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Specific, major histocompatibility complex-unrestricted recognition of tumor-associated mucins by human cytotoxic T cells

(epithelial tumors/multivalent tumor antigen/cell-mediated immunity)

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ABSTRACT **We have previously reported the establishment of cytotoxic T-cell lines, from pancreatic cancer patients, by continuously stimulating tumor-draining lymph node cells with allogeneic pancreatic tumor cell lines. After the preliminary characterization of their phenotype and tumor specificity, detailed studies performed with one of the cell lines, W.D., show that it recognizes a specific antigen, a large and heavily glycosylated mucin molecule, expressed on pancreatic and breast tumors and tumor cell lines. Although this recognition appears major histocompatibility complex (MHC)-unrestricted, the antigen receptor used by the cytotoxic T cell is the α/β heterodimer, typically found on MHC-restricted T cells. The target antigen is atypical, however, in its ability to directly bind and activate the T cells in the absence of self MHC, presumably by abundant and regularly repeated antigenic epitopes. These findings are important because they demonstrate a specific T-cell response against a human tumor-associated antigen. In addition to pancreatic and breast tumors, various mucin molecules are known to be produced by other tumors of epithelial cell origin and could be expected to stimulate similar T-cell-mediated immune responses.**

Cytotoxic T cells (CTLs) reactive with autologous tumor have been found in the blood (1), in tumors (2), and in tumor-draining lymph nodes (3) of patients with various types of cancer. We were interested in studying cell-mediated immune responses to tumor-associated antigens in pancreatic adenocarcinoma patients. Because autologous tumor is generally not available from these patients, expansion of pancreatic tumor-reactive T cells, using the prototype mixed lymphocyte–autologous tumor cell culture, was not possible. However, many pancreatic tumor-associated antigens detected on tumor cells in tissues are also expressed on established pancreatic tumor cell lines (4). This suggested the possibility that allogeneic pancreatic tumor cell lines could be used, in place of autologous tumor, as stimulating antigen in the *in vitro* expansion of pancreatic-tumor-reactive T cells. Tumor-reactive CTLs that kill both autologous and allogeneic melanomas had been reported (5, 6). This approach proved justified and we reported (7, 8) the establishment of tumor-antigen-specific CTLs using allogeneic pancreatic tumor cell lines as stimulating antigen for lymph-node cells from pancreatic cancer patients. The most interesting characteristic of their function was their apparent tumor-cell specificity but lack of major histocompatibility complex (MHC) restriction. In this report, we examine the cell surface molecules involved in this CTL–tumor cell interaction, at the effector cell level, using a cell line W.D., and the target cell level, to better understand the means by which tumor-reactive CTLs may bypass conventional MHC restriction requirements in their recognition of tumor targets.

MATERIALS AND METHODS

Cell Lines and Culture Conditions. Tumor-draining lymph nodes were obtained from a patient with pancreatic adenocarcinoma and the mononuclear cells sedimented on a Ficoll/Hypaque discontinuous gradient. The cells were plated at 5 $\times$ 10^5 cells per ml in complete medium [RPMI medium containing 2 mM glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml)] supplemented with 10% (vol/vol) human serum and interleukin 2 (DuPont) at 5 units/ml. Irradiated (6000 rad; 1 rad = 0.01 Gy) allogeneic pancreatic tumor cells were added, as stimulating antigen, at the time of culture initiation and every 10th day thereafter. The tumor cell lines used were obtained from the following sources: CAPAN-1, CAPAN-2, BT-20, MCF-7, SKBR-3, LS180, LS174T, SW13, K562, and DU-145 were obtained from the American Type Culture Collection; SKMEL-14, DU-MEL-12, DUMEL-92, and DU-OS1 from H. Seigler (Duke University, Durham, NC); T3M4 from T. Okabe (Tokyo); COLO-357 from George Moore (Denver, CO); PT145-P1, QGP-1, PANC-1, RWP-1, and PANC-89 from H. Kalthoff (Hamburg, F.R.G.); CAMA-1, OVCA-420, OVCA-429, OVCA-432, OVCA-433 from R. Bast (Durham, NC); PC-1 from J. Ware (Durham, NC); MF and MT from S. Slovin (Philadelphia). The HPAF tumor cell line has been described (4). Tumor cell lines were cultured in complete medium supplemented with 10% fetal calf serum. The alloreactive T-cell lines, JBn1 and MH3, and the Epstein–Barr virus-transformed B-cell line, JR, were established in our laboratory and described (9).

