Amplification of Virus-Induced Antimelanoma T-Cell Reactivity by High-Dose Interferon-α2b: Implications for Cancer Vaccines

Igor Astsatsurov,1 Teresa Petrella,1 E. Umit Bagriacik, Mark de Benedette, Robert Uger, Gail Lumber, Neil Berinstein, Ileana Elias, Neill Iscoe, Caitlin Hammond, Paul Hamilton, and David E. Spaner2

Division of Molecular and Cellular Biology, Research Institute, [I. A., C. H., D. E. S.] and Department of Radiology [P. H.], Sunnybrook and Women’s College Health Sciences Center; Cancer Vaccine Program, Aventis-Pasteur [E. U. B., M. d. B., R. U., N. B., I. E.]; Toronto-Sunnybrook Regional Cancer Center [T. P., G. L., N. B., N. I., D. E. S.]; and Department of Medicine, University of Toronto [T. P., N. B., N. I., D. E. S.], Toronto, Quebec, M4N 3M5 Canada

ABSTRACT

Purpose: The therapeutic effectiveness of cancer vaccines, composed of tumor antigens that are also self-antigens, may be limited by the normal mechanisms that preserve immunological tolerance. Consistent with this notion, we found that vaccination of melanoma patients with recombinant viral vaccines expressing gp100 (a melanoma antigen also expressed by normal melanocytes) produced only transient increases in noncytotoxic T cells specific for immunodominant gp100 epitopes. To improve the therapeutic effects of these vaccines, IFN-α2b (IFN-α) was administered to some high-risk patients.

Experimental Design: 7 HLA-A*0201+ patients were injected with high doses of IFN-α (20 MU/m² × 20 doses) at various times after completing the vaccination protocol. Clinical toxicity and responses were documented, and the effects on gp100-reactive T cells were measured by IFN-γ enzyme-linked immunospot assays, tetramers of HLA-A*0201 and gp100 epitopes, and cellular cytotoxicity assays.

Results: In patients who had previously responded to vaccination, high doses of IFN-α recalled gp100-reactive T cells with the ability to kill gp100-expressing tumor targets in vitro. Concomitant with the reappearance of these cytotoxic T cells, tumor regression was observed in the two patients with clinically evident metastatic disease.

Conclusions: The finding that high-dose IFN recalls previously activated tumor-reactive T cells with potent killing ability suggests a strategy to maintain antitumor responses initiated by cancer vaccines.

INTRODUCTION

Cancers such as melanoma that are incurable with conventional chemotherapy (1) may be susceptible to vaccines that enhance the activity of tumor-reactive T cells (2). A number of tumor antigens have been identified and used to make specific cancer vaccines. For melanoma, these antigens include members of the MAGE family, tyrosinase, melanA/Mart-1, Trp-2, and gp100 (3–5).

Despite the identification of these target antigens, current vaccines often activate T cells for only a short time without providing strong antitumor activity (6). Possible explanations for this transient activation include: (a) T cells are often only weakly reactive to tumor antigens that are also self-antigens; (b) T cells exposed to these antigens during tumor progression may become anergic; (c) immunoregulatory mechanisms that prevent sustained autoimmune responses may also inhibit antitumor responses; and (d) tumor cells, alone, may not be able to sustain the vaccine-primed antitumor responses (6, 7).

We recently evaluated the effects of viral gp100-based vaccines in melanoma patients with skin or lung metastases or at high risk of developing metastatic disease because of deep primary lesions or LN3 involvement (8). In agreement with others (6), we found that vaccine-induced anti-gp100 T-cell responses were often transient (Table 1). In an attempt to provide additional therapeutic benefits, we administered HDI to some of these patients because survival of such patients can be prolonged by HDI for 1 month followed by low doses of IFN-α (10 MU/m²) s.c. for 48 weeks (9). However, the major therapeutic effect of IFN-α may be provided by the high-dose component because low dose therapy, alone, does not produce this survival benefit (9).

We found that HDI could recall antitumor T-cell responses in patients who had previously mounted immune responses to the viral vaccines. In marked contrast to tumor-reactive T cells activated by the vaccines alone, T cells recalled by HDI killed tumor antigen-bearing targets in vitro and were associated with evidence of tumor regression in vivo. These results lend insight into the mechanism of action of IFN-α in cancer therapy and...
may have implications for the pathogenesis of autoimmune diseases in general. They also suggest that HDI after vaccination with tumor antigens may be an effective immunotherapeutic strategy.

