Human Dendritic Cells Transfected with Renal Tumor RNA Stimulate Polyclonal T-Cell Responses against Antigens Expressed by Primary and Metastatic Tumors*

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ABSTRACT

Although renal cell carcinoma has been shown to respond to immunotherapy, renal cell carcinoma-specific rejection antigens and their corresponding CTL epitopes have rarely been described. The use of dendritic cells (DCs) transfected with mRNA isolated from tumor cells may allow specific immunotherapy even in cancers for which potent rejection antigens have not been identified. Here we show that DCs transfected with RNA isolated from renal cancer tissue are remarkably effective in stimulating tumor-specific T-cell response in vitro but do not cross-react with normal tissue antigens including antigens expressed by renal parenchyma. In contrast, the tumor-specific CTLs lysed allogeneic tumor but not allogeneic normal tissue targets, suggesting the presence of shared albeit unidentified antigens among renal carcinomas. CTL responses against telomerase reverse transcriptase (TERT) accounted in part for the reactivities against allogeneic tumors because renal tumor RNA-transfected DCs stimulated polyclonal CTL responses, which encompassed as a subcomponent a response against TERT. Nonetheless, the tumor-specific CTLs were consistently superior to the CTLs stimulated with TERT RNA-transfected DCs in recognizing and lysing tumor targets, suggesting that tumor-specific CTLs represent a polyclonal response providing more effective antitumor activity than T-cell responses directed against a single antigen in the form of TERT. Tumor RNA-transfected DCs were capable of stimulating T-cell reactivities not only against the primary tumor but also against metastatic tumors, although discrete differences in the antigenic repertoire expressed by these tissues were apparent. Thus, total tumor RNA-transfected DCs may represent a broadly applicable vaccine strategy to induce polyclonal and potentially therapeutic T-cell responses in renal cancer patients.

INTRODUCTION

The incidence of RCC is rising, with approximately 30,000 new cases detected annually in the United States accounting for 11,600 deaths/year (1). About one-third of RCC patients will present with metastatic disease, and one-third of the remainder will eventually develop distant metastases for which no effective standard treatment exists. Like melanoma, RCC has been shown to respond to immunotherapeutic intervention, suggesting that these tumors express specific rejection antigens, which are recognized by the immune system. However, widely expressed and clinically effective renal tumor antigens shared among many RCC patients have not yet been identified. Realizing the limitations of immunization with defined antigens, several studies have provided preliminary evidence that DCs loaded with tumor-derived antigenic mixtures in the form of tumor extracts (2) or cell lysates (3) may represent an efficient strategy to induce antitumor immunity in RCC patients. Although this is promising, it can be predicted that a generalized and widespread application of these approaches will be hampered by the requirement of large amounts of tumor tissue needed for antigen preparation and DC pulsing. This limitation becomes even more relevant because it has been argued that continuous boosting will be necessary to maintain effective antitumor immune responses (4).

Alternatively, it was recently shown that DCs transfected with antigens encoded in tumor mRNA are capable of inducing potent T-cell responses against multiple, tumor-specific epitopes while obviating the need to identify the antigens involved (5). Because functionally intact RNA can be amplified using PCR technology, nonlimiting amounts of antigen could be generated, even from small amounts of tumor tissue, for DC pulsing (6).

The primary objective of this study was to determine whether DCs transfected with autologous RCC RNA are able to stimulate CTL responses in vitro against a broad repertoire of as yet unidentified RCC-specific antigens. We further sought (a) to investigate whether the CTLs generated by stimulation with tumor cell-derived antigenic mixture in the form of RNA would also induce autoimmune reactivities against nontumor cellular antigens and (b) to define whether primary renal tumor-specific CTLs also recognize antigens expressed by metastatic lesions.

Here we show that DCs transfected with RNA isolated from renal cancer tissue are remarkably effective in stimulating tumor-specific T-cell response in vitro but do not cross-react with antigens from normal tissues including antigens expressed by renal parenchyma. Conversely, the tumor-specific CTLs lysed allogeneic tumor but not allogeneic nonmalignant targets, suggesting the presence of shared antigens among renal carcinomas. T-cell responses against TERT accounted in part for the responses against the allogeneic tumor targets because tumor RNA-transfected DCs reproducibly stimulated the formation of TERT-specific CTLs as a component of the polyclonal antitumor response. Although our studies suggest that distinct differences exist in the antigenic repertoire between the primary renal tumor and metastases, total tumor RNA-transfected DCs were capable of stimulating CTLs specific for the primary tumor as well as CTLs against metastatic targets, providing a scientific foundation for further clinical investigation of this approach.

