Therapy-Induced Tumor Immunosurveillance Involves IFN-Producing Killer Dendritic Cells

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Abstract
A unique class of IFN-producing killer dendritic cells (IKDC) resembling natural killer cells has been defined that can recognize and lyse tumor cells through a tumor necrosis factor–related apoptosis-inducing ligand–dependent mechanism. IKDC may mediate the host-dependent antitumor activity of Gleevec/STI571 and other therapeutics that can inhibit the c-kit tyrosine kinase. IKDC represent an important new component of the innate immune system responding to cancer. [Cancer Res 2007;67(3):851–3]

Introduction
Complete and permanent success of cancer therapy depends on the targeting of all tumor cells (including cancer stem cells) or, alternatively, on the direct removal of a part of the tumor, escorted by a “bystander effect” in which the immune system recognizes, attacks, and eradicates remaining tumor cells. Our group found two examples of such a bystander effect promoted by immune cells. First, anthracycline-treated tumor cells could elicit a dendritic cell (DC)–mediated cytotoxic T cell (CTL) response that conferred stable long-term protection against rechallenge with the same tumor (1). This effect depended on the apoptogenic agent, on the form of cell death, on the innate immune system, as well as on the cognate immune system.

Second, inhibition of the c-kit tyrosine kinase by the paradigmatic anticancer drug, Gleevec/STI571, could endow DC with natural killer (NK) cell stimulatory capacity in mice and humans, leading to prolonged survival in patients bearing gastrointestinal sarcoma (2). In attempting to dissect the immune mechanisms leading to drug-induced tumor rejection, we recently discovered a new player in innate immunity that we called “IFN-producing killer dendritic cells” (IKDC), for its ability to produce IFNγ and to recognize tumor cells that are resistant to classic NK cells (3).

Key Findings Leading to the Discovery of IKDC
As recently reported, c-kit tyrosine kinase inhibitors such as Gleevec/STI571 can stimulate a host-dependent antitumor activity involving myeloid DC-primed NK cells (2). With the aim of further improving the NK cell–mediated antitumor effect induced by Gleevec/STI571, we combined Gleevec/STI571 with interleukin 2 (IL-2) and observed enhanced antitumor effects against B16F10 melanoma lung metastases, as compared with either agent alone.

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Analysis of depleting anti-NKR-P1c/NK1.1 monoclonal antibody completely abrogated the tumoricidal activity induced by the combination of Gleevec/STI571 and IL-2, suggesting a role for NK1.1 expressing cells.

Immunohistochemistry of regressing lung metastases in Gleevec/STI571+IL-2–treated mice revealed prominent infiltrates of CD11c+ cells in tumor beds and in surrounding parenchyma, suggesting that DC might be critical effectors of the antitumor effects achieved with the combination therapy (Fig. 1). We further did cytofluorometric analyses of single cell suspensions obtained from lung metastases. Classic NK cells defined as CD3+ B220+ NK1.1+ cells represented about 4% ± 2% of tumor-infiltrating cells and were not significantly expanded by the combination therapy, whereas the number of CD11c+ cells increased 2- to 3-fold in response to Gleevec/STI571+IL-2. Among those CD11c+ cells, classic plasmacytoid DC (pDC) coexpressing CD11c, B220 and Gr1 molecules represented not more than 3% of tumor-infiltrating CD11c+ cells. Importantly, the majority (72% ± 5%) of tumor-infiltrating CD11c+ cells expressed B220 but lacked Ly6C/Gr1, and all tumor-infiltrating CD11c+ cells that expressed NK1.1 molecules were Gr1 negative. Therefore, we focused our further investigation on the subset of CD11c+ cells coexpressing B220 and NK1.1 molecules.

The subset of CD11c+B220+ NK1.1+Gr1− cells (later called IKDC) increased 4-fold during treatment with Gleevec/STI571+IL-2 (Fig. 1) and coexpressed other NK cell markers such as CD49b/Dx5, CD122, NK2D, CD11b, and Ly49, but failed to express CD3, CD4, CD8α, CD25, PDCA-1, and costimulatory molecules. Because up to 50% of the CD11c+B220+ NK1.1+CD49b+ cells coexpressed major histocompatibility complex (MHC) II in tumors, we hypothesized that these CD11c+ cells would belong to the DC lineage.

Phenotype and Morphology of IKDC
In naïve mice, this new distinct cell population, defined as CD11c+B220+ NK1.1+CD49b− cells, represented about 1% to 2% of all splenic CD11c+ cells and was detected in all lymphoid organs, including blood, liver, gut, lung, and skin (3, 4). We could find those cells in several inbred mouse strains, including in Rag−/− IL-2Rγ−/− mice (where they lack NK1.1 molecules but express CD11c, B220, and CD49b), but not in IL-2/IL-15 receptor β chain–deficient mice (3). The phenotypic and functional characterization of CD11c+B220+ NK1.1+CD49b− prompted us and our colleagues to call the new cell “IKDC,” for reasons explained below.

Analyses using light microscopy and transmission electron microscopy highlighted that freshly isolated IKDC have a unique morphology. IKDC do not resemble pDC (with their developed and pathognomonic endoplasmic reticulum), display a smooth plasma membrane with small pseudopodia, a high nucleo cytoplasmic ratio and a dense cytoplasm containing few mitochondria but numerous granules (Fig. 1; refs. 3, 4).
Death receptors were found to be involved in the tumoricidal activity promoted by the combination therapy using Gleevec/STI571+IL-2. B16F10 tumor cells manipulated to express proteins that block the proapoptotic signal transduction complex initiated via death receptors (such as the dominant-negative mutant for Fas-associated death domain or the cowpox virus caspase-8 inhibitor CrmA) were resistant to this combination therapy in vivo.

