Immunization against Endogenous Retroviral Tumor-associated Antigens

Michael H. Kershaw, Cary Hsu, Wallace Mondesire, Libbie L. Parker, Gang Wang, Willem W. Overwijk, Rejean Lapointe, James C. Yang, Rong-Fu Wang, Nicholas P. Restifo, and Patrick Hwu

Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland 20892; [M. H. K., W. M., G. W., W. W. O., R. L., J. C. Y., N. P. R., P. H.]; Howard Hughes Medical Institute-NIH Research Scholars Program, Bethesda, Maryland 20814 [C. H., L. L. P.]; and Center for Cell and Gene Therapy, Department of Immunology, Baylor College of Medicine, Houston, Texas (R. F. W.)

ABSTRACT

Endogenous retroviral gene products have been found in some human tumors, and therefore, may serve as antigens for immunotherapy approaches. The murine colorectal carcinoma CT26 and melanoma B16 have recently been found to express the endogenous retroviral gene products gp70 and p15E, respectively, that can serve as antigens recognized by T cells. To date, though, there has been no demonstration of tumor treatment using an endogenous retroviral protein. In this study, we demonstrate that mice immunized with recombinant vaccinia encoding the gp70 H2-L^A-restricted minimal determinant were protected from CT26 tumor challenge. Splenocytes from mice immunized with vaccinia gp70 specifically secreted IFN-γ in response to gp70 peptide-pulsed stimulators. Although this strategy could protect against subsequent tumor challenge, it was ineffective against established tumors. Therefore, to investigate the treatment of established CT26 or B16 lung metastases, mice were treated with cultured dendritic cells (DCs) pulsed with gp70 or p15E peptide. Significant inhibition of established lung metastases required immunization with peptide-pulsed DCs pretreated with CD40 ligand that has been demonstrated to increase the T-cell stimulatory activity of DCs. The ability to immunize against endogenous retroviral tumor antigens may have relevance in the induction of antitumor immunity for some human cancers.

INTRODUCTION

Many endogenous retroviral elements have been identified in humans. These elements are thought to have arisen from rare infection and integration events of retrovirus into germ-line cells early in evolution. The sequences generally encode defective proviruses, although transcripts and protein products of portions of the provirus have been detected in a number of tissues and tumor cell lines. Their relevance as TAA

Rejean Lapointe, James C. Yang, Rong-Fu Wang, Nicholas P. Restifo, and Patrick Hwu 3

Martin Postdoctoral Research Fellowship (to M. H. K.).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Supported by a National Health and Medical Research Council of Australia C. J. Martin Postdoctoral Research Fellowship (to M. H. K.).

4 These authors contributed equally to this work.

5 To whom requests for reprints should be addressed, at National Cancer Institute, NIH, Building 10, Room 2B-42, 9000 Rockville Pike, Bethesda, MD 20892. Phone: (301) 402-1156; Fax (301) 435-5167; E-mail: Patrick_Hwu@nih.gov.

6 The abbreviations used are: TAA, tumor-associated antigen; HERV, human endogenous retrovirus; DC, dendritic cell; IL, interleukin; β-gal, β-galactosidase; MuLV, murine leukemia virus; APC, antigen-presenting cell.
immunize against the CT26 tumor. Although immunization with recombinant vaccinia could protect against subsequent tumor challenge, treatment of established tumor was not achieved. Therefore, we investigated the effect of immunization with in-vitro generated, bone marrow-derived, peptide-pulsed DCs on established tumor growth. DCs are central to antigen presentation and the initiation of an immune response. In addition, to enhance the immunogenic capacity of DCs against an established tumor we treated DCs with CD40 ligand (19), because interaction of CD40 ligand with CD40 expressed on DCs has been demonstrated to induce IL-12 production and increase T-cell stimulatory activity of DCs (20).

