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Role of Tumor Cell Apoptosis in Tumor Antigen Migration to the Draining Lymph Nodes

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Establishment of an immune response against cancer may depend on the capacity of dendritic cells to transfer tumor Ags into T cell-rich areas. To check this possibility, we used a colon cancer cell variant that yields tumors undergoing complete T cell-dependent rejection when injected into syngeneic rats. We previously demonstrated that immunogenicity of these tumors depended on the early apoptosis of a part of these tumor cells. In this paper we show that fluorescent tumor cell proteins are released from FITC-labeled tumor cells and undergo engulfment by tumor-infiltrating monocytes without a phenotype of mature dendritic cells or macrophages. Fluorescence-labeled mononuclear cells with a phenotype of MHC class II+ dendritic cells are also found in the T cell areas of the draining lymph nodes. Interestingly, no fluorescent cell can be found in lymph nodes after a s.c. injection of Bcl2-transfected apoptosis-resistant tumor cells that yielded progressive tumors. Proliferation of tumor-immune T lymphocytes was induced by dendritic cells isolated from the draining lymph nodes recovered after a s.c. injection of apoptosis-sensitive, but not apoptosis-resistant, tumor cells. These results show that tumor cell apoptosis releases proteins that are engulfed by inflammatory cells in the tumor, then transported to lymph node T cell areas where they can induce a specific immune response leading to tumor rejection. The Journal of Immunology, 2000, 164: 1995–2000.

Most tumors are accepted by the host immune system and progress even when they contain potentially antigenic proteins. Some tumor cell variants raise an immune response that leads to tumor rejection. Analysis of this response may help to understand how most tumors escape immune rejection.

The REGb clone was obtained from a cultured cell line established from a chemically induced colon carcinoma in a BD-IX rat. Whereas the parental cells produce progressive and lethal tumors when injected into syngeneic rats, REGb cells induce tumors that regularly regress after 2 or 3 wk and immunize the host against a further challenge with the parental cell line or its progressive cell variants (1). Using this model, we recently demonstrated that the immunogenic clones differed from the tolerated progressive cell lines of the same tumor origin by the capacity of a part of regressive cells to undergo apoptosis during the first days following their injection into a syngeneic host. Overexpression of the anti-apoptotic protein Bcl-2 in REGb cells prevented the development of an antitumor immune response (2). This suggested that induction of a specific immune response involved the release of antigenic proteins from tumor cells undergoing apoptosis.

Tumor cells cannot activate directly T lymphocytes, as they do not usually express MHC class II molecules nor costimulatory signals such as B7. Thus, tumor Ags can only be presented indirectly to T cells through professional APC, such as dendritic cells (3). This requires that antigenic proteins are shed from tumor cells before undergoing endocytosis by APC. In a model using melanoma cells in mice, phagocytosing cells were observed to be attracted at the injection site and ingest tumor cell fragments (4). It has been recently demonstrated that dendritic cells selectively recognized and captured apoptotic cells and cell fragments liberated following apoptosis (5–7). Moreover, antigenic proteins that were contained in apoptotic bodies and engulfed by dendritic cells were shown to be 1–10,000 times more efficient in generating MHC-peptide complexes than preprocessed peptides (8).

APC have to present the ingested tumor Ags to T cells. The present study was undertaken to determine the pathway that led from tumor cell to tumor Ag presentation to T cells. For that purpose, we labeled proteins from living REGb cells or REGb-bcl2 cells with FITC before injecting these cells into syngeneic animals. We show that FITC-labeled proteins released from apoptotic REGb cells were locally engulfed by tumor-infiltrating inflammatory cells, then found in dendritic-like cells of the draining lymph nodes where tumor Ags can be presented to tumor-specific T lymphocytes. Interestingly, no fluorescent cells were found after FITC-labeled REGb-bcl2 cells injection that did not yield a rejection immune response but rather a progressive tumor. We conclude that apoptosis of tumor cells might facilitate tumor Ag transport from tumor site to draining lymph nodes where these Ags can initiate a specific T cell immune response.

Materials and Methods

Animals and tumor cells

Animals used in these experiments were syngeneic BD-IX strain rats bred in our laboratory by brother-sister mating. Experimental protocols were consistent with the “Guidelines on the Protection of Experimental Animals” published by the Council of the European Community in 1986. The regressive variant DHD-K12/Tsb (REGb) was established from the DHD tumor, a colon adenocarcinoma induced by 1–2 dimethylhydrazine in a
BD-IX rat. REGb-bcl2 cells were transfected with pEB7 expression plasmid containing cDNA encoding human bcl-2 as previously described (2). These tumor cells were cultured in a mixture of Ham’s F10 medium and FBS (10:1 v/v, complete culture medium) as previously described (1).