Cytotoxic Assays, Cold Target Inhibition, and Antibody/Antigen Blocking Studies. Four-hour ^{51}Cr-release assays and calculation of percent specific release were performed as described (10). For cold target inhibition experiments, unlabeled target cells were mixed with labeled tumor target cells, at various unlabeled to labeled cell ratios, and the mixed cells were added to effector cells for a 4-hr cytotoxic assay. Antibody-blocking studies were performed by incubating either target cells or effector cells, as indicated, with monoclonal antibody for 45 min at 37°C (6) prior to their use in a cytotoxic assay. Blocking studies with pancreatic tumor mucin were performed by incubating effector cells with purified mucin for 45 min at 37°C prior to the addition of labeled target cells.

Antibodies and Immunofluorescence Analysis. Antibodies OKT3, OKT4, OKT8, and W6/32 were affinity-purified from

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Abbreviations: MHC, major histocompatibility complex; CTL, cytotoxic T cell; TCR, T-cell antigen receptor; E/T ratio, effector to target cell ratio.

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tissue culture supernatants of hybridomas obtained from the American Type Culture Collection, and DU-PAN-1 antibody was affinity-purified from tissue culture supernatant of a hybridoma described (4). Leu4, Leu7, Leu11, and Leu19 were obtained from Becton Dickinson; βF1 was from T Cell Sciences (Boston); SM3 was from J. Taylor (Imperial Cancer Research Fund, London); and BC1 and BC3 were from I. McKenzie (University of Melbourne, Melbourne, Australia). Immunofluorescence staining and analysis was performed as described (10).

^{125}I-Labeling, Radioimmunoprecipitation, and SDS/PAGE Analysis. ^{125}I-labeling of cells, chemical cross-linking with dithiobis(succinimidyl propionate) (DSP), and solubilization were performed as described (11). Immunoprecipitation was performed using either a control antibody (DU-PAN-1), anti-CD3 antibody (Leu4), or anti-α/β T-cell receptor antibody (βF1). The antigen–antibody complexes were brought down with protein A-Sepharose CL-4B. SDS/PAGE analysis was performed under reducing conditions as described by Laemmli (12).

Proliferation Assay. Proliferation assays were performed by incubating 10^5 responder cells with either 10^4 irradiated stimulator cells or purified pancreatic tumor mucin at a concentration of 1900 DU-PAN-2 units/ml (13) for 48 hr at 37°C, in complete medium supplemented with 10% (vol/vol) human serum but not interleukin 2. The cells were then pulse-labeled with 1 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine for 16 hr at 37°C and harvested using a Skatron cell harvester.

Northern Blot Analysis. Total RNA extraction, gel electrophoresis, and blotting were performed as described (14). Northern blots were hybridized to a 60-base-pair ^{32}P-end-labeled oligonucleotide probe synthesized according to the cloned breast mucin gene tandem repeat sequence (15, 16).

RESULTS

A number of cell lines have been established from lymph nodes of patients with pancreatic adenocarcinoma and their phenotype and function were assayed at various time points in culture. The predominant phenotype of cells, after only 1 month in culture with interleukin 2 and an allogeneic pancreatic tumor cell line as antigen, is in all cases CD3$^+$ CD8$^+$ CD4$^-$ Leu7$^-$ Leu11$^-$ Leu19$^-$, and the predominant activity is a pancreatic-tumor-directed MHC-unrestricted cytotoxicity (7, 8). Extensive analysis of the phenotype and function of a number of these cell lines and clones derived from them will be the subject of another report (Z. Wahab, personal communication). In this report one cell line, W.D., was extensively analyzed to determine the target antigen and the effector cell molecules involved in this tumor-antigen-specific but MHC-unrestricted lysis. Although clonal analysis of W.D. cells was not possible, as repeated attempts to clone the cell line by limiting dilution were unsuccessful, Southern blot analysis examining T-cell antigen receptor (TCR) γ gene rearrangements in W.D. cell line DNA strongly suggests that the line contains a single predominant clone (data not shown).

