells without the need for any additional activation (Fig. 1). In contrast, although NK cells are known producers of IFN-γ, their interaction with target cells alone was not sufficient to induce that factor. However, NK cells could produce IFN-γ when provided with a second activating signal: type 1 IFNs, IFN-α or IFN-β (Fig. 1), the soluble factors commonly produced by virally infected cells (reviewed in Ref. 31). Similar to the recognition of the target cells alone, exposure of NK cells to type 1 IFNs alone was not effective.

Since previous reports have demonstrated that NK cells can both activate and kill DC (16–18, 32–35), we tested the relative ability of NK cells to kill DC as compared with their killing of tumor targets. As shown in Fig. 2A, freshly isolated NK cells effectively killed chromium-labeled K562 cells in short-term cocultures, but were ineffective in killing DC, even at relatively high NK cell:DC ratios. DC killing was still only marginally enhanced in the presence of IFN-α, a long-known enhancer of NK cell killing of virally infected cells (36, 37). To test whether the lack of DC killing resulted from an intrinsic resistance of DC or from poor NK cell activation in the absence of transformed cells, we established triple cultures consisting of NK cells, DC, and K562 cells, selectively using either 51Cr-labeled DC or K562 cells. As shown in Fig. 2B, resting NK cells efficiently killed 51Cr-labeled K562 cells in a manner that was independent of IFN-α. In contrast, 51Cr-labeled immature DC in the parallel cultures were not killed by NK cells, even when IFN-α was present during the assay. These results suggest that, at least during early stages of immune responses, DC are poor targets for NK cell killing.

“Two-signal” activation requirement for NK cells to induce DC maturation

Having established that the primary interaction with NK cells does not result in DC killing, we tested to what extent and under which conditions this interaction can affect the activation state of DC. To this aim, NK cells, K562, IFN-α, and their combinations were added directly to the cultures of day 6 immature DC for 48 h. At
day 8, control DC expressed moderate levels of CD86 and were negative for CD83, a marker of human mature DC (38). Addition to DC cultures of either NK cells or K562 alone or their combination had little impact on DC expression of these activation markers (Fig. 3). DC expression of CD86 was weakly up-regulated by IFN-α (1000 IU/ml) alone, but the level of CD83 expression increased only slightly. In sharp contrast, when NK cells were stimulated with K562 cells in the presence of IFN-α, the cocultured DC showed a uniform induction of CD83 and high CD86 expression (Fig. 3A). In accordance with the poor ability of recently activated NK cells to kill DC (Fig. 2), the yields of such NK cell-matured DC were similar to the yields of control DC in the parallel cultures performed in the absence of NK cells (data not shown).

To determine whether the NK cell-derived DC maturation-inducing signal requires direct cell contact between DC and NK cells or whether it is mediated by soluble product(s), we analyzed the impact of NK cells on bystander DC, separated by a semipermeable membrane in a Transwell system. To this aim, NK cells, with or without K562 cells and IFN-α, were added to the upper chamber of the Transwell inserts, and their impact on day 6 DC present in the bottom chamber was analyzed. Similar to the direct coculture model, the maturation of bystander DC in the bottom chamber was observed only when NK cells were stimulated with both K562 target cells and IFN-α and was prevented when any one of these three factors was omitted (Fig. 3B). The effective induction of the DC maturation in the bystander model suggests a key role for NK cell-produced soluble factors.

Of the known soluble DC maturation factors, only TNF-α was clearly detectable in NK/DC coculture supernatants. In accordance with the essential role of TNF-α, DC maturation was significantly inhibited by the addition of soluble sTNFR, both in the Transwell (Fig. 3B) as well as in the direct coculture (Fig. 3C) models.

Similar requirements for two-signal stimulation of NK cells in the induction of DC maturation, and the similar role of TNF-α in this phenomenon, were also observed when IFN-β was used instead of IFN-α or when K562 cells were replaced by another NK-sensitive target, T2 cells, both in the direct coculture and the Transwell models (data not shown).

