Coupling and Uncoupling of Tumor Immunity and Autoimmunity

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Summary

Self-antigens, in the form of differentiation antigens, are commonly recognized by the immune system on melanoma and other cancers. We have shown previously that active immunization of mice against the melanocyte differentiation antigen, a tyrosinase-related protein (TRP) gp75\(^{\text{TRP-1}}\) (the brown locus protein) expressed by melanomas, could induce tumor immunity and autoimmunity manifested as depigmentation. In this system, tumor immunity and autoimmunity were mediated by autoantibodies. Here, we characterize immunity against another tyrosinase family glycoprotein TRP-2 (the slaty locus protein), using the same mouse model and method of immunization. As observed previously for gp75\(^{\text{TRP-1}}\), immunity was induced by DNA immunization against a xenogeneic form of TRP-2, but not against the syngeneic gene, and depended on CD4\(^+\) cells. Immunization against TRP-2 induced autoantibodies and autoreactive cytotoxic T cells. In contrast to immunization against gp75\(^{\text{TRP-1}}\), both tumor immunity and autoimmunity required CD8\(^+\) T cells, but not antibodies. Only autoimmunity required perforin, whereas tumor immunity proceeded in the absence of perforin. Thus, immunity induced against two closely related autoantigens that are highly conserved throughout vertebrate evolution involved qualitatively different mechanisms, i.e., antibody versus CD8\(^+\) T cell. However, both pathways led to tumor immunity and identical phenotypic manifestations of autoimmunity.

Key words: melanoma • melanocyte • tyrosinase-related protein • T cell • perforin
Materials and Methods

Mice. C57BL/6 mice (6-8-wk-old females) were acquired through the National Cancer Institute breeding program. Homozygotic mice genetically deficient for β2-microglobulin (β2m−/−), MHC II (Abb−/−), and perforin (B6.pfp−/−), all in a C57BL/6 background, were obtained from Taconic Farms, Inc. Immunoglobulin μ chain (μ MT−/−) and gld/gld (B6.gld/gld) mice were acquired from The Jackson Laboratory. These mice were bred and kept in a pathogen-free Memorial Sloan-Kettering Cancer Center vivarium according to National Institutes of Health Animal Care guidelines. All mice entered the study between 7 and 10 weeks of age.

Cell Lines and Tissue Culture. B16F10/LM3 (15) is a pigmented mouse melanoma cell line of C57BL/6 origin, derived from the B16F10 line, provided by Dr. Isaiah Fidler (M.D. Anderson Cancer Center, Houston, TX). The EL-4 cell line was derived from a C57BL/6 mouse lymphoma, and SK-MEL-188 is a human melanoma cell line. Tumor cell lines were cultured as described (15).

Plasmid Constructs. The human TRP-2 (hTRP-2) and mouse TRP-2 (mTRP-2) expression vectors (supplied by Drs. S.A. Rosenberg and J.C. Yang, National Cancer Institute, Bethesda, MD) were previously described (9). These genes were cloned into the PCR3 vector, which was used as a control vector without inserts. The mouse GM-CSF gene, provided by Powderject Vaccines Inc., was cloned into the WRGxEN vector (13).

DNA Immunization. The method of DNA immunization has been reported (13). In brief, plasmid DNA encoding hTRP-2, mTRP-2, or GM-CSF was coated onto 1.0 µm gold bullets. Animals were immunized by delivering gold–DNA complexes using a helium-driven gun (Accell GmbH). Animals were immunized by delivering gold–DNA complexes using a helium-driven gun (Accell GmbH). Animals were immunized by delivering gold–DNA complexes using a helium-driven gun (Accell GmbH). Animals were immunized by delivering gold–DNA complexes using a helium-driven gun (Accell GmbH).

All mice entered the study between 7 and 10 weeks of age. Animals were injected intraperitoneally with hTRP-2 or mTRP-2, or were untreated. Significant protection was observed with hTRP-2 compared with no treatment (P < 0.0001). (C) Mice were immunized three times at weekly intervals with hTRP-2, or mTRP-2, or were untreated. Significant protection was observed with hTRP-2 compared with no treatment (P = 0.001) or with mTRP-2 (P = 0.001). The difference between mice treated with mTRP-2 and untreated mice was not significant (P = 0.16). (D) Immunization with hTRP-2 DNA started 4 d after tumor challenge, or mice remained untreated. A significant therapeutic effect was observed (P < 0.001). (E) Immunization with hTRP-2 plus GM-CSF DNA started 10 d after tumor challenge. Significant therapeutic effect was observed compared with other treatment and control groups (P < 0.01). GM-CSF treatment alone yielded 692 ± 69 metastases, mTRP-2 gave 902 ± 65 metastases, hTRP-2 gave 783 ± 75 metastases, and control null vector gave 705 ± 61 metastases (not shown in figure). Results are shown as mean number of lung metastases ± SEM.
Results

