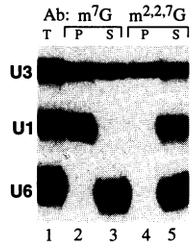

Fig. 3. Trimethylation (5′ cap) of pre-U3 RNA occurs in isolated nuclei free of the cytoplasm. A mixture of m^7G-capped U3 and U1 RNAs and γ-mpppG-capped U6 RNA (made in vitro as in Fig. 2) was injected into oocyte nuclei that had been isolated under mineral oil (*6*). After incubation at 18°C for 6.5 hours, the 5′ caps of the RNAs were analyzed as in Fig. 1B. The RNAs present in the total sample (T, lane 1) and the precipitate (P, lanes 2 and 4) and supernatant (S, lanes 3 and 5) fractions were separated by polyacrylamide gel electrophoresis.

in enucleated *X. laevis* oocytes (*11*). However, we find that trimethylation of U3 RNA in the cytoplasm is much slower than it is in the nucleus; this is in contrast with trimethylation of pre-U1 RNA, which is efficiently trimethylated in the cytoplasm (Fig. 2) (*8*). Because proteins that normally function in oocyte nuclei (but are synthesized and perhaps stored in the cytoplasm) may adventitiously modify U3 RNA in the cytoplasm, the physiological significance of cytoplasmic trimethylation of U3 RNA is unclear.

Differences in 5′ cap modification between U3 RNA and other snRNAs have also been detected in other experiments with hamster (tsBN2) tissue-culture cells. In that case, loss of RCC1 protein resulted in the inhibition of export of several snRNAs from the nucleus. This treatment inhibited trimethylation of pre-U1 and pre-U2 snRNAs but not pre-U3 RNA (*12*). These results indicate that trimethylation of pre-U3 RNA also occurs in the nuclei of mammalian somatic cells.

Although several nucleolar snRNAs have $m^{2,2,7}$G caps (*13*), others do not. For example, some of these RNAs arise from the processing of intronic sequences in pre-mRNAs, (*5, 14*) and have 5′ monophosphate termini (*15*). Moreover, in plants, where pre-U3 RNA is made by RNA Pol III (*16*), the 5′ end is modified by the addition of a methyl group to make a γ-mpppG cap (*17*). This is the same 5′ modification that occurs to U6 RNA (*18*), an RNA that also does not exit the nucleus (*1, 19*).

The discovery that U3 RNA remains in the nucleus where it undergoes trimethylation raises the question of why some precursors of snRNAs are exported from the nucleus and others are not. Generally, RNAs undergo maturation in cell compartments that are distinct from those in which they function. For example, spliceosomal snRNAs, which function in the nucleoplasm, are matured in the cytoplasm. By analogy, U3 RNA, which functions in the nucleolus (*20*), is likely to be matured in the nucleoplasm. So far, the location and identity of proteins (*21*) responsible for the stability, cap trimethylation, or nucleolar localization of U3 RNA are unknown. Several nucleolar snRNAs made by RNA Pol II, such as U3, U8, and U13 RNAs (*13*), have common sequences that are good candidates for modification signals and nucleolar localization signals. We propose that, like U3 RNA, these other $m^{2,2,7}$G-capped nucleolar snRNAs undergo maturation solely within the nucleus.

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Role of Bone Marrow–Derived Cells in Presenting MHC Class I–Restricted Tumor Antigens

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Many tumors express tumor-specific antigens capable of being presented to $CD8^+$ T cells by major histocompatibility complex (MHC) class I molecules. Antigen presentation models predict that the tumor cell itself should present these antigens to T cells. However, when conditions for the priming of tumor-specific responses were examined in mice, no detectable presentation of MHC class I–restricted tumor antigens by the tumor itself was found. Rather, tumor antigens were exclusively presented by host bone marrow–derived cells. Thus, MHC class I–restricted antigens are efficiently transferred in vivo to bone marrow–derived antigen-presenting cells, which suggests that human leukocyte antigen matching may be less critical in the application of tumor vaccines than previously thought.