**NK cells induce stable type 1-polarized DC with enhanced IL-12p70-producing capacity**

To analyze the impact of NK cells on DC functions, immature DC were cocultured with freshly isolated NK cells in the additional presence of K562 target cells and/or IFN-α. After 48 h, the cells were harvested, washed, and stimulated with CD40L-transfected J558 cells. As shown in Fig. 4A, the NK cell-preactivated DC produced greatly increased levels of IL-12p70 after such subsequent stimulation. Similar to the induction of DC maturation, such type 1 polarization of DC was only observed when NK cells were activated both by K562 target cells and IFN-α. Similar effects were again observed also using IFN-β, rather than IFN-α, to costimulate NK cells or when K562 were replaced by another NK-sensitive target, T2 cells (data not shown).

To test whether direct cell-cell contact between NK cells and DC is required to induce the DC1-polarizing effect, we used the Transwell model to analyze the IL-12-producing capacity of bystander DC (bottom chamber) exposed to soluble products released by the differentially activated NK cells present in the upper chamber. Although the effects were slightly less pronounced, compared with the direct coculture model, K562 and IFN-α-activated NK cells were clearly able to polarize bystander DC (Fig. 4A), indicating the dominant role of soluble factors in this phenomenon.

![FIGURE 1](https://www.jimmunol.org/)  
**Two-signal requirement for the induction of IFN-γ production by NK cells.** Differential requirements for the induction of NK cell lytic activity and cytokine production. Negatively selected NK cells were cocultured with K562 target cells in the absence or presence of IFN-α (1000 IU/ml) to analyze the cytolytic activity of NK cells and their ability to produce IFN-γ. **Left,** Cytolytic activity of NK cells against K562 in a 4-h 51Cr release assay does not depend on the presence of IFN-α. **Right,** IFN-γ production by NK cells stimulated with K562 target cells, IFN-α, or their combination. Similar data have been obtained with IFN-β in four different experiments. The concentrations of IFN-γ in 24-h supernatants were tested by ELISA.

**FIGURE 2.** NK cells selectively kill K562 but not DC. **A,** Freshly isolated NK cells were coincubated for 6 h with either 51Cr-labeled K562 cells or with 51Cr-labeled “day 6” immature DC (in the absence of K562). **B,** “Triple cell” 51Cr release assays were performed using freshly isolated NK cells as effectors against K562 cells and DC. Either the K562 cells (■) or DC (○, □) were selectively labeled with 51Cr. Solid symbols represent the addition of IFN-α (1000 IU/ml) to the assay. NK cells were added at increasing numbers while both the 51Cr-labeled target cells and “cold targets” (in B) were each used at a fixed concentration of 1 x 10⁵ cells/well. After 6 h, the percentage of target cell killing was determined by measuring the radioactive 51Cr release to the assay supernatant, compared with detergent lysis.
These same Transwell experiments also demonstrated that the observed enhanced IL-12-producing capacity of the NK cell-polarized DC was not due to persistent IFN-γ production by contaminating NK cells during CD40 stimulation of DC but results from a stable modulation of DC functions.

Both in the direct coculture model (Fig. 4B) and in the Transwell experiments (data not shown), the development of type 1-polarized DC was strongly inhibited by the addition of either sTNFR1 or sIFN-γ receptor, indicating the requirement for both TNF-α and IFN-γ in NK cell-dependent DC1 induction. The ability for either one of these two agents to impede the development of a type 1-polarized DC supports our previous observations that immature DC require a simultaneous exposure to a maturation factor and to IFN-γ to develop the DC1 effector phenotype, whereas the exposure to either factor alone is not sufficient (10, 39). Interestingly, similar to the previously reported DC1 induced by CD8+ T cells (10), NK cell-induced DC1 produced significantly higher levels of IL-12p70 than DC1 induced by high doses of rTNF-α and IFN-γ (Fig. 4B, inset). Apart from the combination of the target cell recognition and IFN-α, also the combination of proinflammatory cytokines, IFN-α plus IL-18 or IL-2 plus IL-18, promoted the NK cell-mediated induction of type 1-polarized DC (Fig. 4C).