The Effector Cells (CTLs). The specificity of W.D. cells was tested using as targets a panel of tumor cell lines derived from a large number of different tissue sites. Interestingly, in addition to 8 of 10 pancreatic tumor cell lines lysed by W.D., 4 of 4 breast tumor cell lines were also lysed (Table 1). The reactivity of W.D. appeared to be limited almost exclusively to pancreatic and breast tumor lines, as only 1 of the 16 other tumor cell lines chosen from a wide variety of other tissue sites was lysed to any appreciable extent, ovarian carcinoma OVCA-420 (Table 1). The specificity of lysis remained restricted to these same tumor targets at effector to target (E/T) ratios as high as 40:1 (data not shown).

Table 1. Specific tumor target lysis by W.D. cells

Tumor	Cell line	% specific lysis*
Pancreatic adenocarcinoma	QGP-1	100
	PT45-P1	100
	PANC-89	98
	CAPAN-1	80
	CAPAN-2	71
	T3M4	57
	HPAF	23
	COLO-357	20
	PANC-1	5
	RWP-1	1
Breast carcinoma	SKBR-3	57
	MCF-7	42
	BT-20	25
	CAMA-1	22
Ovarian carcinoma	OVCA-420	21
	OVCA-429	3
	OVCA-432	0
	OVCA-433	5
Colon carcinoma	LS-180	0
	LS-174T	0
Adrenocortical carcinoma	SW-13	4
Melanoma	DUMEL-12	3
	DUMEL-92	7
	SKMEL-14	3
Myelogenous leukemia	K562	3
Osteosarcoma	DUOS-1	6
Prostate carcinoma	DU-145	1
	PC-1	0
Malignant fibrohistiocytoma	MF	0
Liposarcoma	MT	0

W.D. cells were cultured with CAPAN-2 cells as stimulating antigen and assayed after 1 month in culture at an E/T ratio of 10:1.
*Standard deviation of specific release values among triplicate samples was <5% in all cases.

Cold target inhibition experiments were performed to examine whether the epitope recognized on the breast tumor targets and the ovarian tumor target OVCA-420 was indeed the same as that recognized on the pancreatic tumor targets. At a target to inhibitor ratio of 1:5, all breast tumor cell lines tested inhibited W.D.-mediated lysis of the pancreatic tumor target PANC-89 by at least 50%, similar in magnitude to the effect of adding PANC-89 itself or the pancreatic tumor cell line CAPAN-2 as cold targets. At a target to inhibitor ratio of 1:10, the ovarian tumor cell line lysed by W.D. (OVCA-420) inhibited lysis of PANC-89 by 27%, whereas tumor cell lines which were not lysed by W.D. cells (OVCA-432, K562) inhibited lysis of PANC-89 by only 6% (Fig. 1).

The fact that W.D. cells kill in an antigen-specific, yet apparently non-MHC-restricted manner raised questions regarding the involvement of the TCR in allogeneic tumor recognition and lysis. In addition, the role of monomorphic MHC determinants on the target cell and of T-cell accessory molecule, CD8, which is expressed on these CTLs, needed to be examined. Monoclonal antibodies directed against the TCR-associated CD3 complex (OKT3 Ab), the CD8 molecule (OKT8 Ab), and monomorphic MHC class I determinants (W6/32 Ab) were tested for their ability to inhibit lysis of a pancreatic tumor target CAPAN-2 by W.D. cells. Antibodies to molecules not expressed on the CTL, CD4 (OKT4 Ab) and DU-PAN-1, were used as controls. As is shown in Table 2, preincubation of W.D. cells with OKT3 antibody greatly inhibited their function (up to 72%). Antibody OKT8 had a less dramatic but significant effect (35% inhibition), which equaled the effect of preincubation of the target cell with W6/32 antibody (Table 2). In contrast, the effect of OKT4

