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Melanocyte Lineage-specific Antigen gp100 Is Recognized by Melanoma-derived Tumor-infiltrating Lymphocytes

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Summary

We recently isolated a cDNA clone that encodes the melanocyte lineage-specific antigen glycoprotein (gp)100. Antibodies directed against gp100 are an important tool in the diagnosis of human melanoma. Since the gp100 antigen is highly expressed in melanocytic cells, we investigated whether this antigen might serve as a target for antineoplastic cytotoxic T lymphocytes (CTL). Here, we demonstrate that cytotoxic tumor-infiltrating lymphocytes (TIL) derived from a melanoma patient (TIL 1200) are directed against gp100. HLA-A2.1+ melanoma cells are lysed by TIL from this patient. In addition, murine double transfectants, expressing both HLA-A2.1 and gp100, are lysed by TIL 1200, whereas transfectants expressing only HLA-A2.1 are not susceptible to lysis. Furthermore, the HLA-A2.1+ melanoma cell line BLM, which lacks gp100 expression and is resistant to lysis, becomes susceptible after transfection of gp100 cDNA. Finally, HLA-A2.1+ normal melanocytes are lysed by TIL 1200. These data demonstrate that the melanocyte differentiation antigen gp100 can be recognized in the context of HLA-A2.1 by CTL from a melanoma patient. Gp100 may therefore constitute a useful target for specific immunotherapy against melanoma, provided that no unacceptable cytotoxicity towards normal tissue is observed.
a well-defined melanocytic antigen, the gp100 antigen, and investigated whether this antigen can serve as a target for antimalanoma CTLs.

Here, we demonstrate that the melanocyte differentiation antigen gp100 is specifically recognized by melanoma-derived tumor-infiltrating lymphocytes (TIL) in the context of HLA-A2.1.

**Materials and Methods**

**Cell Culture.** TILs were generated by growth of single cell suspensions of metastatic melanomas with 1,000 U/ml IL-2 (Cetus Corp., Emeryville, CA) and were grown as was described previously (7). Melanoma lines Mel 397 and Mel 624 were obtained and grown as was reported previously (7). HLA-A2.1+ melanoma lines MeWo (12) and BLM (13) and murine P815 transfectants were grown in DMEM (Gibco, Paisley, Scotland, UK) plus 7.5% heat-inactivated FCS (Gibco). JY, K562, and murine EL4 transfectants were cultured in IMDM (Gibco) plus 7.5% FCS. Murine cells were grown in DMEM (Gibco, Paisley, Scotland, UK) for 1 h. Various amounts of effector cells were then added to 2 x 10^5 target cells in triplicate wells of U-bottomed microtiter plates (Costar, Badhoevedorp, The Netherlands) in a final volume of 150 μl. After 5 h of incubation, part of the supernatant was harvested and its radioactive content measured. Target cells were incubated for 48 h with 50 U/ml human (Boehringer, Ingelheim, Germany) or mouse recombinant IFN-γ (TNO, Rijswijk, The Netherlands) before use in chromium release assays.

**Results and Discussion**

In search of gp100-specific CTLs we focused on HLA-A2.1 as a restriction element because of its widespread occurrence in caucasians and its presumptive dominant role in CTL reactivity against melanoma. A HLA-A2.1+ TIL line, TIL 1200, was used for this study. This TIL line expresses TCRα/β, CD3, and CD8 (data not shown).

HLA-A2.1-restricted Killing of Melanoma Tumor Cells by TIL 1200 Corresponds to gp100 Expression. Cytolytic activity of TIL 1200 was analyzed using a panel of human melanoma cell lines. As shown in Fig. 1, TIL 1200 efficiently lysed HLA-A2.1+ Mel 624 and MeWo melanoma tumor cells, which both express gp100, whereas no reactivity towards HLA-A2.1+ gp100+ Mel 397 cells was seen. It is interesting to note that we observed that BLM melanoma cells also are resistant to lysis by TIL 1200 (Fig. 1). Previously, we have shown that BLM cells lack the expression of the gp100 antigen, both at the protein and at the mRNA level (2). Furthermore, HLA-A2.1+ EBV-transformed B cells (JY), which also lack gp100 expression, and K562 cells, were not lysed by TIL 1200 (data not shown). Together, these data demonstrate that TIL 1200 displays HLA-A2.1-restricted killing which correlates with gp100 expression.