**PATIENTS AND METHODS**

**Patients.** Eligibility criteria included a confirmed diagnosis of melanoma with metastases [or high risk (10) of developing metastases], the HLA-A*0201 haplotype [identified by sequence-specific primer-PCR (11) at the Aventis-Pasteur HLA laboratory], age > 18 years, Eastern Cooperative Oncology Group performance status of 0 or 1, and informed, written consent according to national and institutional guidelines. All patients had previously been vaccinated as part of a Phase I trial sponsored by Aventis-Pasteur.

**Aventis Vaccine Trial.** The vaccine used in this trial involved the ALVAC (2)-gp100M recombinant virus (12, 13; made from a second generation Canarypox virus expressing a full-length gp100 gene encoding two epitopes modified for enhanced HLA class I binding) along with the two modified peptide epitopes (described below). The purpose of the trial was to evaluate the toxicity of these reagents and also the feasibility and effectiveness of injecting them directly into inguinal LNs under ultrasound guidance. Four groups of 6–8 HLA-A*0201 melanoma patients (satisfying the criteria described above) were vaccinated using different routes of administration. Patients in group I received ALVAC-gp100 (0.5 ml, 0.5 × 10⁷ plaque-forming units/ml) s.c. on day 1 of a 21-day cycle for three cycles followed by 2 ml of a mixture of the modified gp100 peptides (500 µg/ml of each peptide) s.c. on day 1 of a 21-day cycle for two cycles. Patients in group II received gp100 peptide mixture intranodally daily for 5 days every 21 days for 2 cycles. Patients in group III received ALVAC-gp100 (0.5 ml) on day 1 of a 21-day cycle for three cycles followed by the gp100 peptide mixture (0.4 ml intranodally daily for 5 days every 21 days for two cycles). Patients in group IV received ALVAC-gp100 (0.5 ml intranodally) on day 1 of a 21-day cycle for three cycles followed by the gp100 peptide mixture (0.4 ml intranodally daily for 5 days) every 21 days for two cycles. Full details of this ongoing trial will be published separately. Of the patients in the study of HDI after vaccination reported in this article, M237 was in group I, M136 and M260 were in group II, M302, M246, and M166 were in group III, and M335 was in group IV.

**Treatment with HDI.** HDI (Scherin Canada, Pointe-Claire, Quebec, Canada) consisted of 20 injections of IFN-α (20 MU/m²/day) over 4 weeks (14). The IFN-α dose was reduced by 33% for severe toxicity [grade 3 or 4, defined by the common toxicity criteria (version 2.0) established by the National Cancer Institute Cancer Treatment Evaluation Program (15)].

**Study Design.** Consenting patients still considered at risk for developing progressive disease were administered HDI after completing the Aventis-sponsored vaccine trial. Toxicity was monitored weekly while patients received HDI. Toxicity and disease status (determined by clinical and/or radiological evaluation) were monitored monthly during the 3-month FU period. PBMCs were collected at these times by density gradient centrifugation and kept in liquid nitrogen until used for immunological monitoring.

**Reagents.** HLA-A*0201-restricted peptide epitopes for CTLs (from Aventis Pasteur, Toronto, Ontario, Canada) included FLU (GILGFVFTL; Ref. 16), gp100 epitopes modified to increase class I MHC binding [gp100:209-2M (IMDQVPFSV) and gp100:280-9V (YLEPGPVTV; 17)], and the HIV p17 Gag protein-derived peptide (SLYNVTAVL; Ref. 18). The gp100 peptides (5 mg/ml stock) were dissolved in water and the others (10 mg/ml stock) in DMSO. CDB-FITC antibodies were purchased from PharMingen (San Francisco, CA). Antibodies from BB7.2 [anti-HLA-A2; Ref. 19; obtained from the American Type Culture Collection (Manassas, VA)] were purified and labeled with FITC in our laboratory. Tetramers (20) of HLA-A*0201 complexed to the peptides YELEPGPVTV (Lot No. BL/0757), IMDQVPFSV (Lot No. BL/0755), or GILGFVFTL (Lot No. BL/0839) bound to phycoerythrin-labeled streptavidin were purchased from ProImmune Ltd. (Oxford, United Kingdom). T2 cells were from the American Type Culture Collection.