Fluorescent labeling of tumor cells
FITC (Fluka, Buchs, Switzerland) was stored at 4°C until use. One day before the experiment, 20 mg of FITC was added to 5 ml HBSS (Life Technologies, Paisley, U.K.) and dissolved by stirring overnight in the dark at room temperature. Ten million REGb cells or REGb-bcl2 cells were suspended in 1 ml of complete medium, then incubated for 30 min at 37°C with FITC at a final concentration of 500 μg/ml. Cells were washed until no fluorescence was detected in the supernatant. Upon UV illumination, tumor cells showed a bright greenish-yellow fluorescence.

Western blot analysis
FITC-labeled subconfluent cultured cells were lysed at 4°C for 15 min in lysis buffer (150 mM NaCl, 1 mM KH2PO4, 1 mM EGTA, 1 mM Na2PO4, 5 mM MgCl2, 0.1 mM PMSF, 0.15 U/ml aprotinin, 1 μg/ml pepstatin, and 10% glycerol). The protein concentration was measured in the supernatant by using the micro BCA protein assay (Pierce, Annieres, France). An amount of 50 μg of proteins was separated by SDS-PAGE using a 12% polyacrylamide gel. The gel was analyzed under fluorescent illumination. The results are representative of three separate experiments.

Results

Thymidine incorporation assay
T cell suspensions were prepared from spleens of normal rats or rats immunized by three monthly REGb cell injections. Spleens were pressed through a stainless-steel wire mesh into complete medium (RPMI 1640 with 10% FCS). Pelleted cells from the spleen suspension were passed through nylon wool columns to obtain >90% pure T cells. Enriched T cells from normal or REGb cell-immunized rats were suspended (106 cells/ml) in a round-bottomed 96-well plate (Nunclon, Roskilde, Denmark). They were cocultured in triplicate with mitomycin preincubated dendritic cells (1 × 103 cells/ml) isolated from draining popliteal lymph nodes 7 days after injection of REGb cells or REGb-bcl2 cells in 100 μl Ham’s medium. After 3 and 5 days of incubation at 37°C in 5% CO2, the cells were pulsed with 1 μCi of [methyl-3H]Tdr (57 μCi/mmol; Amersham) for 18 h at 37°C and harvested onto a glass fiber filter with a Skatron harvesting apparatus (Skatron, Lier, Norway). Thymidine incorporation was measured in a beta scintillation counter (model 1409; Wallac, Turku, Finland) and the results from triplicate wells were expressed as mean cpm ± SD. The results are representative of three separate experiments.

Tumorigenicity assays
To study the tumor cell injection site, 1 × 106 REGb cells in 100 μl serum-free Ham’s F10 medium were injected s.c. into the thoracic wall of BD-IX rats. To study the migration of tumor Ag to the lymph nodes, 5 × 104 FITC-labeled tumor cells in 100 μl serum-free, serum-free Ham’s F10 medium were injected s.c. in the hind footpad.

Immunohistochemical studies of tumor cell injection site and lymph nodes
Animals were killed at different times after REGb cell injection. The site of tumor cell injection, and the ipsi and contralateral lymph nodes were resected and either fixed in formaldehyde (4% in PBS) and embedded in paraffin, or embedded in Tissue-Tek (Miles, Elkhart, Indiana) and snap-frozen in methylbutane cooled in liquid nitrogen. On sections stained with Hemalun-Eosin, tumor cells were distinguished from inflammatory cells according to their size, their large nucleus with oversized nucleoles, and their assembling in nodules. An immunohistochemical study of lymph nodes and tumor site was performed in acetonite fixed 5-μm cryostat sections. Mouse mAb to rat monocytes (ED1), MHC class II (OX-17), TCR (R7β), CD4 (W3/25), and CD8 (OX8) and IgG isotype-matched control (R7/3), CD4 (W3/25), and CD8 (OX8) and IgG isotype-matched control sera were purchased from Serotec (Oxford, U.K.). Mouse mAb against rat mature (R7/3), CD4 (W3/25), and CD8 (OX8) and IgG isotype-matched control sera were purchased from Serotec (Oxford, U.K.). Mouse mAb against rat mature tissue macrophages (Ki-M2R) was a gift from Prof. H. H. Wacker (Kiel, Germany). Sections were incubated with mAb, with biotinylated sheep Ab to mouse IgG (Amersham, Little Chalfont, U.K.), then with streptavidin-peroxidase and stained with aminoethylcarbazole. In other sections, tumor tissue macrophages (Ki-M2R) was a gift from Prof. H. H. Wacker (Kiel, Germany). Sections were incubated with mAb, with biotinylated sheep Ab to mouse IgG (Amersham, Little Chalfont, U.K.), then with streptavidin-peroxidase and stained with aminoethylcarbazole. In other sections, tumor cells were stained after incubation with a rabbit polyclonal Ab to cytokeratin (Monosan, Uden, The Netherlands).