MATERIALS AND METHODS

Tissue Procurement and Cellular RNA Generation. PBMCs, renal tumor, and nonmalignant control tissues were collected after obtaining informed consent from human subjects treated on protocols approved by the institutional review board. All primary tumor material was histologically classified as clear cell carcinoma by an experienced pathologist. Tissue samples were transported to the laboratory at room temperature in RNA preservation solution (RNAlater; Ambion, Austin, TX) and then processed for RNA generation. Control RNA was extracted from three histologically distinct, nonmalignant control tissues, which included benign RE, ureteral SM, and PBMCs. Autologous renal tumor cells were generated from short-term primary cultures. Tissues were minced with opposing scalpels, and the resulting tissue fragments were cultured in

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The abbreviations used are: RCC, renal cell carcinoma; DC, dendritic cell; TERT, telomerase reverse transcriptase; RE, renal epithelium; SM, smooth muscle; PBMC, peripheral blood mononuclear cell; nt, nucleotide(s); GFP, green fluorescent protein; IL, interleukin; LN, lymph node; BM, bone metastasis.
DCCs Transfected with Renal Tumor RNA Stimulate CTLs Capable of Killing Autologous Tumor Cells. We first determined whether DCs generated from renal cancer patients and transfected with autologous renal tumor RNA are capable of stimulating CTL responses against an uncharacterized repertoire of renal tumor antigens in vitro. DCs were cultured from leukopheresis-derived monocytic precursors in the presence of IL-4 and granulocyte macrophage colony-stimulating factor as described previously (8). DCs of the immature phenotype (CD3−, CD14+, CD16−/CD56−, CD19−, MHC I−, MHC II+, CD40−, CD86−, CD80−, and CD83−) were used as targets in CTL assays. Total cellular RNA was generated from the following sources: (a) RCC RNA-transfected DCCs as tumor vaccine.

RESULTS

DCs Transfected with Renal Tumor RNA Stimulate CTLs

Capable of Killing Autologous Tumor Cells. We first determined whether DCs generated from renal cancer patients and transfected with autologous renal tumor RNA are capable of stimulating CTL responses against an uncharacterized repertoire of renal tumor antigens in vitro. DCs were cultured from leukopheresis-derived monocytic precursors in the presence of IL-4 and granulocyte macrophage colony-stimulating factor as described previously (8). DCs of the immature phenotype (CD3−, CD14+, CD16−/CD56−, CD19−, MHC I−, MHC II+, CD40−, CD86−, CD80−, and CD83−) were used as targets in CTL assays. Total cellular RNA was generated from the following sources: (a) RCC RNA-transfected DCCs as tumor vaccine.

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These results are representative of two experiments. For stimulations, patient were stimulated twice with autologous DCs transfected with various autologous renal tumor RNA: RCC (patient A), SM (patient B), and PBMCs (patient C). DCs transfected with autologous RCC RNA, SM RNA, or PBMC RNA were used as targets. Using identical experimental conditions, tumor-specific T cells were reacted against their cognate targets (RCC RNA-transfected DCs), K562 cells, or naïve DCs (bottom right panel).