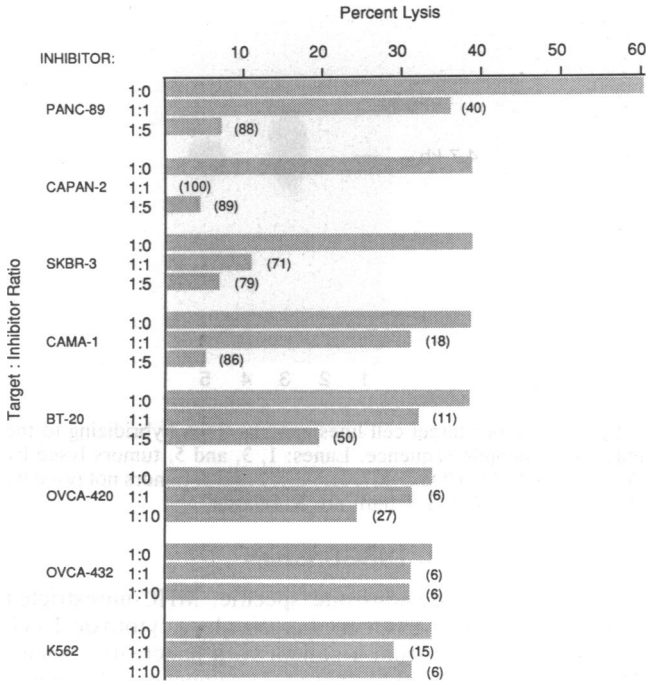

FIG. 1. Competitive inhibition by unlabeled tumor cell lines of W.D.-mediated lysis of ^{51}Cr-labeled pancreatic tumor target cells. Percent inhibition of lysis at each target (labeled) to inhibitor (unlabeled) ratio is indicated in parentheses. The ratio of effector (W.D.) cells to target (labeled PANC-89) cells used was (*i*) 5:1 when unlabeled PANC-89 itself was used as an inhibitor and (*ii*) 3:1 when unlabeled CAPAN-2, SKBR-3, CAMA-1, BT-20, OVCA-420, OVCA-432, and K562 were used as inhibitors.

antibody (18% inhibition) was only slightly higher than that of a control antibody, DU-PAN-1 (9% inhibition).

The inhibitory effect of OKT3 antibody suggested that the lytic activity exhibited by W.D. cells involves the CD3-associated antigen-specific TCR. We were, therefore, interested in identifying the TCR components expressed by these CTLs. After chemical cross-linking of ^{125}I-labeled W.D. cell surface proteins and immunoprecipitation with Leu4 antibody, the CD3-associated polypeptides were identified by SDS/PAGE analysis as TCR α and β chains (Fig. 2, lane 2). The same two chains were brought down when βF1 was used as the precipitating antibody (Fig. 2, lane 3). No reactivity with W.D. cells was observed with antibodies directed against either TCR γ or δ chains by radioimmunoprecipitation or immunofluorescence analysis (data not shown).

The Target Antigen. A number of tumor-associated antigens detected by monoclonal antibodies raised against pancreatic and breast tumors have been identified as mucins (for review, see refs. 17 and 18). We examined the possibility that

Table 2. Specific blocking of W.D.-mediated lysis of CAPAN-2 cells with monoclonal antibodies

E/T ratio	Antibody	% lysis	% inhibition
5:1	—	69	0
	OKT3	19	72
	OKT8	45	35
	OKT4	57	18
	DU-PAN-1	63	9
3:1	—	46	0
	W6/32	31	33

W.D. cells were cultured with T3M4 cells as stimulating antigen and assayed after 1 month in culture at an E/T ratio of 5:1 or 3:1. Results shown are from a single experiment but are representative of results obtained in three experiments. Antibody was used at 5 μg/ml.