Isolation of lymph nodes cells
Rats were killed 1, 2, 3, 4, 7, or 12 days after REGb cell or REGb-bcl2 cell injection in the footpad. Popliteal lymph nodes were taken out, pooled for each experimental group, and mechanically disaggregated through a stainless-steel wire mesh. After washing, lymph node cells were suspended in complete medium. To obtain dendritic cell-enriched populations, the lymph node cells were layered onto a metrizamide (Sigma, St. Louis, MO) gradient (14.5 g/100 ml of RPMI 1640 medium) and centrifuged for 15 min at 600 × g. Cells at the interface were washed once and suspended in complete medium. This population comprised 50–60% dendritic cells that were identified on their distinctive morphology and labeling with ED1 and OX-17 (anti-MHC class II) mAbs.

Cytoluciferometry analysis
Cells were washed in PBS supplemented with 0.5% BSA and 0.01% sodium azide, adjusted to 1 × 106 cells/100 μl. A total of 10,000 events were analyzed for each sample with a FACScan (Becton Dickinson, Grenoble, France) using CellQuest software for determining the percentage of cells that were fluorescent following the engulfment of FITC-labeled proteins.
FITC-labeled cells are found in the draining lymph nodes

We have previously demonstrated that REGb cell injection in syngeneic rats induced a T cell-dependent tumor-rejecting immune response (1). As very few T cells could be detected on the tumor cell site, we searched for a transfer of the antigenic information to the T cell areas of the secondary lymphoid organs. Therefore, FITC-labeled REGb cells were injected into the footpad of BD-IX rats, a site that is drained by the popliteal lymph node. Four days after tumor cell injection in the footpad, a few brightly fluorescent cells could be identified in some ipsilateral popliteal lymph node sections (Fig. 5a). These rare fluorescent cells, which remained detectable up to 12 days after the tumor cell injection, were located in restricted areas of the lymph node, outside of the B cell follicles (Fig. 5a). They were labeled with ED1 mAb (Fig. 5b), whereas they were not labeled with a anti-cytokeratin mAb, an Ab that characterizes epithelial cells such as REGb cells (data not shown). Fluorescent cells demonstrated dendritiform extensions that could constitute a fluorescent network between adjacent cells. Some of these adjacent cells were labeled with an anti-TCR mAb (data not shown). No fluorescence was observed in the contralateral popliteal lymph node, in lymph nodes resected in other sites, or in the spleen of these animals. Interestingly, no fluorescent dendritic-like cells were found in the popliteal draining lymph nodes from rats which received FITC-labeled REGb-bcl2 cells in the footpad 4 days before lymph node resection (Fig. 5c).
Phenotypic characterization of the FITC-loaded cells in the draining lymph nodes

To better characterize the FITC-labeled cells that were identified in the draining lymph nodes, we collected the ipsilateral popliteal lymph nodes of eight BD-IX rats, 4 days after s.c. injection of FITC-labeled REGb cells in the footpad. The lymph nodes were dissociated and their cells were analyzed on flow cytometry. About 5% of these cells were found to be spontaneously fluorescent (Fig. 6). An immunocytological study confirmed that most of the fluorescent cells (Fig. 7a) were labeled with ED1 mAb and expressed MHC class II molecules (Fig. 7b). These data strongly suggested that fluorescein-labeled cells were dendritic cells. However, we cannot strictly establish that the ED1⁺, MHC class II⁺, FITC⁺ cells found in the lymph nodes were dendritic cells, as no specific rat dendritic cell marker is presently available.

The cells from popliteal lymph nodes were enriched on metrizamide gradient in dendritic cells characterized on their morphology and immunofluorescence staining with ED1 and MHC class II mAbs. The fluorescent dendritic cells isolated from the draining lymph nodes of rats that received FITC-labeled REGb cells in footpad were rare, but they could be observed when lymph node cells were put in suspension (Fig. 7, c and d). These fluorescent cells from popliteal lymph nodes were enriched on metrizamide gradient in dendritic cells characterized on their morphology and immunofluorescence staining with ED1 and MHC class II mAbs.

