B7-1 Expression by a Non-Antigen Presenting Cell-derived Tumor

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Abstract

The existence of a naturally occurring immunosurveillance against neoplastic cells is controversial. A difficulty with this concept is that tumor-specific antigen-reactive T cells would not be expected to become activated after encountering tumor cells, since T cells that bind to antigen in the absence of the costimulation provided by antigen-presenting cells may be inactivated. We studied a transgenic model of tumorigenesis where T cells reactive to a particular tumor-specific antigen are lost prior to the development of non-antigen-presenting cell-derived tumors; therefore, the tumors that develop are not subjected to immunosurveillance. We found that a tumor cell line derived from one such tumor expresses the T-cell costimulatory molecule B7-1, the expression of which is normally restricted to antigen-presenting cells. In addition, we found that several immortalized cell lines, which are nontumorigenic and thus have suffered only early genetic events in the tumorigenesis process, express B7. This suggests that a host cell can be induced to express surface B7-1 molecules after suffering an oncogenic insult, which might possibly be a primary mechanism of immunosurveillance against tumors.

Introduction

The hypothesis of immunosurveillance against tumor cells has existed for quite some time (1). This hypothesis suggests that the host is frequently exposed to oncogenic insults, resulting in the transformation of cells; however, many of these cells do not go on to develop into tumors because the host immune system identifies the transformed cells as nonself and rejects them. Indeed, it has been shown that there are TSA4 that are expressed mainly by malignant cells (2, 3). Also, T cells are present in the host that are capable of rejecting the malignant cells; it has been shown that an endogenous immune response to tumor cells can be induced by a variety of manipulations, such as transfecting tumor cells with the interleukin 2 gene (4) or with the gene for the T-cell costimulatory molecule B7-1 (5).

However, direct evidence supporting the existence of an effective immune surveillance process is limited. There is an increase in the incidence of certain malignancies in chronically immunosuppressed people who have undergone bone marrow transplantation, who have congenital immunodeficiency states, and who have AIDS. Many of these types of malignancies that occur have been associated with a viral etiology, suggesting that immunosurveillance to virally induced malignancies exists. However, it has also been suggested that since there is no increase in the incidence of many other common tumors in chronically immunosuppressed people, immunosurveillance to nonvirally-induced tumors may not occur. A similar conclusion has been made based on the observation of nude mice, which lack the development of a normal thymus. These mice are profoundly deficient in T cells and yet do not have an increased incidence of tumors (6). These arguments against the existence of immunosurveillance are indirect. It remains possible that immunosurveillance is intact in immunosuppressed people and nude mice. Immunosuppressed people maintain adequate immune function, for instance, against infectious diseases. Nude mice have natural killer cells and B cells and even produce a limited number of T cells (7). Furthermore, it is possible that immunosurveillance is an efficient process, composed of several back up systems, and is faced with dealing with only a very limited tumor cell challenge. Therefore, immunosurveillance could potentially remain functional in immunosuppressed people and nude mice.

We have been interested in studying immunosurveillance from the perspective of the tumor cell. Here we present evidence that non-APC-derived pancreatic acinar cells can be induced to express the T-cell costimulatory molecule B7-1 after malignant transformation. This may be a mechanism whereby TSA-reactive T cells can become activated, rather than anergic, after encountering a tumor cell.

Materials and Methods

Mouse. B10.BR mice were obtained from The Jackson Laboratory. The rat elastase I promoter/SV40 T antigen transgenic mouse line, 177-5 (8), and the RAG-2-deficient mouse line (9) have been described previously.

Cell Lines. The AT cell line was derived from a spontaneously arising pancreatic tumor from an elastase promoter/SV40 T antigen transgenic mouse that had been back crossed with B10.BR mice. The tumor was mechanically dispersed and placed in collagen I (Vitrogen 100)-coated plates in a reduced serum-containing medium described previously to minimize fibroblast contamination (10). After several weeks, a monomorphic population of cells emerged and grew progressively. The C3H10T/2 cell line was obtained from American Type Culture Collection. The BALB/MK (11) and M2/NEO (12) cell lines have been described previously.

Preparation of Normal Acinar Cells. Pancreatic acinar cells from nontransgenic mice were obtained by digesting 8 pancreases in 1.5 mg/ml collagenase I with shaking at 37°C for 20 min, followed by digestion three separate times with 0.75 mg/ml collagenase I with shaking at 37°C for 5 min.