Despite the presence of tumor antigens capable of being recognized by T cells (*1*), the persistent growth of tumors in their host indicates that T cells specific for their antigens have not been appropriately activated in vivo (*2*). Dissection of the immune response induced by vaccination with either irradiated or genetically modified tumor cells has shown that, as with other antigens, both $CD4^+$ and $CD8^+$ T cells are involved in generating the systemic antitumor response (*3, 4*). Most likely, efficient

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priming of anti-tumor immune responses results from collaboration between activated tumor-specific $CD4^+$ cells and $CD8^+$ cells activated in proximity to each other.

In many tumor vaccine models, the requirement for colocalized activation of helper and cytotoxic T lymphocytes (CTLs) presents an apparent paradox. Although T cell priming is thought to take place in the lymph nodes draining the vaccine site, tumor cells are rarely found in these draining lymph nodes. In the case of MHC class II–restricted $CD4^+$ T cell priming, antigen-presenting cells (APCs) pick up antigens in the extracellular space and carry them to the draining lymph nodes, where they are processed and presented to T cells (*5*). The mechanisms of priming MHC class I–restricted $CD8^+$ cells are less clear. The notion that host-derived APCs can efficiently ingest exogenous antigens for processing and presentation on MHC I to $CD8^+$ cells in vivo has been considered improbable because the defined cellular pathways for MHC I antigen presentation require that the antigen be expressed endogenously in the cytoplasm or endoplasmic reticulum of the cell that presents it (*6*). Thus, for a tumor-specific antigen current models would dictate that the tumor cell must present the MHC class I epitopes itself (*7*).

However, cross-priming experiments have shown that bystander cells can present MHC class I–restricted minor histocompatibility antigens in vivo (*8*). It is unclear whether cross-priming is a minor bystander phenomenon or a critical pathway in the priming of MHC class I–restricted T cells. In the case of nonhematopoietic tumor cells, all of the tumor-specific antigen is expressed by a nonprofessional APC (one that does not have antigen presentation as its main function). We therefore designed a set of experiments to determine the relative role of tumor- versus host-derived APCs in the priming of immune responses to MHC class I–restricted tumor antigens.

We generated a paired set of MHC class I^- and MHC class I^+ tumors. The C57BL/6(B6)-derived B16 melanoma variant B78H1 does not express classical class I heavy chain genes (*9*). Transfection with the genes for the heavy chains of K^b, D^b, or both restores expression of the corresponding class I molecule on the surface (designated MHC class I^+ B78H1) and renders the cells susceptible to killing by H-2^b–specific alloreactive T cells (*9*). We first compared the relative abilities of MHC class I^- B78H1 or MHC class I^+ B78H1 cells to prime mice for the generation of CTLs capable of lysing MHC class I^+ B78H1 target cells in vitro (*10*). Irradiated tumor cells transduced with granulocyte-macrophage colony-stimulating factor (GM-CSF) were used because GM-CSF–producing vaccines generate more potent anti-tumor immune responses than irradiated wild-type vaccines (*4*). Equivalent CTL activity, blockable by monoclonal antibodies to CD8, was induced by both the MHC class I^- and the MHC class I^+ B78H1 variants transduced with GM-CSF (Fig. 1).