MATERIALS AND METHODS

Cell Lines. CT26 is a carcinogen-induced, undifferentiated colon carcinoma of BALB/c mice. CT26.CL25 is a β-gal-expressing cell line derived from CT26 by transduction with a retroviral vector encoding the lacZ gene driven by the Moloney MuLV long terminal repeat (21). B16 is a spontaneously arising melanoma of C57BL/6 mice propagated by Dr. Isaiah J. Fidler (M.D. Anderson Cancer Center, Houston, TX). NIH/3T3 is a mouse fibroblast cell line. 3T3-CD40L cells were generated by transducing NIH/3T3 cells with mouse CD40L cDNA. cDNA for mouse CD40L was isolated by reverse transcription-PCR from anti-CD3-activated mouse splenocytes and cloned into NotI/XhoI sites in the retroviral expression vector pSAMEN (22). The ectropic producer cell line GP+686 expressing CD40L was generated similarly as described previously (23), and supernatant was used to transduce NIH/3T3 followed by selection in G418. CD40L expression on NIH/3T3 was confirmed by flow cytometry. Cell lines were maintained at 37°C/5% CO₂ in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin (all from Biofluids, Rockville, MD), and 1.25 μg/ml Amphotericin B (Fungizone; Life Technologies, Inc., Grand Island, NY).

Peptides. The gp70 peptide used in this study is a 9 amino acid sequence (SPSYVVYHQF) derived from the endogenous MuLV envelope protein gp70 (10). Control H2-L^{a}-restricted peptide was β-gal (TPHPARIGL; Ref. 24). The H2-K^{b}-restricted peptide sequences used were derived from β-gal (DAPITYTNV) and p15E (KSPWFTTL). Peptides used to pulse DCs contained the Tat-linked peptides to be more effectively pulsed onto DCs. 6

Vaccinia Virus Immunization. Recombinant vaccinia vectors were generated by insertion of either the gp70 or β-gal peptide sequence into the viral thymidine kinase coding region as described previously (25). Each peptide sequence in vaccinia was preceded by the ES signal peptide sequence (26). The sequence in vaccinia was preceded by the ES signal peptide sequence (26). The vaccinia vector was evident from the observation that splenocytes from mice immunized with vaccinia β-gal-secreted lower levels of IFN-γ after incubation with gp70-pulsed stimulators.

RESULTS

Immunization with Vaccinia gp70 Induces gp70-specific Splenocytes. Splenocytes from mice immunized with recombinant vaccinia gp70 were assessed for their reactivity to gp70-pulsed targets. Four weeks after immunization, splenocytes were cultured for 6 days with gp70 peptide then cocultured with peptide-pulsed targets. T cells generated from vaccinia gp70-immunized mice produced high amounts of IFN-γ in response to incubation with gp70 peptide-pulsed irradiated splenocytes but not to P1A or β-gal-pulsed targets (Table 1). The requirement for incorporation of the gp70 peptide epitope into the vaccinia vector was evident from the observation that splenocytes from mice immunized with vaccinia β-gal-secreted lower levels of IFN-γ after incubation with gp70-pulsed stimulators.

Recombinant Vaccinia Virus gp70 Protects against CT26 Tumor. Recombinant vaccinia gp70 was next assessed for its ability to induce antitumor effects in vivo. Mice were immunized with HBSS or recombinant vaccinia vector encoding either gp70 or β-gal peptide and challenged 3 weeks later with either CT26 or CT26.CL25 (β-gal-transduced CT26) tumor i.v. Mice immunized with vaccinia gp70 were almost totally protected from lung metastasis formation (Table 2) after challenge with CT26. Protection against the CT26 tumor required the presence of gp70 peptide sequence in the vaccinia vector, because mice immunized with vaccinia vector containing β-gal peptide sequence developed significantly higher numbers of lung metastases (P2 < 0.05). Because CT26.CL25 also expresses gp70, we evaluated the effect of immunization with vaccinia gp70 on CT26.CL25 growth. Significant reduction in CT26.CL25 lung metastases resulted from immunization with vaccinia gp70. Mice were also protected from CT26.CL25 challenge but not CT26 challenge after immunization with vaccinia β-gal, thereby demonstrating the immunizing ability of vaccinia β-gal against β-gal-expressing tumor.

