Innate Direct Anticancer Effector Function of Human Immature Dendritic Cells. I. Involvement of an Apoptosis-Inducing Pathway

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Dendritic cells (DCs) mediate cross-priming of tumor-specific T cells by acquiring tumor Ags from dead cancer cells. The process of cross-priming has been intensively scrutinized with the ultimate goal of promoting enhanced protective or therapeutic immunity. The major sequential steps of this T cell priming process have been well defined (1–10). Thus, DCs first capture and digest dead cancer cells, then process their proteins into peptide epitopes, and finally present these epitopes in the context of the cell surface expressed MHC molecules to TCRs on specific CD4 and CD8 T cells.

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The following Abs were applied in this study: FITC-conjugated anti-human CD3 (IgG1) mouse mAb, PE-conjugated anti-human CD56 (IgG1) mouse mAb, FITC anti-human CD56 (IgG2b) mouse mAb, FITC anti-human CD16 (IgG1) mouse mAb, PE anti-human CD14 (IgG2a) mouse mAb, FITC anti-human CD19 (IgG1) mouse mAb (all from BD Biosciences, San Jose, CA); FITC anti-human CD80 (IgM) mouse mAb and PE anti-human CD86 (IgG1) mouse mAb (Ancell, Bayport, MN); FITC anti-human CD11c (IgG1)
mouse mAb and PE anti-human CD3 (IgG2b) mouse mAb (Caltag Laboratories, Burlingame, CA); FITC anti-human CD40 (IgG1) mouse mAb (Ancell); PE anti-human CD83 (IgG2b) mouse mAb (ImmunoTech, Marseille, France); FITC anti-human HLA-ABC class I (IgG2a) mouse mAb (Serotec, Burlington, Ontario, Canada); PE anti-human HLA-DR (IgG2a) mouse mAb and biotin-conjugated anti-human IL-3R (IgG2a) mouse mAb (BD Biosciences); FITC anti-human TRαβ2 (IgGb) mouse mAb, FITC H1.30 anti-human CD45 (IgG1) mouse mAb, PE H1.30 human DC45 (IgG1) mouse mAb, and conjugated isotype control mAb (Caltag Laboratories); anti-human CD3 (IgG1) mouse mAb, anti-human CD5 (IgG2b) mouse mAb, anti-human CD19 (IgG1) mouse mAb, anti-human CD56 (IgG2a) mouse mAb, anti-human CD16 (IgG1) mouse mAb, and anti-glycoporphin A (IgG1) mouse mAb (DAKO, Carpinteria, CA).

The following cytokines and ligands were used: recombinant human GM-CSF and recombinant human IL-4 (both kindly provided by Schering-Plough Research Institute, Kenilworth, NJ), and adenine nucleotide translocase inhibitor caspase inhibitor Z-Val-Ala-Asp(OMe)-fmk (Z-VAD-fmk; Enzyme Systems Products, Livermore, CA), and pan-caspase-9 inhibitor Ac-LEHD-CHO (Calbiochem, San Diego, CA), pan-caspase-8 inhibitor IETD-fluoromethyl ketone (fmk), caspase-3 inhibitor DEVDD-CHO, caspase-9 inhibitor Ac-LEHD-CHO (Calbiochem, San Diego, CA), pan-caspase inhibitor Z-Val-Ala-Asp(Ome)-fmk (Z-VAD-fmk; Enzyme Systems Products, Livermore, CA), and adenine nucleotide translocase inhibitor bongkrekic acid (Biomol, Plymouth Meeting, PA).

Isolation of monocytess

Monocytes (CD56−CD3−CD14+CD56+) were purified from normal human peripheral blood mononuclear leukocytes (PBMLN) to 95% purity using a modification of the previously described negative immunoselection technique with Ab-coated magnetic beads (14).