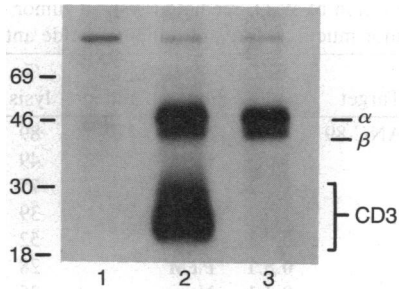

FIG. 2. Immunoprecipitation of TCR α and β polypeptide chains from W.D. cells. The cells were labeled with ^{125}I and cross-linked with dithiobis(succinimidyl propionate) (DSP), and immunoprecipitates were analyzed under reducing conditions by SDS/PAGE. Lanes: 1, control monoclonal antibody (DU-PAN-1); 2, Leu4 monoclonal antibody; 3, βF1 monoclonal antibody. Molecular masses in kDa are indicated.

mucins, being very large, highly glycosylated molecules with multiple antigenic epitopes for monoclonal antibody binding (15), might be capable of directly stimulating W.D. cells, which would help explain the MHC-unrestricted nature of their recognition. Pancreatic tumor mucins were purified as described (19) and compared to whole pancreatic tumor cells in their ability to stimulate proliferation of W.D. cells and specifically block their lysis. Several anti-tumor mucin antibodies, selected for their reactivity with the mucin polypeptide core rather than the carbohydrate determinants, were also tested for their ability to block W.D.-mediated lysis of tumor targets.

As shown in Table 3, purified pancreatic tumor mucins were found to directly stimulate W.D. cell proliferation in the absence of antigen-presenting cells but had no stimulatory effect on an alloreactive T-cell line, JBn1, established from a liver transplant patient and specific for the donor HLA antigens. The mucin-induced proliferation was comparable to that stimulated with intact tumor cells.

In addition to having a proliferative effect, purified pancreatic tumor mucins could also inhibit W.D.-mediated lysis of the pancreatic tumor target PANC-89 by about 50% at each E/T ratio tested but had no such effect on the lytic activity of another control alloreactive CTL, MH3, against its specific target JR (Table 4). Blocking experiments are difficult to perform with an antigen that, at the same time, can directly stimulate the CTLs. At lower E/T ratios the observed effect is thus a net result of a concurrent stimulatory and blocking

Table 3. Antigen-specific proliferative response of W.D. cells

Responder	Stimulator	Proliferative index
W.D.	—	1.0
	CAPAN-2	5.2
	T3M4	4.7
	HPAF	3.1
	K562	0.9
	PTM	3.5
JBn1	—	1.0
	CAPAN-2	0.7
	T3M4	0.6
	HPAF	0.7
	K562	0.6
	PTM	1.0

W.D. cells were cultured with PANC-89 cells as stimulating antigen and assayed after 1 month in culture. Pancreatic tumor mucin (PTM) was used at 1900 DU-PAN-2 units/ml. JBn1 cells are allospecific and were cultured with alloantigen-expressing B cells as stimulating antigen and assayed after 1 month in culture.

Table 4. Inhibition of W.D.-mediated lysis of tumor cells by pancreatic tumor mucin and an anti-mucin peptide antibody

Effector	Target	E/T ratio	Antibody or antigen added	% lysis	% inhibition
W.D.	PANC-89	3.0:1	None	89	—
		3.0:1	PTM	49	45
		1.5:1	None	75	—
		1.5:1	PTM	39	48
		0.8:1	None	52	—
		0.8:1	PTM	28	46
		0.4:1	None	35	—
		0.4:1	PTM	19	46
MH3	JR	3.0:1	None	88	—
		3.0:1	PTM	86	2
		1.5:1	None	74	—
		1.5:1	PTM	72	3
		0.8:1	None	60	—
		0.8:1	PTM	59	2
		0.4:1	None	42	—
		0.4:1	PTM	37	12
W.D.	PANC-89	10:1	None	66	—
		10:1	SM3 antibody	49	26
		1:1	None	22	—
		1:1	SM3 antibody	11	50
W.D.	MCF-7	10:1	None	47	—
		10:1	SM3 antibody	21	55
		1:1	None	19	—
		1:1	SM3 antibody	10	47

Results shown are from a single experiment but are representative of results obtained in three experiments. Pancreatic tumor mucin (PTM) was used at 1900 DU-PAN-2 units/ml. SM3 antibody was used at 20 μg/ml.

activity, and the expected increase in blocking with the decrease in effector cell numbers is seldom seen.