A parallel set of in vivo protection experiments was then done in which mice immunized with either the MHC class I^- B78H1·GM-CSF or the MHC class I^+ B78H1·GM-CSF variant were later subjected to lethal doses of live MHC class I^+ B78H1 tumors (Fig. 2A). Again, equivalent in vivo protection was generated with either the MHC class I^- or class I^+ vaccines. In vivo experiments in which different T cell subsets were depleted confirmed that for both the class I^- and class I^+ vaccines, systemic protection against the MHC class I^+ B78H1 challenge was dependent on $CD8^+$ T cells. Because irradiated wild-type B78H1 cells are moderately immunogenic, it was possible to compare the in vivo immunization efficacy of vaccines that do not produce GM-CSF by decreasing (by 66%) the dose of live tumor cells used in challenging vaccinated animals (Fig. 2B). Irradiated, nontransduced MHC class

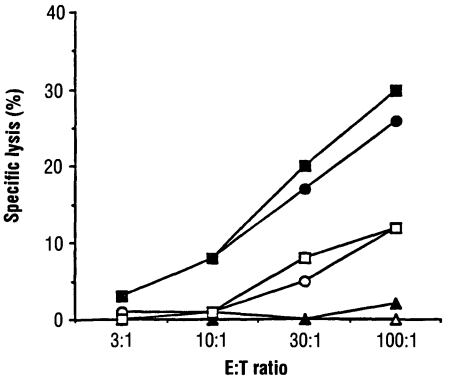

Fig. 1. Generation of CTLs against MHC class I^+ B78H1 cells after immunization with class I^- or class I^+ variants of GM-CSF–transduced B78H1 (B78H1·GM-CSF) cells. C57BL6 mice were immunized subcutaneously in the left flank with 1×10^6 irradiated (50 Gy) cells, either MHC class I^- or MHC class I^+ B78H1·GM-CSF. After 2 weeks, splenocytes were removed and cultured for 5 days with mitomycin C–treated MHC class I^+ B78H1 cells in the presence of interleukin-2. At the end of the culture, live cells were mixed with ^{51}Cr-labeled MHC class I^+ B78H1 targets at different effector-to-target ratios (E:T ratios) with or without the addition of anti-CD8 in a 4-hour, ^{51}Cr release assay. Black squares indicate immunization with class I^+ B78H1·GM-CSF; black circles, immunization with class I^- B78H1·GM-CSF; white squares, immunization with class I^+ B78H1·GM-CSF and block with anti-CD8 (mAb 2.43); white circles, immunization with class I^- B78H1·GM-CSF and block with anti-CD8; black triangles, no immunization; and white triangles, no vaccine with anti-CD8 treatment.