Isolation of DCs

DCs were purified from PBMLN as 90% pure populations of CD14+CD19−CD56−CD16+ cells by the use of a MACS Blood Dendritic Cell Isolation Kit (Miltenyi Biotec, Auburn, CA), as described in the manufacturer’s protocol. To obtain highly purified (i.e., 95%) populations of DCs, the enriched DC populations were simultaneously labeled with FITC-conjugated anti-TRαβ2, anti-CD14, anti-CD19, anti-cD56, and PE-conjugated anti-HLA-DR mAbs and sorted as TRαβ2+CD14+CD56−HLA-DR+ cells using a FACStarplus cell sorter (all mAbs and the cell sorter were from BD Biosciences).

DC cultures

Immature DCs were generated using a slightly modified protocol. To obtain highly purified (i.e., 95%) populations of DCs, the enriched DC populations were simultaneously labeled with FITC-conjugated anti-TRαβ2, anti-CD14, anti-CD19, anti-cD56, and PE-conjugated anti-HLA-DR mAbs and sorted as TRαβ2+CD14+CD56−HLA-DR+ cells using a FACStarplus cell sorter (all mAbs and the cell sorter were from BD Biosciences).

Cell lines and freshly isolated tumor cells

All cell lines were of human origin and mycoplasma free. Normal cell lines included skin keratinocytes (NHEK-Ad), dermal fibroblasts (NHDF-Ad), epidermal melanocytes (HMEM-Neo), mammary epithelial cells (Clonetech, Palo Alto, CA), NIH/3T3 fibroblasts, Walker 256 (MD), HUVEC (Cell Systems, Kirkland, CA), and T cell blasts (PBMLN stimulated for 7 days with 10 μg/ml Con A). Leukemia cell lines used in this study were K562 myeloid leukemia, Daudi Burkitt’s B cell lymphoma, MOLT-4 acute lymphoblastic leukemia, and Jurkat T cell leukemia (American Type Culture Collection (ATCC), Manassas, VA). Solid tumor-derived cell lines included gliomas P303, P388, S2, Pmel 136.34, and Pmel 255.1 (UPCI); breast carcinomas (IgG2a) mouse mAb, anti-human CD16 (IgG1) mouse mAb, and anti-glycoporphin A (IgG1) mouse mAb (DAKO, Carpinteria, CA).

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Isolation of DCs

DCs were purified from PBMLN as 90% pure populations of CD3−TRαβ2+CD14+CD56+CD11b+CD4+HLA-DR+ cells by the use of a MACS Blood Dendritic Cell Isolation Kit (Miltenyi Biotec, Auburn, CA), as described in the manufacturer’s protocol. To obtain highly purified (i.e., 95%) populations of DCs, the enriched DC populations were simultaneously labeled with FITC-conjugated anti-TRαβ2, anti-CD14, anti-CD19, anti-cD56, and PE-conjugated anti-HLA-DR mAbs and sorted as TRαβ2+CD14+CD56−HLA-DR+ cells using a FACStarplus cell sorter (all mAbs and the cell sorter were from BD Biosciences).

DC cultures

Immature DCs were generated using a slightly modified technique (15). Briefly, freshly purified monocytes were resuspended (12.5 × 10^6 cells/2.5 ml) in AIM-V medium containing 2.5% FCS (both from Life Technologies, Long Island, NY), placed into T25 tissue culture flasks (Falcon, BD Labware, Franklin Lakes, NJ) and incubated at 37°C for 2 h. Following this incubation, the nonadherent cells were removed and the adherent cells were washed carefully five times with warm (37°C) RPMI 1640 (Life Technologies) medium. After the last washing, the adherent cell cultures were supplemented with AIM-V 2.5% FCS medium containing 1000 U of IL-4 and 1000 U of GM-CSF and cultured for 5–7 days.

Mature DCs were generated from day 5 immature DCs by an additional 2-day incubation in the presence of IL-4, GM-CSF (1000 U/ml), and CD40L (2 μg/ml). Following the above-described two-step purification of monocytes, including negative selection on immunomagnetic beads and adherence, the isolated adherent DC precursors (monocytes) as well as monocyte-derived DCs were consistently 95% pure populations of CD14+ and CD3−CD14+CD19−CD56−CD16−MHC-ABC−MHC-DR+ cells, respectively.