FIGURE 5. Migration of ED1⁺ cells containing FITC-labeled inclusions in draining lymph nodes. Four days after injection of FITC-labeled REGb cells into the footpad, restricted areas of the draining lymph nodes were labeled yellow-green upon UV illumination (a). On the same section, these fluorescent-labeled areas contained ED1⁺ cells (b) and were located between B cell-containing follicles (F). No fluorescent dendritic-like cells were found in the popliteal draining lymph nodes from rats that received FITC-labeled REGb-bcl2 cells in the footpad 4 days before lymph node resection (c). (Magnification, ×160).

FIGURE 6. Flow cytometry analysis of green-fluorescent cells in the draining lymph node. Four days after footpad injection of FITC-labeled REGb cells, the draining popliteal lymph nodes were resected and dissociated cells were examined on flow cytometry. A peak of green-fluorescent cells (5% in this experiment) was clearly individualized.

FIGURE 7. Characterization of green-fluorescent cells isolated from the draining lymph nodes. The green fluorescent cells (a) were red-fluorescent after labeling with mAbs to MHC class II (b) (arrow). These lymph node cells were enriched on metrizamide gradient in dendritic cells. Green-fluorescent cells are seen upon UV illumination (c) and on phase-contrast examination (d). One cell appeared to undergo apoptosis (arrow). The other cells had dendritic-like cytoplasmic extensions. (Magnification, ×250).
cells (105/ml) isolated from popliteal lymph nodes from rats that received
immune rats were cocultured with mitomycin C-treated semipurified dendritic
cells are engulfed by mononuclear cells at the tumor cell injection site. A few days later, some fluorescence
cells were identified in the draining lymph node. In this later localization, fluorescent cells were cytolysin-negative, ED1-positive,
and MHC class II-positive. These fluorescent cells were in close contact with TCR-positive cells.

As we shown previously, a part of REGb cells underwent apoptosis during the first 2 days following their s.c. injection into syngeneic host (2). These apoptotic cells released cell fragments or apoptotic bodies that contained proteins. We show here that these proteins are transferred in the mononuclear cells that rapidly infiltrate the tumor cell injection site as a part of these cells acquires a fluorescent staining. These ED1⁺ cells are labeled neither with KiM2R mAb, a marker of differentiated tissular macrophages (12), nor with an anti-MHC class II mAb that labels mature dendritic cells. These cells are probably undifferentiated monocytes, and are actively endocytic, as suggested by the strong expression of ED1, a marker of phagolysosomes (13, 14).

It is very likely that tumor Ag presentation by ED1⁺ inflammatory cells to T cells does not occur at the tumor cell injection site. First, accumulation of T lymphocytes is very low at this site, at least during the first week following tumor cell injection, a time where an active immune response is generated because REGb cell rejection often begins as soon as 10–12 days after injection. Furthermore, tumor-infiltrating ED1⁺ monocytes do not express MHC class II molecules, a marker of mature APC. Also, we previously demonstrated that REGb cell rejection depended on B7 costimulatory molecules expression, but that B7-expressing cells were rare or absent in the REGb tumour, whereas they were abundant in the draining lymph nodes (15). Thus, presentation of tumor Ags to T cells does not occur inside the tumor, but rather in the draining lymphoid tissues of the spleen or lymph nodes.

When FITC-labeled REGb cells were injected in footpad, fluorescent cells were actually found in the popliteal lymph node that drains the tumor site, but not in the spleen or other lymph nodes, including contralateral popliteal lymph node. The fluorescent cells were localized in the extrafollicular T cell areas, in close contact with the T cells. The fluorescent cells were neither metastatic REGb cells, because they were not labeled with a polyclonal Ab to keratin that labels epithelial cells, including REGb cells, nor were they mature macrophages because they were not labeled with KiM2R mAb. The fluorescent cells still expressed ED1, but also MHC class II molecules and had a dendritic-like pattern. These dendritic-like cells can result from the differentiation of the fluorescent MHC class II monocytes found at the tumor cell injection site after their migration to the lymph node. Differentiation of monocytes into dendritic cells is well demonstrated and can be induced by various conditions including transendothelial migration (16). However, we cannot exclude an intercellular transfer of antigenic material. Inflammatory monocytes and macrophages can emigrate into the draining lymph nodes (17), in which they can deliver Ags from ingested particles to dendritic cells for presentation to T cells (18). It has also been demonstrated that Ag could be transferred between dendritic cells before presentation to T lymphocytes, a phenomenon which amplifies T cell activation (19). Interestingly, after injection of FITC-REGb-bcl2 cells which were more resistant to apoptosis, no fluorescent cells were found in the draining popliteal lymph node. This suggest that apoptosis-resistant FITC-REGb-bcl2 did not release fluorescent apoptotic

dendritic cells contained abundant vesicles and had cytoplasmic extensions characteristic of the dendritic cells. Some had an apoptotic phenotype.