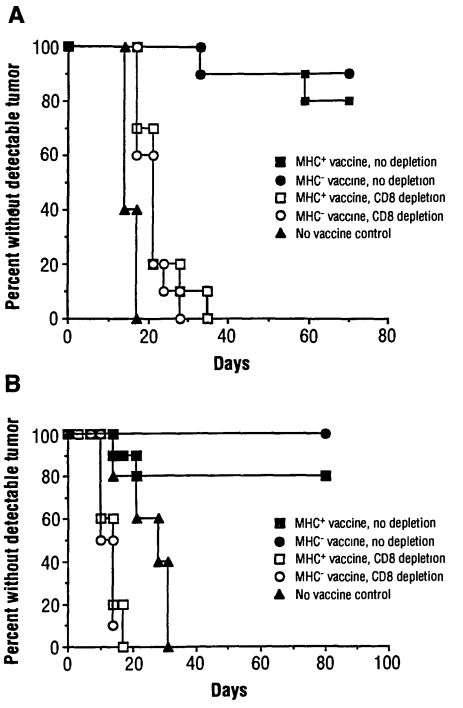

Fig. 2. Protective immunity and survival against MHC class I^+ B78H1 challenge induced by either MHC class I^- B78H1 or MHC class I^+ B78H1 vaccines. (**A**) C57BL/6 mice were injected subcutaneously in the left flank with 1×10^6 irradiated (50 Gy) B78H1·GM-CSF cells (class I^-, circles; class I^+, squares). Animals were challenged 2 weeks later in the right flank with 1×10^6 live MHC class I^+ B78H1 cells. (**B**) C57BL/6 mice were injected subcutaneously in the left flank with either 1×10^6 irradiated (50 Gy) nontransduced MHC class I^- B78H1 or MHC class I^+ B78H1 vaccines. Animals were challenged 2 weeks later in the right flank with 3.3×10^5 live MHC class I^+ B78H1 cells. For the results shown in both (A) and (B), the immunization-challenge experiments were done in intact (black symbols) and CD8-depleted (white symbols) mice. Tumor growth was assessed twice a week by inspection and palpation. The data are presented as Kaplan-Meier plots. Triangles indicate no vaccine control. Cells for injection were harvested from in vitro culture by trypsinization after limited expansion and washed three times in serum-free Hanks' balanced salt solution. All injections were in 0.1 ml given subcutaneously in the left (vaccine) or right (challenge) flank. All experiments included 10 mice per group, and each was repeated at least once. Mice were monitored twice weekly and killed after development of tumors. CD8-depleted mice were generated by twice weekly injection of the CD8 mAb 2.43 beginning 1 week before vaccination (*4*). No $CD8^+$ cells could be detected in the depleted mice by analysis with flow cytofluorometry.

I^- B78H1 and MHC class I^+ B78H1 vaccines protected equivalently against challenge with live MHC class I^+ B78H1 tumor cells, both in a CD8-dependent fashion.

Thus, expression of MHC class I by an immunizing tumor cell was unnecessary for the priming of tumor-specific $CD8^+$, MHC class I–restricted cells, as measured both in vitro and in vivo. Although our results suggest that tumor antigens have been efficiently transferred to host-derived MHC class I^+ cells, it was also possible that the vaccine exclusively primes MHC class II–restricted $CD4^+$ tumor-specific T cells and that activation of CD8 cells is occurring either in the stimulation phase in vitro or at the site of the MHC class I^+ tumor challenge in vivo.

To more definitively address the issue of MHC class I–restricted tumor antigen presentation during priming, we explored a model system in which a specific antigen with known MHC class I epitopes was expressed by the tumor. Influenza nucleoprotein (NP) is a convenient model antigen for this purpose because it has a predominant MHC class I epitope in *H-2*^{b} mice [NP(366–374)] presented by H-2D^b] and a predominant epitope in *H-2*^{d} mice [NP(147–155) presented by H-2K^d] (*11*).

The NP gene was introduced into the BALB/c-derived colon carcinoma, CT26, rendering it sensitive to lysis by H-2^d–restricted, but not H-2^b–restricted, CTLs specific for NP. The expression of NP did not change the in vivo growth characteristics of the tumor. Therefore, the NP gene product appears to resemble natural tumor antigens in that it is capable of being recognized by T cells yet does not alter the in vivo growth properties of the tumor. Although GM-CSF gene transduction enhances the vaccine potency of CT26, irradiated nontransduced CT26 vaccines also induce immune responses (*4*); thus, the specific contribution of local GM-CSF secretion to MHC class I–restricted antigen priming can also be assessed.

Two sets of bone marrow chimeras were produced: BALB/c (*H-2*^{d}) → (BALB/c × B6)F_1 (*H-2*^{dxb}) and B6 (*H-2*^{b}) → (BALB/c × B6)F_1 (*H-2*^{dxb}). Allele-specific antibody staining after reconstitution confirmed that all of the cells derived from bone marrow were donor-derived. Because the recipients are F_1, they are tolerant to both H-2^d and H-2^b alloantigens, and their T cells should be positively selected for both H-2^b and H-2^d restriction. However, the bone marrow–derived APCs were exclusively H-2^d in one case and H-2^b in the other case (*12*).