**In Vitro T-Cell Stimulation.** PBMCs were thawed, washed, and incubated overnight in AIM-V medium (Life Technologies, Inc., Burlington, Ontario, Canada) at 37°C in 5% CO₂. Cells were then adjusted to 2–3 × 10⁶ cells/ml in AIM-V plus 5% AB serum (Sigma; complete media) and plated (1 ml/well) in 24-well polystyrene tissue culture grade plates (Becton Dickinson Labware, Franklin Lakes, NJ) with FLU or both gp100 peptides added at previously optimized final concentrations of 10 or 25 µg/ml, respectively. IL-2 (50 IU/ml; Chiron, Emeryville, CA) was added 3 and 6 days later, and the cells were harvested after 8 or 9 days for ELISPOT or cytotoxicity assays.

**IFN-γ ELISPOT Assays.** ELISPOT assays were performed as previously described (21) with minor modifications. Capture and biotinylated detection antibodies were from the 1-DIK and 7-B6-1 clones, respectively (Mabtech, Stockholm, Sweden). Cultured T cells were reactivated in triplicate wells with FLU or both modified gp100 peptides (final concentrations of 10 and 25 µg/ml, respectively). IL-2 (100 IU/ml) was also included, except in control cultures stimulated by phorbol myristic acetate (20 ng/ml; Sigma) and Ionomycin (1 µg/ml; Calbiochem, San Diego, CA).

**Cellular Cytotoxicity.** T2 targets (5 × 10⁶ cells) labeled with ⁵¹Cr (500 µCi; Perkin-Elmer, Boston, MA) were washed and incubated with 10 µg/ml gp100:209-2M and gp100:280-9V (mixed 1:1) or FLU for 2 h at 37°C. Washed targets (5 × 10³ cells) were cultured with varying numbers of effectors for 4 h, and chromium release assays were then performed as described (22). Total release (TR) was measured by lysis of targets with 10% Triton-X (Sigma), and spontaneous release (SR) was measured without effector cells. Percent-specific lysis was defined as (cpm – SR)/(TR – SR) × 100%.

**Immunofluorescence.** Staining was performed at the end of the in vitro culture period as described previously (22).

**RESULTS**

**Toxicity.** Seven HLA-A*0201 patients (Table 1) received HDI at various times (mean ± SD = 7.2 ± 4.9 months) after a final booster injection of modified gp100 peptides (17, 23). Patients developed typical toxicities associated with HDI, including flu-like symptoms, cytopenias, and liver function test.
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yr)/Sex</th>
<th>Initial disease</th>
<th>Status before HDI</th>
<th>Time from vaccine to FU (mo)</th>
<th>Peak gp100-reactive T-cell frequency before vaccine (spots/10^6)</th>
<th>Peak gp100-reactive T-cell frequency before vaccine (spots/10^6)</th>
<th>Time from last HDI to FU (mo)</th>
<th>gp100-reactive T-cell frequency (spots/10^6) during HDI</th>
<th>Time from vaccination to first infusion of HDI (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M136</td>
<td>52/M</td>
<td>Lung, LN</td>
<td>NED</td>
<td>8</td>
<td>1/10^9</td>
<td>1/10^9</td>
<td>11</td>
<td>1/10^9</td>
<td>1/10^9</td>
</tr>
<tr>
<td>M246</td>
<td>47/F</td>
<td>LN</td>
<td>NED</td>
<td>7</td>
<td>1/10^9</td>
<td>1/10^9</td>
<td>14</td>
<td>1/10^9</td>
<td>1/10^9</td>
</tr>
<tr>
<td>M260</td>
<td>64/M</td>
<td>LN</td>
<td>Clinical regression</td>
<td>6</td>
<td>1/10^9</td>
<td>1/10^9</td>
<td>9</td>
<td>1/10^9</td>
<td>1/10^9</td>
</tr>
</tbody>
</table>

Table 2. Toxicity, treatment delays, and dose reductions in patients receiving HDI after vaccination

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Grade 3&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Grade 2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constitutional symptoms</td>
<td>1/7</td>
<td>3/7</td>
<td>4/7</td>
</tr>
<tr>
<td>Vitiligo</td>
<td>0/7</td>
<td>1/7</td>
<td>1/7</td>
</tr>
<tr>
<td>Elevated liver function tests</td>
<td>1/7</td>
<td>4/7</td>
<td>5/7</td>
</tr>
<tr>
<td>Granulocytopenia/leukopenia</td>
<td>1/7</td>
<td>6/7</td>
<td>7/7</td>
</tr>
<tr>
<td>Neurologic toxicity</td>
<td>1/7</td>
<td>1/7</td>
<td>2/7</td>
</tr>
<tr>
<td>Dose reduction</td>
<td>7/7</td>
<td></td>
<td>7/7</td>
</tr>
<tr>
<td>Dose delay</td>
<td>7/7</td>
<td></td>
<td>7/7</td>
</tr>
</tbody>
</table>