**DC act as carriers of NK-derived Th1-inducing signals**

Although IFN-γ is a known coinducer of IL-12p70 production in monocytes, macrophages, and DC (39–43), the observations of enhanced IL-12-producing capacity of DC activated by NK cells in the Transwell model indicated that this later effect was not simply due to the presence of the IFN-γ-producing NK cells during the CD40L stimulation of DC. Instead, they demonstrated that signals derived from NK cells can induce stable type 1 polarization of DC.

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Since in contrast to NK cells tissue-activated DC are known to efficiently migrate to the T cell areas of the lymph nodes, the above results suggested that polarized DC may act as the principal carriers/conveyors of NK cell-derived immunomodulatory signals for the early polarization of naive Th cell responses within the lymph node.

To directly address the ability of DC to act as carriers of NK cell-derived Th1-inducing signals, we compared the outcome of priming of naive Th cells by nonpolarized DC vs the DC that were exposed to the soluble products of K562 cell- and IFN-α-activated NK cells (in a Transwell system). As shown in Fig. 5, NK cell-polarized DC1 induced strong Th1 responses in naive Th cells, manifested by strongly elevated production of IFN-γ but low production of IL-4, upon subsequent stimulation by the mitogenic

**FIGURE 3.** NK cells require two activating signals to induce final maturation of DC. NK cells (2 × 10^5 cells/well), K562 (2 × 10^6 cells/well), IFN-α (1000 IU/ml), or their combinations were added to day 6 autologous DC cultures (roughly 2–3 × 10^5 cells/well) for 48 h. A, K562- and IFN-α-stimulated NK cells induce DC maturation (shaded histograms) manifested by induction of CD83 and up-regulation of CD86 expression. The data shown in the histograms were obtained after gating on large (and high-HLA-DR-expressing) DC. *, The expression of CD86 and CD83 on DC cocultured with NK cells alone, or NK and K562 cells, were identical as on DC cultured in the absence of any additions and were omitted from the figure for the sake of its clarity. **, The combination of IFN-α and NK cells in the absence of K562 (or K562 and IFN-α in the absence of NK cells) cells were similarly weak inducers of CD86 and CD83 as the low dose (1000 U/ml) of IFN-α alone. At least 10-fold higher doses of IFN-α (10,000–30,000 U/ml) were needed to directly induce high CD83 expression in the absence of K562-stimulated NK cells (data from three experiments not shown). B, Soluble products of K562- and IFN-α-stimulated NK cells induce the maturation of bystander DC in a Transwell system. Addition of sTNFR1 (see Materials and Methods) inhibits the NK cell-induced up-regulation of CD86 on DC (shaded histogram). CD83 expression (data not shown) followed a similar pattern as CD86. C, Addition of sTNFR1 (shaded histogram) inhibits DC maturation also in direct cocultures of DC with K562- and IFN-α-stimulated NK cells. The combination of IFN-α with, selectively, either NK cells or K562 cells resulted in similar levels of CD86 and CD83 expression as IFN-α alone (data not shown).
NK cell-polarized DC1 show enhanced ability to induce virus-specific CD8+ T cell responses

IL-12p70 is a well-known inducer of the activation, survival, and effector functions in CD8+ cells (43). To test whether NK-induced DC1 can act as superior inducers of Ag-specific CD8+ T cell responses, we performed in vitro sensitization experiments using the frequency of Ag-specific IFN-γ-producing CD8+ T cells as a readout. To this aim, CD8+ cells were negatively isolated from HLA-A2* healthy donors and stimulated with the EBV-related HLA-A2-restricted epitope (BMLF1230–238) presented by NK-polarized DC (Transwell system: NK cells stimulated by K562 cells and IFN-α) or, alternatively, by nonpolarized DC (preactivated by IFN-α alone). In accordance with the elevated ability of NK-induced DC1 to induce cell-mediated immunity, the cultures activated by NK-polarized DC1 yielded strongly elevated numbers of IFN-γ-secreting EBV-specific CD8+ T cells (Fig. 6).