TIL 1200 Recognizes HLA-A2.1+ gp100+ Transfectants. To explore whether TIL 1200 recognized a gp100-derived peptide in the context of HLA-A2.1, we cotransfected murine EL4 cells with a genomic fragment encoding HLA-A2.1 together with a plasmid conferring hygromycin resistance. Stable hygromycin-resistant transfectants were selected and analyzed for HLA-A2.1 surface expression by flow cytometry. EL4 cells expressing HLA-A2.1 (EL4 A2.1) were subsequently transfected with pBJ1-gp100neo, which encodes gp100 and confers resistance to G418. Stable transfectants were selected and were screened for gp100 expression using mAb

**Figure 1.** HLA-A2.1-restricted killing of gp100+ melanoma tumor cells by TIL 1200. Lysis of chromium-labeled cells was measured after 5 h. All melanoma cell lines except BLM express the gp100 antigen. One representative experiment out of three.
Figure 2. Lysis of HLA-A2.1+ gp100+ transfectants by TIL 1200. Murine EL4 and P815 cells were transfected with both gp100 and HLA-A2.1 DNA, and human BLM cells were transfected gp100 DNA as described in Materials and Methods. Isolated clones were tested for sensitivity to lysis by TIL 1200. (A and B) Representative examples of experiments performed with four individual EL4 A2.1/gp100 and P815 A2.1/gp100 clones. (C) One experiment out of three, using BLM gp100 clones H2.3 and 5A3.

Lysis of HLA-A2.1+ gp100+ melanocytes rules out the possibility that TIL 1200 recognizes a peptide epitope of gp100 mutated in melanoma tumor cells. This is further demonstrated by the fact that TIL 1200 is reactive with several allogeneic HLA-A2.1 matched, gp100+ melanoma tumor lines. We therefore conclude that TIL 1200 recognizes nonmutated peptide derived from the melanocyte differentiation antigen gp100 expressed by melanoma tumor cells, as well as by normal melanocytes.

Furthermore, our data show that gp100 can be immunogenic, suggesting that melanocyte differentiation antigens may serve as tumor rejection antigens. This hypothesis is strengthened by: (a) the finding that antibodies directed against this type of antigens (e.g., gp75) are present in the serum of melanoma patients (21, 22); (b) the recent observation that several antimelanoma CTLs display cross-reactivity with normal melanocytes (8); and (c) the identification of the melanocyte lineage-specific tyrosinase protein as a target for antimelanoma CTLs (11). Immunogenicity of melanocyte differenti-
lation antigens may also explain the clinical observation that melanoma patients often display local depigmentation of their skin (23). The occurrence of local depigmentation in melanoma patients has been reported to correlate with prolonged survival (23) and may result from the destruction of melanocytes as a consequence of an immune response directed against melanocyte differentiation antigens (24). Other clinical data, indicating that vitiligo (local depigmentation of the skin) is associated with improved survival of melanoma patients (25), are in line with this hypothesis. TILs have been shown to mediate regression of metastatic melanoma in patients treated with TILs plus IL-2 (26). Of particular interest is the fact that therapy with TIL 1200 was associated with objective tumor regression in the autologous patient (Rosenberg, S. A., unpublished data), suggesting that CTLs directed against melanocyte differentiation antigens can cause tumor regression in vivo.

Since melanocyte differentiation antigens such as gp100 are widely expressed in melanoma tumor cells, they may constitute a useful target for specific immunotherapy against melanoma. Although no unwanted toxicity was observed in the autologous patient upon TIL 1200 treatment, adverse side effects upon gp100 immunization, such as the destruction of normal melanocytic cells and retinal pigment epithelium cells, remain a possibility and require further study in animal models. Furthermore, it will be important to determine how widely T cell-mediated gp100 reactivity is observed in melanoma patients.

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