Table 1 Immunization with vaccinia gp70 generates gp70 peptide-reactive splenocytes

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>None</th>
<th>Fresh splenocytes</th>
<th>gp70-pulsed splenocytes</th>
<th>P1A-pulsed splenocytes</th>
<th>β-gal-pulsed splenocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinia β-gal</td>
<td>1343</td>
<td>2088</td>
<td>2708</td>
<td>1978</td>
<td>3555</td>
</tr>
<tr>
<td>Vaccinia gp70</td>
<td>1208</td>
<td>1536</td>
<td>20,543</td>
<td>1048</td>
<td>9526</td>
</tr>
</tbody>
</table>

6 Unpublished observations.
DCs Pulsed with gp70 Peptide Can Treat Established Lung Metastases. Although immunization with recombinant vaccinia could protect against CT26, these methods were ineffective against established tumors (not shown). Therefore, we used peptide-pulsed cultured DCs in an attempt to treat established pulmonary metastases. In addition, because DC function is highly dependent on the level of activation (28), we also studied the use of CD40L-activated DCs in the treatment of established tumor. Mice were injected with $1 \times 10^6$ CT26 tumor cells i.v. followed 3 days later by peptide-pulsed DCs. DCs were incubated 24 h before peptide pulsing in the presence or absence of CD40L. Mice that received gp70-pulsed DCs had significantly less lung metastases than mice receiving either $\beta$-gal-pulsed DCs or HBSS alone (P2 < 0.01; Fig. 1), thereby confirming the requirement for gp70 to achieve therapeutic effect. An additional significant reduction in lung metastases over that achieved by gp70-pulsed DCs was observed by preincubation of DCs with CD40L (P2 < 0.01). $\beta$-Gal-pulsed DCs failed to reduce lung metastases regardless of whether DCs were preincubated with CD40L or not.

DCs Pulsed with p15E Peptide Can Treat Established Lung Metastases. To confirm the antitumor effectiveness of endogenous retroviral peptide-pulsed DCs, we next investigated the use of another retroviral antigen, p15E, in a different tumor model, B16 melanoma, in C57BL/6 mice. Mice were injected i.v. with $3 \times 10^5$ B16 melanoma cells to establish pulmonary metastases. Three days later mice received $4 \times 10^5$ peptide-pulsed DCs pretreated with CD40L. The effect of injection of IL-2 was also studied in this tumor model, because IL-2 has been demonstrated to augment antitumor effects in other studies (29). When lungs were examined 12 days later, it was clear that mice receiving p15E-pulsed DCs and IL-2 developed significantly fewer metastases than mice receiving HBSS or control $\beta$-gal-pulsed DCs (Fig. 2). The requirement for coadministration of IL-2 in this model was also evident, because mice receiving p15E-pulsed DCs without IL-2 developed similar numbers of lung metastases as control mice receiving HBSS only. This experiment was repeated with similar results.

**DISCUSSION**

In this study we investigated the use of endogenous retroviral antigens as immunogens in the CT26 colon carcinoma and B16 melanoma tumor models in mice. Previous studies demonstrated an antitumor response in BALB/c mice after immunization with CT26 modified to secrete granulocyte macrophage colony-stimulating factor, and this response was found to be at least partially directed against a peptide epitope of gp70 (10), but attempts to immunize with this peptide alone were unsuccessful. More recently, immunization with a modified gp70 peptide has been demonstrated to protect mice against tumor challenge (15). In the MC38 and B16 tumor models CTL can also be generated against another endogenous retroviral antigen, p15E (16, 17). In this paper, we describe for the first time active immunization against endogenous retroviral antigens in a treatment setting.

Other endogenous retroviral elements described previously include mouse mammary tumor virus-related sequences with $\leq 14$ copies present in the genome. Mouse mammary tumor virus-related proteins can be detected in many tissues, but expression is highest in organs with exocrine function including mammary tumors and normal mammary gland (30). Human endogenous retrovirus transcripts have been found in some human cancer cell lines (5–7), and recently an endogenous retroviral antigen recognized by T cells has been identified in some human melanomas. The association of endogenous retrovirus protein expression with some malignancies may make these viral proteins a potential target for cancer vaccines.

Recombinant vaccines based on pox viruses have been demonstrated to inhibit tumor growth in mice (18, 31). In addition antitumor effects have been demonstrated in mice using adenoviral or other viral
IMMUNIZATION AGAINST A RETROVIRAL TUMOR ANTIGEN

vectors (32). Indeed, we found in this study that recombinant vaccinia in our initial melanoma clinical trials using recombinant vaccinia and adenoviral vectors for immunization, we have found that preexisting neutralizing antibody may limit the effectiveness of these strategies (33). The use of recombinant fowlpox viruses may be effective at addressing this problem, because most patients do not have a preexisting neutralizing antibody against fowlpox.