Xenogenic hTRP-2 DNA immunization induces tumor rejection. hTRP-2 has 90% homology and 83% identity to the amino acid sequence of C57BL/6 mTRP-2. DNA immunization with xenogeneic hTRP-2 decreased BALB/c LM3 lung metastases by \( \approx 90\% \) (\( P < 0.0001 \)) in tumor protection experiments (Fig. 1, A–C). There was no significant evidence of tumor immunity after immunization with syngeneic mTRP-2 DNA compared with untreated mice or mice injected with control null vector (Fig. 1, A and C).

To assess the potency of DNA immunization using xenogeneic hTRP-2 DNA, mice were immunized 4 d after tumor challenge or 10 d after tumor challenge, when lung metastases were numerous and macroscopic. Immunization at 4 d decreased metastases by \( \geq 80\% \) (\( P < 0.001 \); Fig. 1 D). Therapeutic effects were observed 10 d after tumor challenge using immunization with hTRP-2 DNA plus recombinant mouse GM-CSF DNA as an immune adjuvant. Vaccination significantly decreased lung metastases by approximately half (\( P = 0.004 \); Fig. 1 E). No significant decrease in lung metastases was observed after treatment with hTRP-2 or mTRP-2 DNA, or GM-CSF DNA alone (Fig. 1 E, see legend), although there was a trend towards decreased metastases with GM-CSF alone that did not reach significance (\( P > 0.05 \)). These results showed a requirement for xenogeneic antigen and the adjuvant effect of GM-CSF in the treatment of established tumors.

Xenogeneic hTRP-2 DNA vaccination induces autoantibodies and autoreactive CTLs. We next determined whether immunization with mTRP-2 or xenogeneic hTRP-2 generated antibody and CD8\(^+\) T cell responses against syngeneic mTRP-2. 6 of 12 mice immunized with hTRP-2 had detectable IgG antibodies (IgG1 and IgG2b isotype) against mTRP-2 (data not shown). No autoantibodies against syngeneic mouse TRP-2 were generated after immunization with mTRP-2 (0/12). Generation of autoantibodies after immunization with hTRP-2 required both CD4\(^+\) and CD8\(^+\) T cells, because no autoantibodies were detected in mice deficient in MHC class I (0/11) or II molecules (0/10).

CTL responses against TRP-2 were detected after immunization with xenogeneic hTRP-2, but not syngeneic mTRP-2 DNA. Specifically, CD8\(^+\) CTLs from draining lymph nodes (supraclavicular nodes), stimulated in vitro for 5 d, recognized an MHC class I H-2K\(^b\)-restricted peptide of TRP-2 (Fig. 2 [9]). Interestingly, the H-2K\(^b\)-restricted peptide of mTRP-2, TRP-2\(_{181-188}\), is identical between mouse and human TRP-2, including the immediate flanking amino acid residues. Thus, this self-peptide in the context of self–TRP-2 is immunogenic.

Tumor rejection requires CD4\(^+\) and CD8\(^+\) cells, but not B cells or NK cells. These results suggested that either antibody or CTL responses, or both, mediated tumor rejection. Roles for critical cell types were investigated by immunizing β2m\(^{-/-}\) mice deficient in MHC class I and CD8\(^+\) T cells, MHC II\(^{-/-}\) mice deficient in MHC class II and CD4\(^+\) T cells, IgM\(^{-/-}\), and IgG\(^{-/-}\) mice deficient in mature B cells, and mice depleted of NK1.1\(^+\) cells, including NK cells (Fig. 3). Both MHC class I and II molecules were required for tumor rejection, supporting a central role for both CD8\(^+\) and CD4\(^+\) T cells. Neither NK cells nor B cells were necessary for tumor immunity. Noticeably, mice deficient in B cells developed fewer baseline metastases compared with wild-type C57BL/6 mice, and were completely free of any detectable tumor after treatment with hTRP-2 (12 of 12 mice). This phenomenon of enhanced T cell-dependent tumor rejection associated with B cell deficiency has been reported previously (10). These results showed that T cell immunity, including both CD8\(^+\) and CD4\(^+\) T cells, was required for tumor rejection, but antibodies were not.