In the first set of experiments these chimeras, as well as nonchimeric (BALB/c × B6)F_1 (*H-2*^{dxb}) mice, were immunized with irradiated CT26-NP. After immunization, splenic T cells were removed and stimulated with either NP(147–155) or NP(366–374) plus *H-2*$^{dxb}F_1$ splenocytes. The T cells were then tested for lysis of ^{51}Cr-labeled NP(147–155)-sensitized P815(*H-2*^{d}) targets or NP(366–374)-sensitized MC57G(*H-2*^{b}) targets. With this experimental design, distinct results are predicted depending on whether the tumor cell itself is the APC for MHC class I–restricted NP epitopes or whether host bone marrow–derived cells are the APCs for MHC class I–restricted NP epitopes. If the CT26 tumor cell is the exclusive APC, then in all circumstances the CTLs generated will be specific for the NP(147–155) epitope. In contrast, if bone marrow–derived APCs are the exclusive APCs in the priming of the CTL response to NP, the CTLs generated should be exclusively specific for NP(147–155) in the *H-2*^{d} → *H-2*^{dxb} chimera and for NP(366–374) in the *H-2*^{b} → *H-2*^{bxd} chimera. If both the tumor cell and host bone marrow–derived cells can act as APCs for the MHC class I epitopes on NP, then a mixed result is expected.

The specificity of NP-specific CTLs was determined exclusively by the MHC haplotype of the bone marrow donor (Fig. 3A). In the CT26-NP–immunized F_1 animals, both specificities were seen. In the *H-2*^{d} → *H-2*^{dxb} chimera, all of the CTLs were specific for the NP(147–155) epitope. In the *H-2*^{b} → *H-2*^{bxd} chimera, where the MHC haplotype of the bone marrow–derived APCs is exclusively H-2^b and different from the haplotype of CT26 (*H-2*^{d}), all of the NP-specific activity was directed at the D^b-restricted NP(366–374) epitope. Similar results were seen whether or not the GM-CSF–producing vaccine was used (Fig. 3B), which indicates that presentation of MHC class I–restricted tumor antigens by bone marrow–derived cells was not a property solely related to the effects of paracrine GM-CSF secretion. When MHC class I^- B78H1·NP·GM-CSF cells were used to immunize (BALB/c × B6)F_1 mice, CTLs specific for both the NP(366–374) epitope and the NP(147–155) epitope were generated (*13*). Similar results were obtained when responder T cells were purified, ruling out the possibility that the apparent restriction of antigen presentation to bone marrow–derived MHC alleles was a result of selective presentation of NP nonapeptides by APCs from the immunized chimera relative to the F_1 splenocytes added to the in vitro stimulation phase of the assay (*14*).

Although earlier studies have shown that thymic epithelial elements that are radioresistant mediate positive selection (*15*), it was formally possible that in our