Dendritic cells recovered from the draining lymph nodes stimulates tumor-immune T cells
To demonstrate the ability of the dendritic cells recovered from the draining lymph nodes to present REGb cell tumor Ags to T cells, dendritic cells were enriched on metrizamide gradient centrifugation from the popliteal lymph nodes of rats that have received a REGb cell or a REGb-bcl2 cell injection in the footpad 7 days before lymph node resection. Thymidine incorporation of tumor cells was not used in this experiment to prevent T cell stimulation by FITC-haptenized peptides presented by dendritic cells. Spleenic T cells from untreated rats and rats preimmunized by three monthly injection of REGb cells were collected to be cultured in the presence of dendritic cells isolated from the popliteal lymph node. T cell response was evaluated by measuring thymidine incorporation. Thymidine incorporation was maximal on day 3 of the mixed dendritic cell T cell culture. T cell response is significantly higher when tumor-immune T lymphocytes were cultured in the presence of dendritic cells isolated from the popliteal lymph node of rats injected with REGb cells than in the presence of dendritic cells isolated from rats injected with REGb-bcl2 cells (Fig. 8). These results indicate that FITC-labeled proteins are transferred in the monocytic cells that rapidly infiltrate the tumor cell injection site as a part of these cells acquires a fluorescent staining. These ED1⁺ cells are labeled neither with KiM2R mAb, a marker of differentiated tissular macrophages (12), nor with an anti-MHC class II mAb that labels mature dendritic cells. These cells are probably undifferentiated monocytes, and are actively endocytic, as suggested by the strong expression of ED1, a marker of phagolysosomes (13, 14).

FIGURE 8. Dendritic cells obtained from draining lymph nodes after footpad injection of REGb cells stimulated proliferation of tumor-immune T lymphocytes. Splenic T cells (10⁵/ml) from naive or REGb tumor-immune rats were cocultured with mitomycin C-treated semipurified dendritic cells (10⁵/ml) isolated from popliteal lymph nodes from rats that received REGb cells or REGb-bcl2 cells in the footpad 7 days before lymph node resection. Thymidine incorporation into T cells was measured 3 days after the beginning of the mixed culture. These results are representative of three separate experiments.

Our results clearly show that proteins from immunogenic tumor cells are engulfed by mononuclear cells at the tumor cell injection site. Then, these proteins are found in dendritic-like cells of the draining lymph node T cell areas.

We could directly visualize this migration of tumor cell proteins by labeling them covalently with FITC in the living tumor cells ex vivo. Skin painting with FITC has been used for labeling Langerhans cells and studying their migration to the draining lymph nodes (9, 10). FITC has also been used for labeling lymphocytes ex vivo, before their i.v. injection, to follow their migration and distribution in various tissues (11). In the present study, FITC-labeled REGb tumor cells were injected s.c. in syngeneic rats. We observed that FITC-labeled proteins were engulfed by ED1⁺, MHC-class II-negative mononuclear cells that infiltrated rapidly the tumor cell injection site. A few days later, some fluorescent cells were identified in the draining lymph node. In this later localization, fluorescent cells were cytolysin-negative, ED1⁺, and MHC class II-positive. These fluorescent cells were in close contact with TCR-positive cells.

Discussion
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bodies that could be engulfed by mononuclear cells and transported to the draining lymph nodes. The question of whether tumor-infiltrating ED1 phagocytic cells migrate to draining lymph nodes, differentiate into mature dendritic cells, and prime host lymphocytes T directly, or are destroyed by the host effector cells after which Ags are taken up and represented by dendritic cells has not yet been investigated and additional experiments are needed to address this issue.

A more direct approach of the capacity of dendritic-like cells isolated from tumor-draining lymph nodes to present tumor Ags to immune lymphocytes was a proliferation assay following mixed isolated from tumor-draining lymph nodes to present tumor Ags to yet been investigated and additional experiments are needed to address this issue.

The present work determines the main steps leading from the proteins enclosed in an immunogenic tumor cell to tumor Ag presentation to T cells. We have previously found that one of these steps, apoptosis of a part of tumor cells early after cell injection, was required for induction of an immune response and tumor rejection. We presently demonstrate that tumor cell protein release during this apoptosis was followed by protein capture by inflammatory cells and presentation to Ag-specific T cells in the draining lymph node.

References