<sup>a</sup> The delivery of HDI was modified for each patient on the basis of common toxicity criteria (15), with grade 4 being the most severe, necessitating stopping treatment. A 33% reduction of dosage occurred after the first treatment interruption and a 66% reduction from baseline dose occurred after the second. No patients had a third treatment interruption that would also have required removal from treatment.

abnormalities, which lasted only during the time of HDI administration (Table 2). One patient (M160) developed neuropsychiatric symptoms, requiring the institution of antidepressants, which also cleared within a week of stopping HDI. One patient (M335) developed vitiligo around skin deposits of melanoma (described below). Dose reductions and treatment delays attributable to toxicity were required for all seven patients (Table 2), which is higher than the 33% incidence reported for 396 patients treated with HDI, alone, in the E1694 Intergroup trial (15).

Recall of Vaccine-Induced gp100-Reactive T Cells by HDI. The requirement for HLA-A*0201 expression allowed the frequency of gp100-reactive T cells to be measured in both ELISPOT assays for IFN-γ production (21, 24) and by flow cytometry using tetramers of recombinant HLA-A*0201 folded around the modified gp100 peptides (25). No patient had circulating gp100-reactive T cells in these assays before HDI (Fig. 1, “FU” dot plots, Fig. 2, a and c; data not shown). Increased frequencies (>1/10^6 cells) of gp100-reactive T cells had been observed in 4 patients (M302, M237, M166, and M335) at some point during the vaccine protocol (Table 1, column 9 and Fig. 1, “Vaccine” dot plots) but decayed rapidly (Figs. 1, “FU” dot plots, and 2, a and c; data not shown). In these patients, increased frequencies of gp100-reactive T cells were found again by the second week of HDI (Table 1 column 10; Figs. 1, “HDI” dot plots, and 2, a and c). Treatment with HDI did not recall gp100-reactive T cells if measurable anti-gp100 responses had never been achieved with vaccination (Table 1, columns 9 and 10; patients M136, M246, and M260). Failure to detect gp100-reactive T cells was not attributable to technical problems associated with cryopreservation and culture conditions because FLU-reactive cells [found in 60–70% of patients because of previous exposure to influenza (16, 26)] could be elicited (Fig. 2, b and d).

Clinical Responses. All patients completed HDI without disease progression. Two patients (M166 and M335) developed evidence of regression of metastatic melanoma after HDI as described below.

Association of Increased gp100-Reactive T Cells and Clinical Responses after HDI in M166. M166 presented with a 0.6-mm primary skin lesion. A mesenteric metastasis was resected 6 years later. No other metastatic disease was evident until he was considered for the melanoma vaccine study 18
months later and found to have a gluteal mass (Fig. 3a, arrow). The initial bidimensional measurements of this mass (by magnetic resonance imaging) were 2.1 × 3.6 cm and, although it was not biopsied, its appearance was most consistent with a soft tissue metastasis related to melanoma. Moreover, the high T1 signal was consistent with melanin within the mass. A decision was made to observe the mass during vaccination because of the difficult nature of the surgery required for its resection. The mass was somewhat smaller (1.6 × 3.7 cm; Fig. 3b, arrow) after active vaccination but improved considerably after HDI (1.1 × 1.2 cm; Fig. 3c, arrow) and had not progressed at the last FU visit, 14 months later.

A specific T-cell response was induced in M166 by the gp100-based vaccine (Table 1, Figs. 1a and 2a). During vaccination, gp100-reactive CD8+ T cells comprised 1% of the total cells in an 8-day culture of PBMCs, primed with gp100:209-2M and gp100:280-9V (Fig. 1a, “Vaccine” dot plot). After vaccination, gp100-reactive T-cell frequencies fell (Fig. 2a) and disappeared by the time that HDI was instituted (Figs. 1a, “FU” dot plot, and 2a). One week after HDI, the frequency of IFN-γ-producing gp100-reactive T cells increased to ~1/1000 (Fig. 2a) and tetramer-staining CD8+ T cells comprised 4.2% of the culture (Fig. 1a, “HDI” dot plot). FLU-reactive CD8+ T-cell frequencies were relatively constant despite HDI and the changing gp100-reactive T-cell frequencies (Fig. 2b).