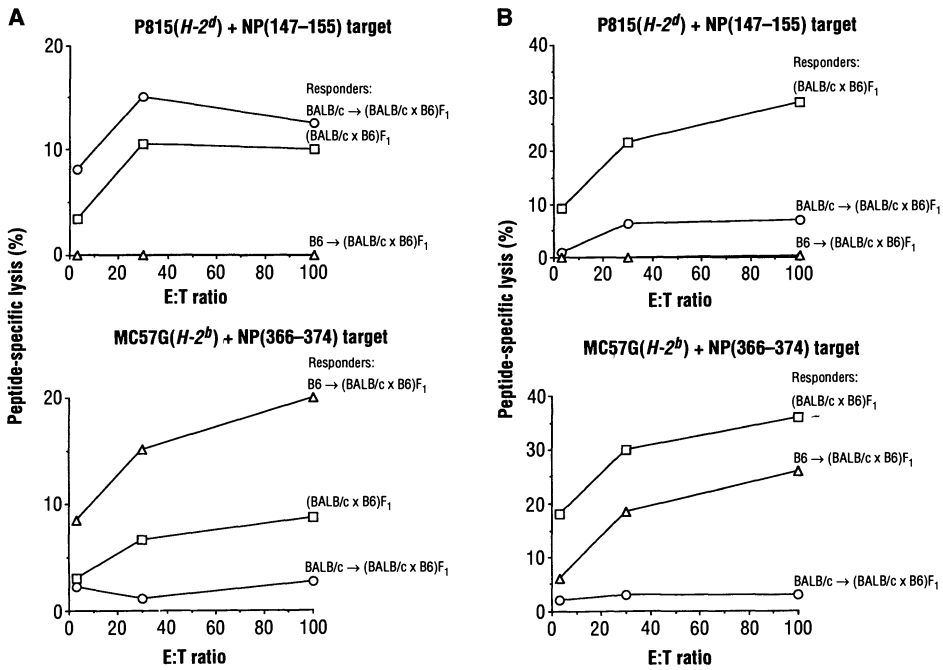

Fig. 3. Epitope specificity of T cells from mice immunized with CT26·NP. (BALB/c × B6)F_1 mice (squares), BALB/c (*H-2*^{d}) → (BALB/c × B6)F_1 (*H-2*^{dxb}) chimeras (circles), or B6 (*H-2*^{b}) → (BALB/c × B6)F_1 (*H-2*^{dxb}) chimeras (triangles) were immunized with either CT26·NP (**A**) or CT26·NP·GM-CSF (**B**). Splenocytes from these mice were then stimulated with either NP(147–155) (top graphs) or NP(366–374) (bottom graphs) + (BALB/c × B6)F_1 splenocytes. Cells were then mixed with ^{51}Cr-labeled P815 targets and NP(147–155) (*H-2*^{d}, top graphs) or MC57G targets and NP(366–374) (*H-2*^{b}, bottom graphs). Peptide-specific lysis represents the difference between the percent specific lysis of the target cells with the MHC-matched NP peptide and the percent specific lysis of the target cells with the MHC-mismatched peptide. In all cases, lysis of targets in the presence of the MHC-mismatched NP peptide was <5% at all effector:target ratios. Data are representative of three separate experiments for (A) and two separate experiments for (B).

chimeras the results obtained were due to selection of the T cell repertoire by bone marrow–derived thymic stromal elements. If such were the case, CD8 responses to NP epitopes presented by MHC class I alleles that were not expressed by bone marrow would not be observed because T cell precursors with those specificities were never selected during thymic development. To rule out this possibility, the $H\text{-}2^b \rightarrow H\text{-}2^{dxb}$ chimeras were tested for their ability to be primed against H-2^d–restricted DBA/2 minor histocompatibility antigens after injection with DBA/2 splenocytes (*16*). The H-2^d–restricted DBA/2 minor histocompatibility antigen responses were observed, which indicates that H-2^d–restricted T cells developed in our $H\text{-}2^b \rightarrow H\text{-}2^{dxb}$ chimeras (Fig. 4). In contrast to tumor antigens expressed by nonhematopoietic tumors, the minor histocompatibility antigens were already expressed endogenously by bone marrow–derived APCs within the immunizing splenocyte population. They can therefore be presented by the MHC class I antigen of these APCs using the classical endogenous pathway without requiring cell transfer.

The data presented here indicate that the priming of an immune response against an MHC class I–restricted antigen that is expressed exclusively in nonhematopoietic cells, such as a tumor antigen, involves the transfer of that antigen to a host bone marrow–derived cell before its presentation to $CD8^+$ T cells. Thus, the cross-priming phenomenon is not a minor bystander effect but rather may represent an important mechanism for antigen presentation during in vivo priming. Our data provide an explanation for the ability of tumor variants that have lost their MHC to prime against tumor antigens normally presented by the down-regulated MHC allele (*17*). Our results are not specific to immunization with GM-CSF–transduced tumor vaccines, which suggests that transfer of MHC class I–restricted tumor antigens to host bone marrow–derived APCs may be a critical feature of many types of tumor vaccines. It has been proposed that the priming of $CD8^+$ CTLs by bone marrow–derived APCs would be detrimental, because the APCs themselves would be killed (*18*). However, if the critical priming APCs were continuously generated by the differentiation of circulating precursors, then they would be expendable. Support for such an idea comes from the demonstration that GM-CSF can induce circulating precursors in both mice and humans to differentiate into dendritic cells (*19*).