Discussion

Several reports have indicated that as early as 3 days after immunization with active virus, as opposed to inactive proteins, Th cells in the draining lymph nodes develop Th1-like vs Th2-dominated cytokine profiles associated with different isotype patterns of B cell responses (44, 45). NK cells have been long known to rapidly accumulate at the site of pathogen entry and in the marginal zone of the spleen, acting as sentinels against viral infections (27, 46). Recently, IL-2-pretreated NK cells have been shown to activate DC (16–18), but their ability to modify the pattern of the DC-induced primary immune responses of naive T cells have been unknown.

In this study, we report that the interaction of NK cells with CD83+ immature DC results in the induction of memory/effector type 1-polarized DC1 that serve as carriers of the NK cell-derived helper for the induction of Th1 responses and the induction of Ag-specific IFN-γ-producing CD8+ T cells. Our data demonstrate that NK cells have different and more tightly regulated activation requirements for such DC-mediated “helper function” compared with their traditional cytolytic “effector” function.
The ability of NK cells to act as a cellular switch affecting the character of immune responses may help to understand the complex pattern of the regulatory functions of type 1 IFNs, common mediators of antiviral immunity with a paradoxical therapeutic efficacy in such contrasting conditions as autoimmune diseases vs cancer and infectious diseases (47–53). Although IFN-α and IFN-β show direct Th1-inducing activity in humans and in mice, due to their ability to activate STAT-4 (IL-12-like effect) (54–58) and to enhance the expression of IL-12Rβ2 subunit (59), paradoxically, they were also shown to suppress the production of IL-12 (60–63).

We observed that, in accordance with previous reports (61, 62), the exposure of DC to type 1 IFNs alone induces only partial DC activation and does not result in the development of DC1 effector phenotype. Similarly, the contact of resting NK cells with a potential target in the absence of an alarm signal (i.e., costimulatory cytokines) results in the elimination of the target, but does not lead to the induction of the NK cell helper function.

In contrast, NK cell-target cell interaction in the context of a viral infection and the resulting production of IFN-αβ, e.g., by natural IFN-producing cells/plasmacytid DC (36, 37, 64), or in the context of later-acting proinflammatory cytokines, such as IL-2 and IL-18, allows NK cells to contribute both to the elimination of target cells and to the development of type 1-dominated immunity by inducing type 1-polarized “memory/effector” DC1. Such costimulatory interplay among NK cells, type 1 IFNs, and DC in the development of the Th1- and CTL-mediated type 1 immunity highlights the general applicability of the “two-signal principle” for the activity of the immune system. The “two-signal” activation requirements of DC (i.e., CD40L and IFN-γ; see Refs. 10 and 42) and NK cells (i.e., target and IFN-α) allow both of these cell types to function as “logical AND gates” of the immune system, utilizing the rules of Boolean logic (65, 66), to support (or not) the development of cell-mediated responses, a powerful but potentially self-damaging form of immunity.