**Association of Increased gp100-Reactive T Cells and Clinical Responses after HDI in M335.** M335 presented with a 0.65-mm primary skin lesion. Six years later, she developed right inguinal LN involvement that was treated with resection and levamisole (27). Subsequent skin metastases were treated with resection followed by HDI and 10 months of low-dose IFN-α. One year later, she developed a right axillary LN metastasis that was treated with resection and radiation. Shortly thereafter, melanoma recurred in the skin and dermis of the right breast and chest wall and was treated with mastectomy and local radiation. Before vaccination, multiple small melanotic skin metastases covered the right chest without other detectable systemic disease (Fig. 3, d and g). While actively being vaccinated, she developed a 4-cm mass in the mastectomy scar (data not shown), extensive adenopathy in the cervical region, and left axilla with multiple nodes measuring up to 13 mm (Fig. 3e), and multiple lung nodules (the largest being 7 mm; Fig. 3h). Although not confirmed by histology, the appearance of these lesions was most consistent with progression of metastatic melanoma. HDI was instituted, leading to rapid improvement of the chest, axillary (Fig. 3f), and lung (Fig. 3i) metastases. The innumerable small lung nodules became barely discernable, and the 7-mm nodule at the left lung base measured <1 mm. Metastatic nodules remained in the skin but, interestingly, vitiligo had formed around some of them (data not shown), suggesting autoimmune destruction of neighboring melanocytes (28). This patient has been subsequently maintained on s.c., low-dose IFN-α for a year without evidence of additional disease progression.

As was the case with M166, gp100-reactive T cells increased transiently after vaccination but disappeared by the time of initiation of HDI (Figs. 1b and 2c). However, 2–3 weeks after starting HDI (and concomitant with the clinical response (Fig.
gp100-reactive T-cell frequencies increased to ~1/667 in the ELISPOT assay (Table 1, column 10; Fig. 2c) and tetramer-staining CD8\(^+\) T cells increased to ~7% of cultured PBMCs (Fig. 1b). Elevated responses in these assays were subsequently maintained for at least 2 months (Fig. 2c).

HDI Alters the Quality of the Antitumor T-Cell Response. The differences between the frequencies of gp100-reactive T cells during vaccination and recalled by HDI (Table 1, columns 9 and 10; Figs. 1 and 2, a and c) did not seem to be of sufficient magnitude to account for the clinical effects observed in M166 and M335. We wondered if the quality of the T cells recalled by HDI had changed to account for these clinical effects. Type 1 responses (that result in activation of CTLs able to kill tumor cells) are generally considered to be required for optimal antitumor immunity (29). Although IFN-\(\gamma\) production is a surrogate marker for CTLs (24), we examined directly the ability of gp100-reactive T cells from M166 and M335 to kill antigen-bearing targets. Because melanoma cell lines from these patients were not available, gp100 peptide-loaded T2 cells were used as targets. T2 cells express complexes of peptides and HLA-A*0201 molecules on their cell surface only when HLA-A*0201 binding peptides are provided because of a defective transporter associated with antigen-processing system (30–32). If gp100-reactive T cells are unable to kill peptide loaded T2 cells, it seems unlikely they could kill autologous melanoma cells with a lower surface density of gp100 peptide-HLA-A*0201 complexes.

Despite similar frequencies of tetramer binding and IFN-\(\gamma\) producing gp100-reactive T cells, there were striking differences in the killing of gp100-peptide-loaded T2 cells before and after HDI. Tumor-reactive T cells activated by vaccination alone were unable to kill gp100 peptide-loaded T2 cells (Fig. 4, graphs “After vaccine”). However, gp100-reactive T cells during and after HDI from both patients were potent killers of gp100 peptide-loaded T2 cells (80% lysis at an E:T ratio of 10:1; Fig. 4). This level of killing was comparable with that observed at the same time with FLU-stimulated T cells and FLU peptide-loaded T2 targets (Fig. 4, graph “Flu-After vaccine”). Direct addition of IFN-\(\alpha\) to the cultures did not increase gp100-specific CTL activity (data not shown).

DISCUSSION
In this study, we have shown that HDI alters both the quantity and the quality of autoreactive T cells that recognize...
tumor antigens. Specifically, both the number of melanoma-reactive T cells and their ability to kill tumor targets were increased by the administration of HDI after vaccination with viruses that expressed gp100.

