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Age-dependent tolerance to an endogenous tumor-associated antigen

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Summary Immunologic tolerance to endogenous antigens reduces antitumor responses. Gp70 is an endogenous tumor-associated antigen (TAA) of the BALB/c-derived colon carcinoma CT26. We found that expression of *gp70* mRNA is detectable in tissues of mice 8 months of age and older. We showed that expression of gp70 establishes immunologic tolerance and affects anti-tumor immunity in a similarly age-dependent manner using gp70-deficient mice. We found that tumors grew in all gp70-sufficient mice, while approximately half of gp70-deficient mice controlled tumor growth with endogenous T-cell responses. Protection in gp70-deficient mice correlated with more robust gp70-specific CTL responses, and increased numbers and avidity of responding antigen-specific T cells after vaccination. We conclude that immunosurveillance may decline with age due to increased or de novo peripheral expression of endogenous TAAs.

Introduction

Many factors decrease immunosurveillance of tumors by cytotoxic T lymphocytes (CTL) impairing effective antitumor responses [reviews [1–4]]. Tumor-associated macrophages, myeloid-derived suppressor cells, and regulatory T cells reduce effector functions of tumor-specific CTL. In addition, tumor cells secrete inhibitory cytokines and produce other factors associated with chronic inflammation that reduce antitumor responses. Finally, most tumor-associated antigens (TAAs) are derived from non-mutated self proteins, resulting in deletion of CTL with high avidity for TAAs. Thus, the T cells that may be most effective against tumors are deleted during negative selection in the thymus [5–7].

Experiments using transgenic mice expressing T-cell receptor (TCR) genes and transfected TAAs have elucidated many of the obstacles that prevent the development and function of tumor-reactive T cells. The influence of antigen-specific tolerance varies depending on the number and source of T cells [8,9]. Examination of transferred cognate

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transgenic T cells into tumor-bearing hosts has determined the conditions for optimal effector function and may have general clinical applications [reviewed in Ref. [10]], but the function of a diverse T-cell repertoire differs from that of a T-cell clone [11]. In polyclonal systems, T-cell function varies with the diversity of the repertoire, the avidity for antigen-presenting cells, and the precursor population size [12–14]. Thus, it is not clear that the rules established from experiments using monoclonal T-cell systems with high affinity TCRs are applicable to polyclonal T-cell repertoires. The role of the antigen in controlling the T-cell response to tumors is also unclear. Many studies use transplantable tumors transfected with model antigens so that either transferred T cells or endogenous responses can be monitored [15,16]. Although the endogenous T-cell responses to TAAs are typically weak and less amenable to experimentation [17] tumor model systems that employ the endogenous T-cell repertoire and natural TAAs may provide more relevant results to guide the design of tumor immunotherapies.

T-cell responses to TAAs encoded by endogenous retroviruses have been detected in both humans and mice [18,19]. Gamma-, or Type C, retroviruses are the most common retroviral elements in the human genome [20] and several examples have also been identified in mouse tumors [21]. In one example, the *gag* gene from Friend leukemia virus encodes an H-2D^d-restricted CTL target on the leukemia cell line, FBL-3 [22]. The ecotropic endogenous Murine leukemia virus (MuLV) also encodes TAAs from both gp70 (SU) and p15E (TM) proteins of the *env* gene. Both I-A^b and I-E^b-restricted peptides from MuLV have been identified in LB27.4 cells, a hybridoma of A20 lymphoma cells (H-2^d) and BW5147 cells (H-2^b) [23]. A CTL epitope p15E$_{604\text{-}611}$/H-2K^b was also identified from these cells [24] as well as from B16 tumor cells [25]. In CT26 tumor cells, a unique glycoslylated I-E^d-restricted epitope contributes to the CD4+ antitumor response [26], and CD8+ T cells respond to the dominant antigen gp70$_{423-431}$/H-2L^d (the AH1 antigen) [18]. *Gp70* mRNA and T-cell responses to gp70-derived antigens are also detectable in other murine tumor cells and models including the 4T1 mammary carcinoma [27], A20 lymphoma [28], and B16 and S91 melanoma cells [18,29]. These findings suggest that the shared gp70 antigen is a bona fide TAA, since these epitopes are derived from a non-mutated self-antigen that is up-regulated during the transformation process. Thus, gp70 is an ideal antigen for the study of the importance of self-tolerance in antitumor immune responses directed against TAAs.