Murine anti-mucin monoclonal antibodies were obtained from various sources and each was tested for its ability to inhibit W.D.-mediated lysis. One antibody, SM3, reported to recognize an epitope present within the first 7 amino acids of the 20 amino acid core peptide of breast tumor mucin molecules (20), effectively inhibited W.D.-mediated lysis of both pancreatic and breast tumor cell lines (Table 4). Other monoclonal antibodies tested, including one reactive with a pancreatic mucin (DU-PAN-2) (19) and one directed to a different epitope present within the last 10 amino acids of the breast tumor mucin core peptide (BC3) (21), had no such inhibitory effect (data not shown).

Having evidence to suggest that the epitope recognized by W.D. cells may be in the same region of the peptide seen by the SM3 antibody, we compared pancreatic tumor lines lysed by W.D. cells with those that are not lysed, for SM3 epitope expression. Immunocytochemical staining of pancreatic and breast tumor cell lines with SM3 antibody revealed that four of four pancreatic tumor lines lysed by W.D. (CAPAN-1, T3M4, QGP-1, and PANC-89) and four of four breast tumor cell lines lysed by W.D. (SKBR-3, MCF-7, BT-20, and CAMA-1) express the SM3 epitope, whereas two of two pancreatic tumor lines not lysed by W.D. (RWP-1 and PANC-1) fail to express the SM3 epitope (data not shown). In addition, Northern blot analysis indicated that three tumor cell lines lysed by W.D. (pancreatic tumor cell lines COLO-357 and HPAF, and the breast tumor cell line BT-20) express an RNA transcript hybridizing with an oligonucleotide probe comprising a 60-base-pair tandem repeat sequence of the cloned breast mucin gene (15, 16) (Fig. 3). In contrast, two tumor cell lines not lysed by W.D. (the pancreatic tumor cell line PANC-1, and the colon tumor cell line LS180) both fail to express such a transcript.

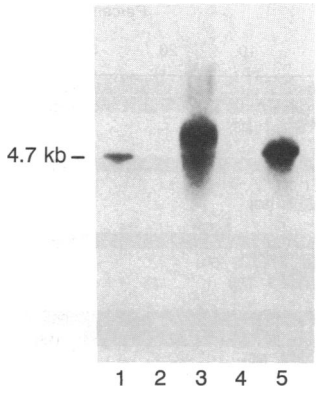

FIG. 3. Tumor target cell lines express RNA hybridizing to the mucin core peptide sequence. Lanes: 1, 3, and 5, tumors lysed by W.D. cells (BT-20, COLO-357, HPAF); 2 and 4, tumors not lysed by W.D. cells (PANC-1, LS-180). kb, Kilobases.

DISCUSSION

In this report we demonstrate specific, MHC-unrestricted recognition of tumor-associated mucins by a cytotoxic T-cell line, W.D., derived from a patient with pancreatic cancer. The epitope recognized by these CTLs appears to be shared by breast and pancreatic tumor target cells. Experiments that demonstrated a significant inhibitory effect of anti-CD3 antibody on CTL function strongly suggested that the CD3-associated TCR mediates recognition and lysis by W.D. cells, and specific immunoprecipitation experiments identified the receptor as an α/β heterodimer. Although the α/β TCR has generally been thought to recognize nominal antigen only in association with self MHC, a phenomenon known as MHC-restricted recognition (22), exceptions to this general rule of T-cell recognition have been reported. In particular, T cells reactive with a variety of tumor cell types appear to utilize the CD3-associated α/β TCR for recognition and lysis of both autologous and allogeneic tumor cell targets (23, 24). In addition, it has been demonstrated that classical α/β TCR-bearing T-cell clones can directly bind and be activated by nominal antigen in the absence of self MHC molecules, if the nominal antigen is present in highly multivalent form (25).