**Role of IKDC in Tumor Immunotherapy**

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However, the combination therapy maintained a significant antitumor activity in TNF−/− mice. Conversely, the efficacy of Gleevec/STI571+IL-2 was completely abolished in mice treated with a neutralizing antitumor necrosis factor–related apoptosis-inducing ligand (TRAIL) antibody (Ab), establishing the pivotal role of TRAIL in the tumoricidal activity. After the combination therapy with Gleevec/STI571+IL-2, we detected the membrane expression of TRAIL on splenic IKDC and NK cells. Tumor cells transfected with CrmA were not significantly killed by IKDC, underlining the importance of death-receptor signaling for its antitumor activity. Furthermore, our TRAIL-blocking experiments in vitro confirmed that tumor killing was mainly dependent on TRAIL.

Because we found that both IKDC and NK cells could kill in a TRAIL-dependent manner, we investigated whether one of these cell types might be capable to prevent tumor outgrowth in effector-deprived hosts such as B16F10-bearing Rag−/− IL-2γ−/− mice. When B220−NK1.1−CD49b+ (IKDC) versus B220−NK1.1−CD49b+ (NK) cells from Gleevec/STI571+IL-2–treated mice were inoculated into established B16F10 tumors, only IKDC could significantly impair tumor outgrowth. Similar findings were achieved when Dx5−1−Ab− cells were adoptively transferred into tumor beds (3).

As TRAIL expression is controlled by IFN type II (5), we further examined the regulation of IFNγ secretion by IKDC and NK cells. Strikingly, IKDC stimulated IFNγ in vitro with tumor cells produced large amounts of IFNγ. Stimulation of IKDC with Gleevec/STI571+IL-2 also resulted in the secretion of IFNγ. Remarkably, IKDC could produce IFNγ in response to a broad array of allogeneic or syngeneic transformed cells except the transporter for antigen processing–deficient RMA-S cells, whereas NK cells failed to do so in the absence of exogenous stimulation, albeit exhibiting lytic activity against RMA-S cells. It is of note that IKDC did not secrete IFNγ in contact with healthy tissues such as primary hepatocyte or thymocyte cultures.

In conclusion, IKDC might mediate their tumoricidal activity by IFNγ secretion and TRAIL-dependent direct tumor cell lysis.

**Chief Implications**

This review describes the discovery of a novel DC subset called IKDC, a multitasking chimera sharing the phenotypic and functional properties of both DC and NK cells (3, 4, 6). Indeed, lytic IKDC express MHC class II and CD86 molecules and secrete large amounts of IFNγ in contact with transformed cells (whereas NK cells fail to do so), IKDC display the cardinal feature of DC, i.e., the capacity of antigen presentation following activation with TLR9 ligands as published by Chan et al., who characterized and described IKDC in parallel to our group in BALB/c mouse (4). These data open up the following prospects:

First, we propose that IKDC constitute a privileged link between the innate and cognate arms of antitumor immunity.

The current paradigm for T cell priming following tissue destruction relies on a two-step process whereby the tumor cell death event is somewhat dissociated from the antigen presentation in lymph nodes. Hence, apoptotic tumor cells might release endogenous danger signals that could activate conventional DC (cDC). The CTL or NK attack of tumors could liberate tumor antigens available for cDC and promote the translocation or secretion of endogenous alarms that could activate surrounding cDC (7). Alternatively, a one-step process could be envisioned in which a single cell would be able to kill tumor targets, uptake apoptotic debris, and initiate its differentiation towards a bona fide antigen-presenting cell. Such a scenario is conceivable because Chan et al. showed that IKDC express L-selectin and CCR7 and can traffic from blood to T cell areas of lymphoid organs, where they exhibit a DC phenotype (losing NK2D2 and acquiring CD40; ref. 4). Previous reports alluded to some DC subtypes endowed with cytolytic functions in humans and rodents. In the RIP-LCMV mouse model of virally induced diabetes, Homann et al. identified a subset of “NK/DC,” defined as CD11c−CD49b+ cells, capable of preventing autoimmune disease in the setting of anti-CD40L blocking Ab (8). Pillarisetty et al. reported of NK/DC, defined as CD11c−NK1.1+ cells, with simultaneous NK and DC functions (9). However, the precise nature of the cell capable of both, killing and antigen presentation, has not been established before we published our study.

Second, our data suggest that the combination therapy with Gleevec/STI571+IL-2 (which involves IKDC that trigger TRAIL-dependent apoptosis of tumor cells) could be implemented in the clinic, in particular, in TRAIL-sensitive cancers that are resistant to the direct antiproliferative effect of Gleevec/STI571. Consequently, identifying the human counterparts of mouse IKDC remains an important challenge.

Third, the implication of the host immune system in antitumor effects mediated by chemo- or radiotherapy is being actively investigated (10). Certain cell death modalities might electively trigger innate and/or adaptive immune responses, thus eliciting an antitumor response mediated by the host (1, 11). The challenge is now to transpose these data to the human system and to decipher the exact role of IKDC in the immune system’s fight against cancer.

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