To determine the role of gp70 expression in the T-cell response to the CT26 transplantable tumor, we produced a gp70-deficient mouse. In young mice under 6 months of age, we found that gp70-deficient mice elicited more gp70-specific T cells that exhibited greater binding avidity than those elicited in gp70-sufficient mice. These T-cell responses were associated with prevention of tumor growth in about half of the gp70-deficient mice. These results suggest that, in spite of the suppressive factors and heterogeneity of tumors that influence the quality of antitumor responses, successful antitumor CTL responses can be elicited in the absence of endogenous TAA expression in normal tissues. Furthermore, vaccination of gp70-sufficient mice over 8 months of age did not produce detectable TAA-specific T cells, although responses to a foreign antigen were readily detected. We also detected *gp70* mRNA expression in normal tissues of gp70-sufficient mice, particularly in mice older than 8 months. We propose that the age-related increase in expression of gp70 and subsequent T-cell tolerance occur with other TAAs and cognate T cells. These results may explain the variable results observed in the clinic with immunotherapy directed against some TAAs and may suggest criteria for selection of some TAAs over others for more successful immunotherapy.

Materials and methods

Generation of BALB.B6 env−/− (gp70−/−, gp70-deficient) congenic mice and littermate controls (gp70+/+, gp70-sufficient)

All animal experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of National Jewish Medical and Research Center. CB6F1/Cr [(BALB/cAnNCr × C57BL/6NCr)$_{F1}$] male and BALB/cAnNCr female mice were purchased from the NCI-Frederick Animal Production Program, bred together, and the offspring were screened by PCR for heterozygosity surrounding the MuLV integration containing a functional gp70 gene at 28.71 Mb of Chr 5 in BALB/c mice using D5MIT387 and D5MIT148 (Chr 5, 28.7 and 32.3 Mb, respectively; NCBI, build m36). Mice were screened using 139 primer sets (Table 1) that flank simple sequence length polymorphisms (SSLPs) distinguishing the two parental strains. Most of the congenic mice used in these experiments were backcrossed five generations with the details of the breeding/selection scheme presented in Table 1. The mice used in Fig. 3a were backcrossed three generations and Fig. 5a were backcrossed 16 generations to BALB/c; after the fifth backcross, only D5MIT387 and D5MIT148 were used in the screening. We define young mice as 6 months of age and under, and middle-aged mice as 8–12 months of age.

PCR screening of genomic DNA

Genomic DNA from tail tissue was extracted after 16 h at 55 °C in lysis buffer (500 mM KCl, 100 mM Tris pH 8.3, 15 mM $MgCl_2$, 4.5% NP-40, 4.5% Tween 20) and 0.2 mg/ml proteinase K. The resulting solution was cleared by centrifugation, and then extracted with equal volumes of phenol, phenol/chloroform, and chloroform. The DNA was ethanol precipitated and then dissolved overnight in water. The DNA concentration was adjusted to 125 ng/μl.

For the genome-wide screen of the N1 generation, PCR reactions were set up using a Multiprobe II Robotic Handling System (Parkard Bioscience Co.). Ten microliters PCR reactions included PCR buffer, 5 nM each fluorescent primer (labeled with Fam, Hex, or Tet), 100 nM each nucleotide, 1 mM $MgCl_2$, 0.5 units Taq, and 20 ng DNA. Reactions were performed in a Hybrid TouchDown Thermal Cycler [94 °C 1.5 min (first cycle only), 30 cycles of 94 °C for 30 s, 55 °C for 1 min, and 72 °C for 1 min]. Pools of up to eight loci distinguished by fluorescent tags and fragment sizes were combined and resolved by automated sequencers at the University of Maine sequencing facility. Samples