FACS Analysis. The optimal titer of antibodies to be used for FACS analysis was determined for each antibody used. Cells (50,000) were incubated in V-bottomed 96-well plates with the CTLA-4-immunoglobulin fusion protein (13) or the biotin-labeled anti-murine B7/BB1 monoclonal antibody, 1G10 (PharMingen), at 4°C in PBS with 2% FCS and 0.1% sodium azide for 30 min. This was followed by staining under the same conditions with FITC-labeled goat anti-human IgG (Tago, Inc.) for the case of the CTLA-4-immunoglobulin fusion protein or streptavidin conjugated to PE (PharMingen) for the case of biotin-labeled antibody. Labeled cells (10,000) were then subjected to FACS analysis.

Tumorigenicity Assay. AT tumor cells cultured in vitro as monolayers were trypsinized to make a cell suspension. Cells (2 × 106) in a volume of 0.2 ml HBSS were injected s.c. in the flank of each of three individual mice for each experiment. Tumor size over time was estimated by palpation and measurement with calipers.

Reverse Transcription-PCR. Total RNA was extracted from monolayers of cultured AT tumor cells by the acid guanidinium thiocyanate-phenol method (14). cDNA was synthesized from 10 mg of total cellular RNA by reverse transcription with Moloney murine leukemia virus reverse transcriptase.
(GIBCO-BRL) using random hexamers as primers. SV40 T antigen-, B7-1-, elastase-, or γ-actin-specific cDNA was amplified in separate reactions using PCR with primers chosen from separate exons of each respective gene. The primers used are as follows: T antigen, sense 5'-CATGATCATTATAGTG-GCTGGGCTG-3' and antisense 5'-CTCTCAACCTGATTTGGAGG-3'; B7-1, sense 5'-ATGGCTTCAATTTGCA-3' and antisense 5'-CTAAAG- GAAGCGGTCT-3'; elastase, sense 5'-AATAACTATGCTCCAGGTTTG- GTTCCGC-3' and antisense 5'-CTGGCATCCAGCGGACTCCATTC-A-3'; and γ-actin, sense 5'-CCCAGAGTCTTACCCGGAGG-3' and antisense 5'-CAGACTGATCTTGCCTGCTC-3'. These primer combinations allowed us to distinguish by size the origin (DNA or spliced mRNA) of the PCR products. The PCR mixture contained 50 mM Tris (pH 9.0), 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 100 ng of each primer, and 1 unit Taq polymerase (Perkin-Elmer/Cetus). After an initial denaturation at 94°C for 5 min, amplification was carried out for 30 cycles at 94°C for 30 s, 55°C for 1 min, and 72°C for 1 min, followed by a final elongation step at 94°C for 2 min, 55°C for 2 min, and 72°C for 10 min. The PCR products were resolved on a 2% agarose gel and stained with ethidium bromide.

Results and Discussion

Part of the reason for a lack of direct evidence to support immunosurveillance is the difficulty in testing this concept. Spontaneously arising tumors that are capable of being eliminated by an immunosurveillance mechanism, by definition, do not grow progressively and, therefore, cannot be studied. To circumvent this problem, we took advantage of a transgenic mouse model of tumor development where thymic tolerance to a TSA causes the loss of T cells reactive to this antigen prior to the development of tumors (15). The TSA is the oncoprotein SV40 T antigen, and the gene coding for this protein was introduced as a transgene driven by the rat elastase promoter (8). Pancreatic acinar cell expression of this protein results in the development of pancreatic tumors that grow progressively. This allowed us to study tumor cells that may normally have been eliminated by immunosurveillance but grow progressively in this system because the mouse specifically lacks functional effector cells reactive to SV40 T antigen.

An additional difficulty with the immunosurveillance hypothesis is that TSA-derived peptides associated with MHC class I molecules on non-APC surfaces could be presented in tolerogenic fashion. T cells require costimulation in addition to signaling through the T-cell receptor for activation (16). This costimulation is achieved by the binding of CTLA-4 or CD28 molecules on a T-cell surface to B7 molecules on the surface of an APC (13). In contrast, if a T-cell receptor of a T cell binds to a peptide/MHC molecule complex in the absence of costimulation, the T cell is rendered unresponsive (17). Therefore, since B7 expression is restricted to APCs, TSA-specific T cells would not be expected to be activated after encountering non-APC-derived tumor cells. Therefore, the hypothesis that we examined in the SV40 T antigen transgenic mouse model is that the clearance of tumor cells by an immunosurveillance mechanism occurs because the tumor cells can be driven to express T-cell costimulatory molecules on their cell surfaces. This would cause TSA-reactive T cells to become activated, rather than anergic, when they encounter tumor cells.