Tumor-associated mucin was selected as a candidate target antigen for MHC-unrestricted recognition by W.D. cells based on its highly multivalent nature and on its expression on tumor cell types lysed by W.D. The antigenic multivalency of mucin molecules observed with monoclonal antibodies has been supported by cloning and sequencing of breast mucin cDNA. The cDNA clones were found to contain a 60-base-pair highly conserved, tandemly repeated sequence with each repeat containing a number of immunogenic epitopes (15, 16). A correlation was indeed found to exist between mucin gene (and mucin epitope) expression by a tumor cell and its susceptibility to recognition and lysis by W.D. cells. Pancreatic tumor mucin was found to have a stimulatory effect on the CTL proliferation as well as an inhibitory effect on CTL-mediated lysis, as would be expected for a molecule specifically bound by the antigen receptor. In addition, SM3, a monoclonal antibody reported to react with an epitope on the mucin protein core, blocked W.D.-mediated recognition and lysis of both pancreatic and breast tumor targets. Because the epitope recognized by SM3 resides within the amino acid sequence tandemly repeated in tumor-associated mucin, T-cell recognition of these epitopes is thought to result in TCR cross-linking and T-cell activation, in the absence of antigen processing and presentation in the context of self MHC molecules.

Although blocking effects of W6/32 antibody indicate that MHC class I molecules on the target cell participate to some

degree in the W.D.–tumor cell interaction, comparable blocking effects with anti-CD8 antibody suggest that this participation may involve accessory interactions of CD8 molecules with monomorphic determinants on class I MHC molecules. With regard to the specific T-cell interaction with its target antigen, however, it appears that TCR recognition of polymorphic self MHC determinants is not required for multivalent antigen recognition, as is the case for specific recognition of univalent antigens.

MHC-restricted recognition has often been suggested as a mechanism by which CTLs are "directed" to target cell surfaces. It is interesting to speculate that tumor cell production of high levels of circulating mucin, such as is known to occur in pancreatic and breast cancer (21, 26), may help tumor cells to escape immune destruction by engaging tumor-specific T-cell clones in the periphery with the soluble antigen.

Our observation that a patient can mount an immune response against tumor-associated mucins brings up a number of important questions. Is the tumor-associated mucin seen by these CTLs different from mucin produced by normal pancreatic or breast ductal epithelial cells, and, if it isn't, why would the immune system view it as foreign? There are a number of monoclonal antibodies, including the SM3 antibody, that are more strongly reactive with tumors than with normal tissue, suggesting the appearance of new tumor-associated epitopes (27). On the other hand, it may be possible that mucins are normally not accessible to the immune system as they are secreted directly into ducts. Malignant transformation of the mucin-producing cells releases high levels of these molecules into the circulation, which then act as endogenous antigens.

In either case it might be possible to manipulate this immune response or reduce circulating tumor mucins to effect the elimination of the tumor or of the metastases after the primary tumor is removed. This however calls for better insight into post-operative treatments that are now based on aggressive chemotherapy and tend to completely destroy an already established immune response.

Note Added in Proof. Since the submission of this manuscript the cloning and sequencing of human colon mucin cDNA was reported (28). The tandemly repeated peptide is 23 amino acids long and its sequence (Pro-Thr-Thr-Thr-Pro-Ile-Thr-Thr-Thr-Thr-Thr-Val-Thr-Pro-Thr-Pro-Thr-Pro-Thr-Gly-Thr-Glu-Thr) differs entirely from the sequence of the breast mucin repeat (Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala-His-Gly-Val-Thr-Ser-Ala). This difference is reflected in the lack of recognition of colon mucins by W.D. cells.

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