Cell lines and freshly isolated tumor cells

All cell lines were of human origin and mycoplasma free. Normal cell lines included skin keratinocytes (NHEK-Ad), dermal fibroblasts (NHDF-Ad), epidermal melanocytes (HMEM-Neo), mammary epithelial cells (Clonetech, Palo Alto, CA), NIH/3T3 fibroblasts, Walker 256 (MD), HUVEC (Cell Systems, Kirkland, CA), and T cell blasts (PBMLN stimulated for 7 days with 10 μg/ml Con A). Leukemia cell lines used in this study were K562 myeloid leukemia, Daudi Burkitt’s B cell lymphoma, MOLT-4 acute lymphoblastic leukemia, and Jurkat T cell leukemia (American Type Culture Collection (ATCC), Manassas, VA). Solid tumor-derived cell lines included gliomas P303, P388, S2, Pmel 136.34, and Pmel 255.1 (UPCI); breast carcinomas (IgG2a) mouse mAb, anti-human CD16 (IgG1) mouse mAb, and anti-glycoporphin A (IgG1) mouse mAb (DAKO, Carpinteria, CA).

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then transferred from the gel onto uncharged nylon membrane (Millipore, Bedford, MA) and BrdU-labeled DNA was detected by standard Western blotting procedures using sequential incubations of the membrane with primary anti-BrdU mAb (Boehringer Mannheim-Roche), biotin-conjugated secondary goat anti-mouse Ig polyclonal Ab (Jackson Immuno-Research Laboratories, West Grove, PA), streptavidin-HRP, HRP substrate, and ECL reagent (Trevigen, Gaithersburg, MD).

Flow cytometry
All analyses were performed using a FACScan (BD Biosciences) flow cytometer. Phenotypes of monocytes and DCs were determined by direct two-color staining with fluorochrome-conjugated mAbs specific for the lineage markers of T cells, B cells, NK cells, monocytes, and DCs, as previously described (21).

Statistical analysis
LU/g/10⁷ effector cells were determined using the formula 10³(T × Xₚ), where T is the number of target cells and Xₚ is the estimated E:T ratio at which 20% of the target cells were killed. Statistical analyses of results were performed using the Wilcoxon’s signed-rank pair tests. To determine whether the data provide evidence for differences in profiles of percent killing as a function of E:T ratio, multivariate permutation methods were used. Differences were considered significant when the value of p was < 0.05.

Results
Killing of cancer cells by DCs
First, we examined whether in vitro generated, monocyte-derived DCs could mediate death of cancer cells and, if they could do so, whether death was the result of cell necrosis or apoptosis. We produced highly pure populations (95%) of CD3⁻/CD14⁻/CD19⁻/CD56⁻ immature and mature DCs from purified CD14⁺ blood monocytes by in vitro culture and stimulation with GM-CSF plus IL-4 and/or GM-CSF plus IL-4 plus CD40L, respectively. The two DC populations displayed predictably distinct morphology (data not shown), distinct phenotypes (i.e., immature DCs were CD83⁻/CD19⁻/CD19⁻/CD56⁻/mature and mature DCs were CD14⁺/CD80⁺/CD86⁺/CD40⁺/MHC class I⁺/MHC class II⁺), while mature DCs were CD83⁺ and CD80high/CD86high/CD40high/MHC class Ihigh/h/MHC class IIhigh (Fig. 1), and distinct functions (i.e., immature DCs exhibited high phagocytic and intermediate MLR-inducing activities, while mature DCs had low phagocytic and high MLR-inducing activities) (Ref. 3 and data not shown).