To facilitate the study of the tumors that develop in the transgenic mice, the AT cell line was established from one of these tumors. This cell line expresses SV40 T antigen, as demonstrated by immunofluorescent staining (data not shown). These cells express the MHC class I molecule H-2 Kk and can functionally process and present T antigen to SV40 T antigen-reactive T cells (15). We found that the AT cell line grew progressively when transplanted onto mice that lack T cells, including RAG-2-deficient mice (Fig. 1) and nude mice (data not shown). When AT cells were transplanted onto immunocompetent mice, however, tumors grew for approximately 7 days and then regressed. Histological examination of tumors growing on immunocompetent mice revealed extensive necrosis and a mononuclear infiltrate. These data suggest that the AT tumor cell line is capable of eliciting a lymphocyte-mediated rejection.

This immune response appears to occur because the AT cells express surface T-cell costimulatory molecules. AT cells stained positively with the CTLA-4-immunoglobulin fusion protein (Fig. 2B), which binds to both the B7-1 and B7-2 molecules (18). The AT cells were also found to be B7-1 positive by staining with the anti-B7-1 monoclonal antibody, 1G10 (Fig. 2C). The AT cell line was found to be heterogeneous, composed of some cells which express high levels of B7-1.
of B7 and others which are B7 negative. To eliminate the possibility that the AT cell line was contaminated with professional APCs, the cell line was cloned by limiting dilution. Some individual clones were found to be B7 negative and others were B7 positive. Reverse transcription-PCR was performed on RNA extracted from a B7-positive clone using B7-1-specific primers, and as expected, B7-1-specific mRNA was found to be expressed (Fig. 3). This B7-positive clone was also found to express T antigen and elastase, demonstrating that it is derived from a pancreatic acinar cell. Normal pancreatic acinar cells, from which the AT cell line is derived, were, however, B7-1 negative, as shown by FACS analysis of cells from a collagenase-disrupted pancreas, after staining with the CTLA.4-immunoglobulin fusion protein (Fig. 2A). These data demonstrate that the neoplastic transformation of cells can result in the expression of B7-1 on the surface of cells that normally do not express this molecule. It was recently reported that B7 is expressed on some melanomas (19), supporting the possibility that this phenomenon may occur in a variety of different tumors.

SV40 T antigen expression is not likely to be the sole event in the AT cell line that results in B7 expression. NIH/3T3 cells transfected with the SV40 T antigen gene resulted in T-antigen expression but no expression of B7 (data not shown). Furthermore, it is known that tumorigenesis is a multistep process requiring several sequential genetic alterations. Therefore, some as yet undefined combination of genetic alterations in the tumorigenesis process is likely to be responsible for B7 expression in the AT cells. It would, in fact, be expected that transformed cells at a late stage in the tumorigenesis process may have attained the ability to down-regulate B7 in order to escape immunosurveillance. We and others (20) have demonstrated that several tumor cell lines, which are tumorigenic on immunocompetent mice, are B7 negative. Conversely, according to our hypothesis, it would be expected that transformed cells at an early stage of tumorigenesis would express B7. Since it has been suggested that immortalization is an early step in the tumorigenesis process, we looked for B7 expression by immortalized cell lines that are non-tumorigenic. We found that the murine tracheobronchial epithelial cell line M2/NEO, the murine skin epithelial cell line BALB/MK, and the murine embryo-derived fibroblast cell line C3H10T1/2 all express surface B7 molecules (Fig. 4). Not all immortalized cell lines express B7, however; for example, NIH/3T3 cells were found not to be B7 negative (data not shown).

In summary, we show that upon oncogenic transformation, B7-1 can be expressed by cells that do not normally express this molecule. We believe that this may be an important process in immunosurveillance. B7-1 expression can alter the cell surface, which is the context in which TSAs are presented to T cells, from an inherently tolerogenic environment to an activating environment. This would then permit the immune-mediated killing of the tumor cells and provides a mechanism by which immunosurveillance against tumors could occur.

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References


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