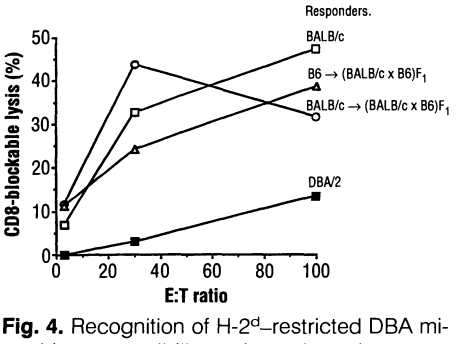

Fig. 4. Recognition of H-2^d–restricted DBA minor histocompatibility antigens by splenocytes from C57BL6 ($H\text{-}2^b$) → (BALB/c × B6)F_1 ($H\text{-}2^{dxb}$) chimeras. BALB/c ($H\text{-}2^d$) (□), DBA/2 ($H\text{-}2^d$) (■), BALB/c ($H\text{-}2^d$) → (BALB/c × B6)F_1 ($H\text{-}2^{dxb}$) (○), and B6 ($H\text{-}2^b$) → (BALB/c × B6)F_1 ($H\text{-}2^{dxb}$) (△) chimeras were immunized with DBA/2 splenocytes. T cells from these mice were subsequently stimulated with DBA/2 splenocytes in vitro. Cells were then mixed with ^{51}Cr-labeled P815($H\text{-}2^d$) in a 4-hour CTL assay. The percent CD8-blockable lysis was the difference between the percent of overall lysis and the percent lysis of the target cells in the presence of mAb 2.43 (anti-CD8).

Because of the common difficulty in obtaining large amounts of autologous tumors, many investigators have proposed and undertaken the development of allogeneic tumor vaccines (*22*). The rationale behind this strategy comes from the identification of common tumor antigens, a finding well-documented in human melanoma. For many of the allogeneic tumor vaccine protocols, patient enrollment has been restricted to individuals sharing at least one human leukocyte antigen (HLA) class I allele with the vaccine. In most cases, the shared HLA allele is HLA-A2, which is expressed by more than 50% of Caucasians. Our results raise the possibility that for these vaccine strategies, it may not be necessary to match MHC alleles because the appropriate epitopes would be selected by the host's APCs, which are MHC-matched to the individual's tumor. In considering the use of HLA-mismatched tumor vaccines, the ultimate immune response can be modified by allogeneic effects not present in our experiments. Recent data, however, suggest that allogeneic effects may enhance anti-tumor immune responses (*23*).

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10. B78H1 cells [L. Graf, P. Kaplan, S. Silagi, *Somatic Cell Mol. Genet.* **10(2)**, 139 (1984)] were cultured in vitro in RPMI media, supplemented with 10% fetal calf serum (FCS), penicillin-streptomycin (50 U/ml), L-glutamine (2 mM), sodium pyruvate (1 mM), and nonessential amino acids (0.1 mM) and grown at 37°C in 5% CO_2. MHC class I^+ variants were generated by the introduction of both K^b and D^b heavy chain complementary DNAs as a coprecipitate with calcium phosphate and then screened by flow cytofluorometry (*9*). Production of GM-CSF was achieved by retroviral transduction of the MHC variants with MFG GM-CSF and was quantitated as described (*4*). GM-CSF transductants produced between 100 and 200 ng of GM-CSF per 10^6 cells for 24 hours. CTL assays were performed with splenocytes. MHC class I^+ B78H1 stimulators were added after incubation with mitomycin C (50 μg/ml for 1 hour at 37°C, then three washes in RPMI and 10% FCS). Recombinant murine interleukin-2 (10 U/ml) was added to each well. After 5 days, ^{51}Cr release assays with and without blocking by CD8 antibodies were done (*4*).
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12. On day 0, (BALB/c × B6)F_1 female recipient mice (from the National Cancer Institute; 6 to 8 weeks old) were irradiated with 10 Gy (units of absorbed dose of ionizing radiation). On days 0 and 2, the