Initially, immature DCs were tested for killing ability against PCI-13 SCCHN cell targets using a variety of apoptosis-specific assays (Fig. 2). In all the assays applied, DCs mediated significant anticancer cytotoxic activity, effective at low E:T cell ratios (i.e., 0.1:1–3:1). Thus, DCs were capable of inducing in cancer cells substantial fragmentation and release of [³¹]cr (Fig. 2A), the formation of typical DNA ladders over 2–8 h of coculture (Fig. 2B), and a significant increase of TUNEL incorporation into nuclear DNA following 24 h of the cellular interaction (Fig. 2C). These results unequivocally demonstrated that DCs effectively induced apoptosis-specific internucleosomal fragmentation of DNA into 200-bp fragments and their oligomers in the nuclei of cancer cells. Next, we demonstrated that DCs induced ~30% of cancer cells to become reactive with annexin V (Fig. 2D) but only 5% of cancer cells to be stained with PI (data not shown), after 4 or 24 h of incubation. Therefore, this interaction provoked apoptosis-specific phosphatidyserine externalization on the tumor cell membrane. DCs also induced a large proportion of cancer cells to become nonreactive with MTT (Fig. 2E), DiOC₆(3) (Fig. 2F), or rhodamine 123 (data not shown) within 3–24 h of their coinoculation. These results showed that DCs effectively promoted apoptosis-specific mitochondrial damage and dysfunction in cancer cells by affecting mitochondrial dehydrogenases (assessed in MTT assay) and/or mitochondrial transmembrane potential (18), as measured in

DiOC₆(3) and rhodamine 123 assays. DCs also lysed 20–30% of cancer cells in either 24- or 72-h ⁵¹cr release (Fig. 2G) or NuMA release (Fig. 2H) assays, respectively. These results indicated that DCs mediated disruption of cancer cell membrane and disintegration of nuclear proteins, respectively, at later time points of the interaction. Notably, no killing of tumor target cells was detected in assays measuring necrosis, such as 4-h ⁵¹cr release (17) or PI uptake (data not shown). Therefore, a substantial proportion of cancer cells that are exposed to DCs appears to be killed, and this killing is mediated by apoptotic mechanism(s).

Signal pathways and role of caspases in DC-induced apoptosis of cancer cells
The cell stress-induced mitochondrial pathway and the death receptor-transduced pathway are main signaling mechanisms of apoptosis (22, 23). The former involves an early activation of the caspase-9 (22), while the latter involves an early activation of the caspase-8 (23). Each of two pathways is able to activate the caspase-3 and other common effector caspases. In addition, they can activate and amplify each other. To assess the potential role of two apoptotic signaling pathways and activation of caspases in DC-mediated killing of cancer cells, we examined the use of relevant caspases and involvement of mitochondria by applying the specific inhibitors (Table I). We found that pharmacological inhibitors of the caspase-8 (IETD-fmk), caspase-9 (Ac-LEHD-CHO), mitochondrial permeability transition pore (bongkrekic acid), caspase-3 (DEVD-CHO), and pan-caspases (Z-VAD-fmk) inhibited a notable proportion of DC-mediated killing of cancer cells (49, 41, 52, 57, and 67%, respectively). These data show that the two main apoptotic pathways and activation of caspases have critical roles in DC induction of apoptosis in cancer cells. The findings also indicate that in the course of DC-mediated killing of cancer cells either of two pathways is primarily and independently activated or one of the pathways is primarily activated while the other is secondarily activated.
Kinetics of DC-mediated tumor cell death

To determine the time dependency of killing of cancer cells by DCs, we performed kinetic experiments. In MTT assays, we observed substantial killing of cancer cells by 30 min and an increase of the activity for up to 3 h of coincubation (Fig. 3). After 3 h, no further increase was evident. Similarly, in DNA laddering assays, the bands of 200-bp DNA fragments and their oligomers were readily visible after 2 h, reaching peak levels after 8 h of coincubation. Therefore, the killing of tumor cells mediated by DCs was very rapid.

