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

By Alexander B. H. Bakker, Marco W. J. Schreurs, Annemiek J. de Boer, Yutaka Kawakami,* Steven A. Rosenberg,* Gosse J. Adema, and Carl G. Figdor

From the Division of Immunology, The Netherlands Cancer Institute, Antoni van Leeuwenhoek Huis, Plesmanlaan 121, 1066 CX, Amsterdam, The Netherlands; and *Surgery Branch, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

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 antimelanoma 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.

The melanocyte lineage-specific antigens recognized by mAb NKI-beteb are among the best diagnostic markers for human melanoma available to date (1). NKI-beteb reacts with melanoma cells throughout tumor development and does not cross-react with other tumor cell types or normal cells, except for melanocytes and pigmented cells in the retina. The antigen recognized by mAb NKI-beteb is an intracellular glycoprotein of approximately 100 kD (gp100) (1). Recently, we isolated a cDNA clone termed gp100-c1, which not only confers reactivity to mAb NKI-beteb, but also to two other mAbs used in diagnosis of malignant melanoma, HMB-50 and HMB-45 (2). Characterization of the gp100-c1 cDNA revealed that gp100 is a type I transmembrane glycoprotein highly homologous to another melanocyte-specific protein Pmel17 (Adema, G. J., A. J. de Boer, A. M. Vogel, W. A. M. Loenen, and C. G. Figdor, manuscript submitted for publication).

Although the mAbs recognizing gp100 are widely used for diagnostic purposes, they are less well applicable for therapeutic use since gp100 is predominantly expressed intracellularly. However, it has now been clearly demonstrated that intracellular proteins can be processed and presented as peptides in association with MHC molecules to T lymphocytes (3, 4). Therefore, the gp100 antigen is a potential target for cellular immune responses against melanoma. CTLs recognizing melanoma tumor cells in a TCR-dependent and MHC-restricted manner have been isolated from tumor-bearing patients (for a review see reference 5). These CTLs often display cross-reactivity with allogeneic HLA-A2.1-matched melanomas, implying that shared melanoma antigens presented in the context of HLA-A2.1 are recognized (6, 7). Recently, it has been reported that some CTLs not only react with melanoma tumor cells, but also with normal melanocytes, suggesting recognition of differentiation antigens expressed by melanocytes (8).

Several approaches have been followed to unravel the nature of the antigens recognized by antimelanoma CTLs (9, 10). However, the identity of the antigens remains largely unknown. So far, only a genetic approach applied by Van der Bruggen et al. (9) and Brichard et al. (11) resulted in the identification of antigens recognized by CTL (9, 11). We have chosen to follow a reversed approach in that we focused on...
Recognition of gp100 Antigen by Melanoma-derived CTLs

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 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% FCS. Murine cells were grown in the presence of 5 x 10^{-5} M [3H]thymidine and were labeled with 20 ng of [3H]thymidine/mL. JY, K562, and murine EL4 transfectants were grown in IMDM (Gibco) plus 7.5% FCS. JY, K562, and murine EL4 transfectants were grown in the presence of 5 x 10^{-5} M [3H]thymidine and were labeled with 20 ng of [3H]thymidine/mL.

Chromium Release Assay. Chromium release assays were performed as described previously (7). Briefly, 10^6 target cells were incubated with 100 μCi Na^25CrO_4 (Amersham International, Bucks, UK) for 1 h. Various amounts of effector cells were then added to 2 x 10^6 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 TCR-α/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.
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 a 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 antimalanoma 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 differentiation-
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.

We thank Drs. E. J. Baas, H. te Riele, and R. De Waal Malefijt for kindly providing plasmids pBA2, pGK-hyg, and pBJ1-neo, and P. Coule for transfection of P815 A2.1 cells. We are indebted to Drs. E. Daenen and D. J. Ruiter for providing normal melanocytes. We thank M. A. van Halem for secretarial assistance.

This work was supported by grants from the Dutch Cancer Society (NKI 91-10 and NKI 91-11).

Address correspondence to Dr. Alexander B. H. Bakker, Department of Tumor Immunology, University Hospital Nijmegen St. Radboud, Philips van Leydenlaan 25, 6525 EX, Nijmegen, The Netherlands.

Received for publication 24 November 1993.

References