Fig. 2. Autologous DCs transfected with autologous tumor RNA stimulate CTLs that recognize antigens shared with other RCC patients. We next investigated whether autologous renal tumor RNA-transfected DCs are capable of stimulating CTL responses against antigens shared among other renal tumors as well as against autologous tumor antigens. In the context of vaccination protocols, antigens in the form of allogeneic renal tumor RNA may represent an attractive alternative because well-characterized and generic vaccines could be generated. As seen in Fig. 3, left panel, DCs transfected with autologous RCC RNA (patient B) were capable of eliciting strong CTL responses against their cognate, but not against PBMC RNA-transfected DC targets. Interestingly, these CTLs (patient B) were also effective in recognizing and lysing autologous DC targets transfected with RCC RNA isolated from another RCC patient (patient A), whereas DC targets transfected with PBMC RNA from patient A were not lysed. Similar observations were made in another set of experiments (Fig. 3, right panel) in which renal tumor-specific CTLs were stimulated using DCs transfected with RCC RNA isolated from patient C. These CTLs lysed cognate DC targets and autologous DCs transfected with allogeneic RCC RNA (patients A and B) but did not lyse DCs transfected with (allogeneic) PBMC RNA extracted from these patients.
These experiments show that under the experimental conditions used, tumor RNA-encoded allogeneic MHC molecules did not stimulate an allogeneic CTL response. This observation can be explained as follows: first, it has been shown that expression levels of histocompatibility antigens by primary RCC are low (13). Thus, the copy number of mRNA encoding for MHC antigens within the allogeneic tumor RNA pool is expected to be minimal, and only insufficient quantities of allogeneic MHC antigens may be expressed as proteins and transported to the DC cell surface, precluding effective presentation. Second, allogeneic MHC-derived peptides are competing for presentation with those from degraded self-MHC, which are present in high numbers in the DC cytoplasm, making effective presentation unlikely. Therefore, the lysis seen with the allogeneic RCC can be solely attributed to renal tumor antigens common between the patients analyzed and not to an allogeneic CTL response.

These experiments further suggest that these patients express, in addition to shared tumor antigens, patient-specific antigens unique to the parental tumor because the responses against the allogeneic targets (shared antigens only) were consistently less efficient than the responses against the autologous targets (patient specific and shared). At this point, it is unclear whether these antigens are shared among many or all RCC patients. The complexities inherent to human studies limited our observations to a small number of patients in whom these cross-reactivities between autologous RCC RNA vaccines and allogeneic RCC RNA-transfected DC targets were identified. On the other hand, similar observations were made by us in patients with prostate cancer in whom prostate tumor RNA-transfected DCs stimulated CTLs that were capable of recognizing tumor targets as well as (allogeneic) LNCaP prostate cancer cells.4

RNA-transfected DCs Stimulate Formation of TERT-specific CTLs. Unfortunately, unlike those seen in melanoma, attempts to identify and isolate widely expressed RCC-specific tumor antigens have not been very successful. However, it has recently been shown that the polypeptide component of telomerase (TERT) can be an attractive candidate for a broadly expressed rejection antigen for many cancer patients (14). Therefore, we sought to determine whether TERT-specific CTLs could account, at least in part, for the cross-reactivities observed among renal tumors. DCs were transfected with either renal tumor RNA derived from patient D or with TERT mRNA generated by in vitro transcription of the plasmid pGEM4Z/TERT/A64. Both DC preparations were used for CTL priming as well as targets in cytotoxicity assays. Fig. 4. left panel, shows that total renal tumor RNA-transfected DCs stimulated T-cell responses that recognized and lysed tumor targets, whereas DC targets transfected with control RNA species (PBMC RNA or GFP RNA) were not lysed. Renal tumor-specific CTLs were comparable to TERT-specific CTLs stimulated with TERT RNA-transfected DCs in recognizing and lysing TERT-expressing targets, indicating that the levels of TERT RNA in the total tumor RNA pool were sufficient to stimulate TERT-specific CTLs. These data also show that in addition to the characterized and single tumor antigen TERT, other as yet unidentified antigens are involved in this response. Most importantly, the tumor-specific CTLs were consistently superior to CTLs stimulated with TERT RNA-transfected DCs in recognizing and lysing tumor targets, suggesting that tumor-specific CTLs represent a polyclonal response providing more effective antitumor activity than T-cell responses directed against a single antigen in the form of TERT.