Ability of in vivo generated immature DCs to kill cancer cells

To evaluate the in vivo relevance of our in vitro findings, we examined whether freshly isolated, noncultured, normal blood donor DCs could kill cancer cells. The DCs directly obtained from PBMNL had a typical immature DC phenotype. Thus, they

Table I. DCs induce apoptosis in cancer cells via death receptor and mitochondrial signaling pathways

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3:1 E:T ratio</td>
</tr>
<tr>
<td>DMSO</td>
<td>20.6</td>
</tr>
<tr>
<td>IETD-fmk (100 μM)</td>
<td>13.0</td>
</tr>
<tr>
<td>Ac-LEHD-CHO (50 μM)</td>
<td>13.9</td>
</tr>
<tr>
<td>Bongkrekic acid (50 μM)</td>
<td>12.7</td>
</tr>
<tr>
<td>DEVD-CHO (50 μM)</td>
<td>10.9</td>
</tr>
<tr>
<td>Z-VAD-fmk (100 μM)</td>
<td>8.3</td>
</tr>
</tbody>
</table>

PCI-13 cell targets were labeled with \(^{3}H\)thymidine and preincubated with the indicated reagents at 37°C for 1 h. After that, target cells were mixed in vitro generated immature DCs in three different E:T ratios and 3-h \(^{3}H\)thymidine release cytotoxicity assays were performed. The presented results are from a representative experiment of six similar experiments performed. The data are mean percentages of cytotoxicity obtained in triplicate and LU\(_{50}/10^7\) DCs. DMSO concentration in both control and experimental wells was 0.5%. IETD-fmk, Caspase-8 inhibitor; Ac-LEHD-CHO, caspase-9 inhibitor; bongkrekic acid, mitochondrial permeability transition pore inhibitor; DEVD-CHO, caspase-3 inhibitor; and Z-VAD-fmk, pan-caspase inhibitor.
were CD3− TCRαβ− CD14− CD19− CD16− CD56− CD11b− CD4+ HLA-DR+ (Fig. 4A and data not shown). They expressed low levels of CD80, CD86, and CD40, and moderate levels of MHC class I and class II molecules, but did not express the CD83 marker of mature DCs (Fig. 4A). Assessments of the anticancer cytotoxic activity of these DCs in 3- (Fig. 4B) and 24-h (data not shown) MTT assays revealed that they were also able to induce death in cancer cell targets. Therefore, in vivo and in vitro generated immature DCs were similar in their ability to mediate death of cancer cells. These observations suggest that this DC effector function might be physiologically relevant.

Susceptibility of cell targets to killing by DCs

To assess the range of target cells that are susceptible to killing by DCs, we next tested cytotoxicity of in vitro generated immature DCs against several normal and cancer cell lines and freshly isolated cancer cells, using 3-h MTT, 1-h [3H]thyminidine release, and 24-h 51Cr release assays. Six normal cell lines, 36 malignant cell lines, and four different samples of fresh SCCHN cells, directly obtained from surgical specimens of primary tumors of head and neck cancer patients, were evaluated in total (Fig. 5). Normal proliferating endothelial cells, 35 of 36 tested leukemia and cancer cell lines, and four of four freshly isolated cancer cell samples were efficiently killed by DCs, in marked contrast to normal fibroblasts, keratinocytes, melanocytes, mammary epithelial cells, and autologous T cell blasts, which were not harmed by immature DCs. The only cancer cell line found to be relatively resistant to killing by DCs was PCI-30 SCCHN. These data demonstrate that immature DCs are promiscuous anticancer cytotoxic cells, which selectively kill cancer cells and spare normal cells.

Tumoricidal capacity of immature DCs vs monocytes and mature DCs

In contrast to immature DCs, their direct precursors, freshly isolated blood monocytes, did not show cytotoxic activity against cancer cells in our experiments (data not shown). Therefore, the anticancer killing ability appears to be acquired by immature DCs. Next, we tested the anticancer cytotoxic activity of mature DCs and compared it with that of immature DCs. In all three cytotoxicity assays used, including the 1-h [3H]thyminidine release, 24-h MTT, and 24-h 51Cr release assays, mature DCs exhibited significantly greater cytotoxic reactivity against cancer cells (Table II). However, the levels of killing by mature DCs were 4- to 4.9-fold lower than those observed for donor-matched immature DCs. This indicates that maturation of DCs, known to be accompanied by their multiple phenotypic and functional changes, including that of Ag-presenting activities, is also accompanied by a significant decrease of their tumoricidal function.