As a single agent, IFN-α is thought to inhibit melanoma cell proliferation by directly regulating gene expression (9). IFN-α may also affect antigen presentation by increasing MHC expression on both melanoma cells and professional APCs (33). The effects on vaccine-induced antitumor responses, described here, suggest that IFN-α may also act by recalling the responses of T cells that have been naturally activated by tumor antigens.

How HDI increases the number of vaccine-induced tumor-reactive T cells is not entirely clear. Increased presentation of gp100 antigens—directly by residual melanoma cells or indirectly by professional APCs—could restimulate recently vaccine-activated gp100-reactive T cells. Alternatively, IFN-α could prevent the death of gp100-reactive T cells that are chronically activated by residual melanoma cells (34) and increase their numbers (35). In mice, IFN-α causes antigen-independent proliferation of CD8+ T cells (36) by stimulating IL-15 production from stromal cells (37). Similar effects in humans could cause the reappearance of recently vaccine-activated T cells in the blood and would be consistent with the fact that we could not mimic the in vivo results by directly adding IFN-α to cell cultures.

The more potent CTL responses observed in vitro after HDI (Fig. 4) were mirrored in the clinical responses of the patients. M335, especially, had suffered disease progression after both single agent IFN-α and during vaccination but showed evidence of tumor regression when HDI was administered after vaccination (Fig. 3). It is not clear how the tumor-reactive T-cell population that arose during vaccination was altered by HDI to promote more effective killing of tumor cells. Simple activation of the cellular lytic machinery by IFN-α (38) is unlikely to account for the phenomenon because increased killing was noted 8 days after the cells had been removed from exposure to IFN-α in vivo. Moreover, the addition of IFN-α to in vitro cultures was not able to induce specific killing by noncytotoxic gp100-reactive T cells (data not shown).

As a result of the treatment in vivo with IFN-α, the tumor-
reactive T-cell population appeared to become dominated by T cells with sufficiently strong reactivity against tumor antigens to mediate CTL activity. This change in the character of the responding T-cell population may be analogous to affinity maturation of an immune response (39) or result from competition between different gp100-reactive T-cell clones for effective activation by the IFN-γ/H9251-treated APCs (40). Alternatively, only the most potent gp100-reactive T cells may survive the effects of inflammatory cytokines induced by IFN-γ, as we recently described for T cells differentially activated by superantigens (41).

Appropriate surrogate markers are important for monitoring the effectiveness of cancer vaccines (42). IFN-γ ELISPOT assays and flow cytometric enumeration of defined tumor antigen-reactive T cells with peptide-MHC tetramers (43) are often used as surrogate markers, as in this study. However, the observation that the quality of the tumor-reactive T-cell population was clearly different after HDI [despite similar frequencies of gp100-reactive T cells in ELISPOT and tetramer assays (Table 1 and Fig. 1, respectively)] suggests that additional studies are required to properly gauge the results of a clinical vaccine. Killing assays (Fig. 4), using autologous tumor cells as targets, would seem to be an excellent assay to gauge these results.

The observations reported in this article suggest that HDI may improve the effectiveness of clinical cancer vaccines by both focusing the responses induced by vaccines onto potent CTLs able to kill tumor cells and maintaining the duration of activity of these cells. The optimal dose and timing of IFN-γ remain to be determined. It is possible that lower doses of IFN-γ [with only modest activity as a single agent (9)] may be more effective as a vaccine adjuvant. Similarly, the patients who mounted recall responses to gp100 (M302, M237, M166, and M335) began HDI 3, 8, 6, and 1.5 months after their last vaccination, respectively (Table 1), whereas the patients who did not (M136, M246, and M260) began HDI 8, 7, and 17 months later. Although these numbers of patients are too small to draw definite conclusions about the timing of IFN-γ, it would seem logical to prescribe it fairly soon after completing a vaccination protocol.

Our observations may also have bearing on the well-known
association of autoimmune diseases with infections (44). In the same way that strong autoimmune responses to gp100 (45) may sometimes lead to sufficient activation of autoreactive T cells to cause clinically evident autoimmune.

REFERENCES


Amplification of Virus-Induced Antimelanoma T-Cell Reactivity by High-Dose Interferon-α2b: Implications for Cancer Vaccines

Igor Astsaturov, Teresa Petrella, E. Umit Bagriacik, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/9/12/4347

Cited articles
This article cites 45 articles, 25 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/9/12/4347.full.html#ref-list-1

Citing articles
This article has been cited by 9 HighWire-hosted articles. Access the articles at:
/content/9/12/4347.full.html#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.