DCs Transfected with RNA Extracted from a Primary Renal Tumor Stimulate CTLs that Recognize Metastatic Lesions. Novel vaccine protocols should be judged not only by their activity against the primary tumor but also, and more importantly, by their activity against distant metastases, which frequently escape immunological recognition (15). However, the preclinical assessment of lytic CTL activity against primary or metastatic tumor targets is hampered by the requirement to simultaneously culture both primary or metastatic tumor cells from the same patient. As a potential solution to this obstacle, we used tumor RNA-transfected DCs to assess the efficacy of vaccination with (RNA-encoded) primary tumor antigens against metastatic targets. Autologous DCs were transfected with cellular RNA extracted from the following sources and used to stimulate CTLs from PBMCs: (a) primary renal tumor; (b) regional LN metastasis; and (c) BM. These RNA-transfected DCs were used for CTL priming and as cellular targets. Additional targets consisted of DCs transfected with GFP RNA, TERT RNA, or autologous PBMC RNA. As shown in Fig. 5A, RCC-transfected DCs stimulated CTLs that were not only effective in recognizing and lysing primary tumor targets (RCC-transfected DCs) but were also (albeit less effectively) effective in recognizing and lysing LN RNA-, BM RNA-, and TERT RNA-transfected DC targets, whereas control targets were not lysed. DCs transfected with LN RNA (Fig. 5B) stimulated CTLs that were equally effective in recognizing and lysing primary renal tumor or both metastatic tumor targets, whereas BM RNA-transfected DCs (Fig. 5C) lysed preferentially their cognate targets (BM RNA-transfected DCs) and were somewhat less effectively in lysing primary tumor, LN, and TERT targets. The likely interpretation of these observations is that, in fact, small, but distinct differences in the antigenic repertoire of primary renal tumors and distant metastases exist. Despite these differences, renal tumor RNA-transfected DCs are capable of stimulating CTL activities against antigens expressed by metastatic tumors as well as antigens present on primary tumors. Our findings also highlight the potential of this approach to generate potent anticancer vaccines from metastatic tumor tissue, if the primary tumor has been surgically removed, and metastatic tumor tissue represents the only source for vaccination, as is often the case in RCC.

DISCUSSION

In this study, we have shown that human renal tumor RNA-transfected DCs are capable of inducing potent CTL responses directed against a broad spectrum of unidentified primary and metastatic renal tumor antigens. The tumor-specific CTLs were capable of recognizing and lysing RNA-transfected DCs and primary cultured renal tumor cells with similar efficacy. Thus, tumor RNA-transfected DCs may not only represent a potent strategy for CTL priming but may...
tumor antigens expressed by nonrelated renal cancers, suggesting the presence of shared antigens among RCC. CTLs stimulated against the allogeneic tumor RNA-encoded MHC molecules apparently did not contribute to the recognition of these targets because autologous DCs transfected with allogeneic RE or PBMC RNA were not lysed under these experimental conditions. An important novel aspect of this study is the demonstration that the antitumor response stimulated by tumor RNA-transfected DCs encompasses a subcomponent a response against TERT, suggesting that telomerase, which is overexpressed in almost every renal cancer and particularly in renal metastases, may represent a potential candidate for antigen-specific immunotherapy for RCC patients. On the other hand, we show that the polyclonal responses stimulated by tumor RNA-transfected DCs are superior to the responses against the characterized and single antigen TERT in recognizing and lysing tumor targets. These polyclonal CTLs may not only be more potent but may also optimize protection against various subclones of the primary tumor that may appear as metastases during the course of disease.

Consistent with this expectation, we show that DCs transfected with RNA isolated from the primary renal tumor provide CTL activities not only against their cognate targets but also, and more importantly, against metastatic targets. The recognition of the metastatic tumor targets by the primary tumor-specific CTLs was somewhat less than that against primary RCC targets, suggesting that, in fact, minor but distinct differences in the antigenic repertoire between both tumor types exist. Consistent with these findings, CTLs stimulated by BM RNA-transfected DCs were more effective in recognizing and lysing their cognate metastatic targets than primary tumor targets, suggesting that an antigenic shift may occur during differentiation of primary tumors into the metastatic phenotype.

Our demonstrated ability to reproducibly stimulate tumor-specific CTLs in many RCC patients that cross-react against the antigens expressed by metastatic tumors may point to the conclusion that tumor RNA-transfected DCs precisely mirror the antigenic distribution expressed by individual tumors as well as account for their intrinsic heterogeneity with potential antigen shift or loss in some tumor cells. This feature may optimize vaccination against the many and unidentified antigens on renal tumor cells and thus may provide protection against primary and metastatic tumors in the tumor-bearing host.

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REFERENCES


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