Xenogeneic Immunization Induces Autoimmunity. T cells also require T cells. Signs of autoimmune, manifested as depigmentation, were observed in mice immunized with hTRP-2, but not generally in mice immunized with syngeneic mTRP-2 (Fig. 4). Depigmentation appeared 4–5 wk after starting immunization over depilated and shaved areas of the mouse coat, spreading to unshaved areas in most mice. Autoimmunity also required T cells, but not antibodies or NK cells, showing that tumor immunity and autoimmunity were coupled by a requirement for class I and II MHC expression leading to a requirement for T cells.

Requirement for Perforin in T Cell–dependent Autoimmunity. CTLs have been proposed to be critical effector cells that mediate tumor rejection. Cytotoxicity of T cells can be mediated by exocytic granules in-
of NK1.1. Results are shown as mean ± SEM. Significant protection was observed in NK depleted tumorigenicity in different mouse strains or under different conditions, and differences in lung metastases in the no treatment groups reflect immunoglobulin-deficient (wt) mice treated with hTRP-2 (h) or mTRP-2 (m) DNA. In addition, groups of mice (12–15 per group) were immunized with hTRP-2 DNA as described in the legend to Fig. 1. Significant tumor protection was observed in pfp-/− (P = 0.00002) and gld/gld (P < 0.0001) mice.

Discussion

TRP-2 is recognized by CTLs of patients with melanoma (7, 11, 12), and has also been defined as a potential tumor-rejection antigen in C57BL/6 mice (9). Thus, TRP-2 provides a model for a differentiation antigen with relevance to a human cancer. Xenogeneic DNA vaccination is one strategy to immunize against potentially weak self-antigens. The approach using a xenogeneic source of antigen is well known to produce autoimmunity, but has also been used to induce tumor immunity against gp75TRP-1 and another melanocyte/melanoma differentiation antigen gp100 (13, 14, 16–18). Other strategies have shown that syngeneic mouse gp75TRP-1 expressed in insect cells (16) or by vaccinia virus (14) can also induce tumor immunity and trigger depigmentation. Expression of the syngeneic protein in the context of xenogeneic cells that may package the antigen in insoluble inclusion bodies (e.g., insect cells) or with strongly immunogenic viral proteins are alternative strategies, at least for inducing antibody-dependent immunity against tyrosinase family antigens.

As noted above, the CTL response directed against the H-2Kb-restricted peptide of mTRP-2, mTRP-2161–188 is remarkable because this peptide is identical between mouse and human TRP-2, including the immediate flanking amino acid residues. It is possible the distant amino acid residues alter processing of this peptide in hTRP-2, allowing more efficient presentation. Alternatively, amino acid differences in other peptides of hTRP-2 may provide T cell help, which in turn is sufficient to trigger CTL responses to the mTRP-2161–188 self-peptide. The observation that only hTRP-2 DNA (but not mTRP-2 DNA) induced CD8+ T cell responses suggests that T cell tolerance was broken by xenogeneic DNA immunization, although we recognize this could reflect differences in efficiency of processing.

Tumor Immunity and Autoimmunity: Two Means to the Same End. Immunity against gp75TRP-1 led to tumor protection and to depigmentation that was indistinguishable from autoimmunity induced by immunization against TRP-2 (13). Similar results were observed by Naftzger et al. (16) and Overwijk et al. (14) after immunization with syngeneic gp75TRP-1 expressed by baculovirus in insect cells or in vaccinia virus, respectively. In gp75TRP-1 systems, tumor immunity and autoimmunity were mediated by autoanti-
We are grateful to Yoichi Moroi for his advice and assistance, Jeffrey Puccio for technical assistance, and Polly Gregor and Paul Chapman for useful discussions. We thank Powderject, Inc. for providing the gene gun apparatus used for particle bombardment to deliver DNA and GM-CSF DNA vector.

These studies were performed in the Swim Across America Laboratory. This work was supported by grants CA56821 and CA59350 from the National Cancer Institute, The Louis and AnneAbrons Foundation, The Dillman Endowment, The Kleberg Foundation, and the R ubin Foundation also provided support for R. Srinivasan, W.G. Hawkins, and R. D yall. J.D. W olchok was supported in part by the Charles A. Dana Foundation and National Cancer Institute grants CA09512 and CA09207. W.B. B owne and J.D. W olchok were previously H-C Fellows; they also received support from National Cancer Institute grants CA47179 and CA09501.

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Submitted: 9 September 1999 Accepted: 21 September 1999

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