Killing of cancer cells by DCs is mediated by both cell membrane-bound and secreted proteins

To examine the mechanisms underlying the cytotoxic activity of DCs against cancer cells, we evaluated the cytotoxic activity associated with parafomaldehyde-fixed DCs and DC culture-conditioned medium. It has been previously shown that cell fixation eliminates secretory activity and preserves cell membrane-bound cytotoxic ligands (17), while conditioned cell culture media may contain secreted soluble forms of cytotoxic ligands (24). We observed that both fixed DCs and DC culture-conditioned medium were cytotoxic for cancer cells (Table III). However, these cytotoxic activities were lower than those of control unmanipulated DCs. Similarly, inhibition of cellular secretion by elimination of extracellular Ca2+, using the Ca2+ chelation with EGTA or EDTA, only partially suppressed DC-mediated killing of cancer cells (data not shown). In contrast, the apoptotic activity of DC supernatants was efficiently inhibited by the proteases papain or trypsin (Fig. 6). The inhibition showed a dose dependency and was complete in the presence of ≥250 μg of the proteases per 1 ml of DC supernatant, whereas it gradually decreased at lower concentrations of the enzymes. These data suggest that the mediators of immature DC-associated tumoricidal activity are both cell membrane-bound and secreted proteins.
DC-mediated cytotoxicity in normal individuals

To determine whether the observed anticancer apoptosis-inducing activity is a general function of DCs, we analyzed the killing ability of in vitro generated, monocyte-derived, immature DCs obtained from the peripheral blood of 25 different normal blood donors. We found that DCs isolated from all individuals evaluated were cytotoxic against cancer cell targets (Fig. 7). These data show that tumoricidal activity is an essential function of normal human immature DCs. We also observed a significant individual variability in the tested population of effectors obtained from different donors. This suggests that DC-mediated killing is genetically and/or environmentally determined.

Discussion

Our data provide novel information that human nonactivated immature DCs not only uptake death cancer cells and process tumor Ags at the periphery (1–9) but also selectively induce apoptotic death of cancer cells without damaging normal cells. In particular, our findings suggest that DCs induce rapid and marked internucleosomal DNA fragmentation, cell surface expression of phosphatidylserine, mitochondrial damage, and activation of caspases, followed by a later-stage disintegration of the cell membrane and degradation of nuclear proteins in tumor cells. The rapid induction of both mitochondrial and DNA damages as well as the observation that DCs kill target cells in MTT assays before development of internucleosomal DNA fragments suggest an ordered progression of tumor cell destruction. These data are consistent with a mechanism in which DCs either simultaneously or subsequently induce mitochondrial damage and DNA fragmentation. In addition, our data show that both the caspase-8- and caspase-9-related apoptosis signaling pathways are simultaneously induced in tumor cells by DCs, indicating that signal transductions via TNF family death receptors and/or cell stress (22, 23) are involved in apoptotic killing of tumor cells by DCs. In a companion manuscript (25), we...

Table II. Immature and mature DCs differ in their effectiveness to mediate apoptosis against cancer cells

<table>
<thead>
<tr>
<th>Effector Cells</th>
<th>1-h [3H]thymidine release</th>
<th>24-h MTT</th>
<th>24-h 51Cr release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature DCs</td>
<td>281.3</td>
<td>6827.9</td>
<td>3056.9</td>
</tr>
<tr>
<td>Mature DCs</td>
<td>57.1</td>
<td>1697.9</td>
<td>650.6</td>
</tr>
</tbody>
</table>

* Targets were PCI-4B SCCHN cells. All differences between the results obtained with two types of DCs were statistically significant (p < 0.001). The presented data are from a representative experiment of three similar experiments performed.