Although our data indicate that IFN-α and NK cell ligands present on the surface of such target cells as K562 cells and T2 cells synergistically induce the DC1-polarizing activity in NK cells, the exact nature of the NK cell receptors and their ligands involved in this phenomenon remains to be determined. Similarly, although TNF-α and IFN-γ appear to be dominant mediators of the NK cell-polarizing activity, it remains to be determined whether other NK cell-derived or autocrine DC-derived factors are involved. Interestingly, although the measurable levels of these factors in NK-DC cocultures were within the picogram per milliliter range (Fig. 1 and data not shown), similar to the previously reported CD8+ T cell-induced DC1 (10), NK cell-induced DC1 produce significantly higher levels of IL-12p70 than DC1 induced by high doses of rTNF-α and IFN-γ (Fig. 4B, inset). These differences may either result from participation of additional factors in NK-DC interaction, or, alternatively, may reflect a particular kinetics of TNF-α and IFN-γ release, optimal for DC1 induction. Although the production of IFN-γ was restricted to NK cells and could not be demonstrated in DC interacting with NK cells in any of the tested conditions (intracellular staining) nor in DC stimulated by LPS or CD40L, the TNF-α production could be observed not only in NK cells but, to a lesser extent, also in DC (data not shown). This opens a possibility that DC-derived TNF-α, induced by NK cell-derived TNF-α (or other factors) in a positive feedback mechanism (67), may contribute to the NK cell-induced modulation of DC functions. Prospective mouse studies using TNF-α-deficient animals should allow us to compare the respective contributions of NK- and DC-derived TNF-α.

Similarly, we cannot distinguish whether the higher levels of IL-12 production by DC1 induced by NK cells directly, as compared with our Transwell system (Fig. 4A), reflect the participation of membrane-bound molecules, as postulated for the NK cell-induced DC maturation (15–17), or simply reflect a lesser distance between the cells in the direct coculture model. The molecular basis for “the two-signal” requirement for the induction of the helper function of NK cells is being currently analyzed.

Although the current set of experiments did not demonstrate any significant killing of DC by freshly activated NK cells, even at relatively high (10:1) NK:DC ratios, it is possible that some activation stages of NK cells, or particular NK cell subsets, will mainly kill DC or inactivate them, resulting in suppression of an immune response. Although poly(LC)-, EBV-, or IL-2-preactivated NK cells can kill DC at higher E:T ratios (16, 17, 32, 36, 68), it remains unclear under which conditions this may occur in vivo and what would be the net impact of NK-dependent DC activation and killing upon the magnitude and character of a physiologic immune response.

Already in 1987, it was suggested that NK cells play a positive role the induction of CTL (69). In two recent sets of in vivo experiments, murine NK cells have been shown to be critical for the induction of tumor-specific CTL (70) and for a Th1 bias associated with acute allograft rejection (71), indicating that both in the cancer and transplant settings, NK cells play a supportive rather than any inhibitory role in the induction in Ag-specific type 1 immunity. Another set of mouse studies have demonstrated that NK cells can support the induction of Th1-type Ab isotypes against polyoma virus and can counteract the induction of (Th2-associated) eosinophilia in the course of respiratory syncytial virus infection (72, 73). In accordance with a helper role of NK cells, NK cell-deficient patients and NK cell-deficient animals suffer from recurrent infections, especially with herpes viruses and mycobacteria (19–24, 74–76), suggestive of their inability to mount protective memory responses (20). Although a deficit in NK cell functions can be associated with atopic eczema (22), the general question of a possible deficit in NK cell-dependent DC1 polarization in atopic individuals remains to be addressed.

The current data may add to a better understanding of the poor effectiveness of immune responses against cancer, as opposed to effective antiviral immunity. During an early phase of tumor growth, NK cells can contribute to the elimination of transformed...
tumor cells, but due to the absence of a second, e.g., IFN-α-dependent, signal, NK cells are not induced to exert any “helper activity” and thus they do not activate nor polarize local DC. In effect, despite the ability of NK cells to recognize the transformed cells and to control their initial growth, they cannot support the development of tumor-specific type 1 immunity, resulting in the eventual loss of the control of tumor growth. This is in contrast to viral infections, where efficiently activated local NK cells contribute to the induction of adaptive Ag-specific responses, resulting in virus elimination.

The current observations suggest that increasing the availability of IFN-α at the tumor site, promoting the interactions of vaccine-carrying DC with NK cells, or using activated NK cells or their soluble products to modulate DC ex vivo before their use as a vaccine may constitute effective strategies in the immunotherapy of cancer patients.

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