Other means of initiating an immune response include the use of peptides derived from TAAs, which has been demonstrated to induce protective immunity in mice (34) and increase tumor-reactive T-cell precursors in melanoma patients (35). However, in some circumstances, administration of tumor-associated peptide has been demonstrated to induce tolerance and results in no protection against a tumor (36, 37). In general, in mice, vaccination with TAA peptide alone does not invoke a strong antitumor response.

Effective immunization is dependent on sufficient antigen-gaining access to significant numbers of APCs. DCs are effective APCs that express high levels of MHC and costimulatory molecules (38). One means of providing sufficient numbers of antigen-bearing APCs is by ex vivo expansion of DCs to large numbers (39) followed by pulsing with tumor peptide and delivery to the host. Tumor growth inhibition has been observed in mice receiving DCs pulsed with tumor antigen peptide (40) or transduced with tumor antigen genes (27). Our initial studies determined that DCs pulsed with gp70 endogenous retroviral peptide had a significant effect on 3-day CT26 lung metastases, although tumor inhibition was not complete. Activation of DCs with CD40L followed by pulsing with gp70 augmented the antitumor effect above that seen with gp70-pulsed DCs alone. CD40 is a 50,000 dalton protein expressed on a variety of cells including B cells, monocytes, and DCs. Ligation of CD40 results in activation of DCs as demonstrated by increases in expression of costimulatory molecules (28). It is likely that the activation of DC by CD40L in this investigation resulted in increased stimulation of endogenous peptide-specific T cells leading to increased antitumor effects. Although we demonstrated a correlation between the generation of peptide-reactive T cells and increased antigen immunity in the vaccinia studies described here, we did not determine this in the DC studies. It would be interesting to investigate this correlation in future studies involving immunization with peptide-pulsed DCs. In light of recent findings concerning the use of modified peptide epitopes in the generation of antigen immunity (15) it would be interesting to determine whether peptide modification could additionally enhance the antitumor effects seen in our system.

The ability to immunize against endogenous retroviral antigens was confirmed in a second tumor model in another strain of mice (B16 melanoma in C57BL/6). In this part of the study CD40L-activated DCs pulsed with a peptide of p15E-produced tumor inhibition but only when mice also received IL-2. The reason for the requirement of IL-2 in the treatment is not clear, although it is likely that it was necessary for the optimal generation and activation of tumor-reactive T cells. Interestingly, IL-2 administration was not necessary for effective immunization of mice in the gp70/CT26 system described here. This may be attributable to inherent differences in the immunogenicity of the gp70 antigen in BALB/c mice compared with the p15E antigen in C57BL/6 mice. It is possible that the antitumor effect of immunization may be enhanced in the gp70/CT26 system with the addition of IL-2.

In summary, in this study we have demonstrated the ability to immunize against endogenous retroviral antigens. The use of recombinant vaccinia containing an immunodominant epitope of gp70 could significantly protect mice from subsequent tumor challenge, although no treatment of established lung metastases was observed. However, a significant treatment effect of established lung metastases was observed when either gp70 or p15E peptide was pulsed onto in vitro-generated, bone marrow-derived DCs, which were used to treat mice. The activation/differentiation state of DCs seems to be important in the generation of antitumor activity, because an additional increase in therapeutic effect was demonstrated after earlier in vitro treatment of DCs with CD40L. Because endogenous retroviral sequences may be antigenic in human tumors, this may have significance in the development of cancer vaccines.

ACKNOWLEDGMENTS

We thank Deborah R. Surman for help in virus preparation.

REFERENCES


7923


Immunization against Endogenous Retroviral Tumor-associated Antigens

Michael H. Kershaw, Cary Hsu, Wallace Mondesire, et al.

*Cancer Res* 2001;61:7920-7924.

Updated version Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/61/21/7920

Cited articles This article cites 38 articles, 22 of which you can access for free at: http://cancerres.aacrjournals.org/content/61/21/7920.full#ref-list-1

Citing articles This article has been cited by 9 HighWire-hosted articles. Access the articles at: http://cancerres.aacrjournals.org/content/61/21/7920.full#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.