Table III. Fixed DCs and DC culture conditioned media are cytotoxic for human cancer cells

<table>
<thead>
<tr>
<th>Effector</th>
<th>PCI-13</th>
<th>PCI-4A</th>
<th>PCI-4B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated DCs</td>
<td>32.6 (1374.9)</td>
<td>52.7 (6219.0)</td>
<td>41.6 (2789.0)</td>
</tr>
<tr>
<td>Fixed DCs</td>
<td>13.1 (168.3)</td>
<td>36.4 (1802.4)</td>
<td>25.4 (1278.0)</td>
</tr>
<tr>
<td>DC supernatant</td>
<td>27.8</td>
<td>44.8</td>
<td>29.7</td>
</tr>
</tbody>
</table>

* Immature DCs, either untreated or following fixation with 1% paraformaldehyde, were tested for cytotoxicity using MTT assays, as previously described (17). DC conditioned media were collected from cultures of immature DCs (1 x 10^6/ml) following their incubation for 48 h. The assays were performed in triplicates. Data are mean percentages of killing obtained using a 3:1 E:T ratio and a 1:4 dilution of the conditioned culture media. SD of the results were <10% of the means. In parentheses are LU20/10^7 DCs, calculated on the basis of mean percentages of killing obtained in four E:T ratios (3:1, 1:1, 0.1:1, and 0.01:1). The differences between killing mediated by untreated and fixed DCs were statistically significant (p < 0.005). The presented results are from a representative experiment of two similar experiments performed.
The killing of cancer cells mediated by DCs represented an infrequent, largely promiscuous (i.e., with respect to tumor target range), selective, and essential anticancer activity. This finding that mature DCs are more effective at mediating antitumor activity by destroying newly forming tumor blood vessels.

FIGURE 6. Tumoricidal activity of DC supernatants was inhibited by proteases. DC supernatants were obtained by 48-h culture of in vitro generated day-5 immature DCs in serum-free AIM-V medium supplemented with GM-CSF plus IL-4. A part of the supernatants was coinoculated with trypsin or papain at indicated various concentrations for 60 min at 37°C. Following this enzymatic treatment, the supernatants were mixed with FCS at a final concentration of 25% and incubated for 30 min at 37°C, to neutralize the excess of proteases. Cytotoxic activity of the supernatants was then assessed against PCI-13 target cells using 24-h MTT assays. The presented results are from a representative experiment of four similar experiments performed. The data are mean percentages of cytotoxicity ± SD of triplicates.

mediating the receptor-ligand interactions responsible for this DC-mediated apoptotic death of tumor cells. Previous studies have indicated that certain subsets of rat DCs (26) and activated human DCs might be cytotoxic for rare tumor cell targets (11, 12). From these studies it was not clear whether the killing of cancer cells mediated by DCs represented an infrequent in vitro artifact, a rudimentary activity, or a major activity of DCs. Furthermore, the mechanisms and biological significance of such activity were not clear. Our current data and those presented in a companion manuscript (25) support the conclusion that human nonactivated immature DCs are cytotoxic in vitro in low E:T ratios for the large majority of cancer cell lines and freshly isolated cancer cells, but not for normal cells. In addition, our data demonstrate that antitumor activity of DCs is a constitutive function of these effector cells in normal individuals. Therefore, cytotoxic activity mediated by DCs is an efficient, largely promiscuous (i.e., with regard to tumor target range), selective, and essential antitumor effector function. Our finding that DCs also kill proliferating “normal” endothelial cells may suggest that DCs are also capable of delineating the receptor-ligand interactions responsible for this DC-mediated apoptotic death of tumor cells.

FIGURE 7. Antitumorcytotoxicity is a general function of DCs in normal individuals. The antitumor cytotoxic activity of cultured, monocyte-derived, immature DCs obtained from 25 different normal individuals was tested against PCI-13 target cells in 3-h MTT assays. The data are reported in both mean percentages of cytotoxicity (E:T ratio, 3:1) (A) and LU (LU20/107 DCs) (B) for each individual tested. Each dot represents the data from a given individual and the horizontal bars represent the overall mean values for all donors evaluated.

References