F_1 recipient mice were injected intraperitoneally with the NK1.1 monoclonal antibody PK136 to inhibit Hh barrier rejection (*9*). Bone marrow of either female BALB/c or C57BL/6 mice was depleted of T cells with monoclonal antibody (mAb) J1j (anti–Thy-1), mAb C3PO (anti-CD2), mAb RL172 (anti-CD4), and mAb 3.155 (anti-CD8). Approximately 2×10^6 to 4×10^6 bone marrow cells were injected into recipient F_1 mice. Four months after the bone marrow reconstitution, one mouse from each of the two bone marrow constructs was killed. Splenocytes were stained for MHC class II molecules I-E^d and I-A^b with mAb 14.4.4 [immunoglobulin G2a (IgG2a)] and mAb Y-3P (IgG2a), respectively, to confirm complete reconstitution with donor bone marrow. The CT26·NP cell line was produced by infection of CT26 with a Moloney-based defective recombinant retrovirus, provided by E. Gilboa [J. V. Fetten, N. Roy, E. Gilboa, *J. Immunol.* **147**, 2697 (1991)], containing the NP gene from the PR8 influenza strain and a neomycin-resistance gene. Infected cultures were selected in G418 (400 μg/ml). Individual colonies were tested for NP expression by NP mAb staining and lysis by NP + H-$2K^d$–specific CTL lines. A clone positive for both antibody staining and lysis was chosen for further experiments. Cells (1×10^6 CT26·NP + 1×10^6 CT26 wild-type or 1×10^6 CT26·NP + 1×10^6 CT26·GM-CSF) were irradiated (50 Gy) and injected subcutaneously in the left flank of the chimeras. Spleens were removed from the mice 2 weeks after the initial immunization, and splenocytes were cultured in vitro with either NP(147–155) peptide or NP(366–374) peptide in the presence of interleukin-2 and splenocytes from a (BALB/c × C57BL/6)F_1 mouse. After a 7-day in vitro incubation, the splenocytes were harvested and plated in triplicate on a 96-well V-bottom microtiter plate at various effector-to-target ratios. Surrogate target cells, P815(*H-2*^{d}) and MC57G(*H-2*^{b}), were labeled with ^{51}Cr and added to the effector cells (3000 cells/well) in the presence of synthetic NP(147–155) peptide (500 pg/ml) or NP(366–374) peptide (50 pg/ml). After 4-hour incubation of the cell mixture at 37°C and in 5% CO_2, the media were harvested and counted on a gamma counter.

13. A. Y. C. Huang *et al.*, unpublished data. B78H1·NP produced a small but measurable titer of transmissible helper virus. Thus, in the case of B78H1·NP, in vivo virus transmission cannot be ruled out as a potential mechanism for NP presentation on host-derived MHC class I molecules. In contrast to B78H1·NP, CT26·NP did not produce any transmissible helper virus; thus, in vivo virus transmission does not account for the results shown in Fig. 3.
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16. Naive C57BL6 (*H-2*^{b}) → BALB/c × B6 (*H-2*^{dxb}) female chimeric mice were injected intraperitoneally (IP) with 1.5×10^7 live DBA/2 splenocytes from a female mouse in 0.1 ml of 1× Hanks' balanced salt solution. As controls, naive female BALB/c (*H-2*^{d}) mice, BALB/c (*H-2*^{d}) → BALB/c × B6 (*H-2*^{dxb}) chimeric mice, and DBA/2 mice were also injected IP with the same number of live DBA/2 splenocytes. Two mice were used in each group in one single experiment. Two weeks after immunization, splenocytes from immunized mice were stimulated, and P815 (derived from DBA/2 mice) targets were labeled with ^{51}Cr and added to the effector cells at a concentration of 3000 cells/well.
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Cloning of a T Cell Growth Factor That Interacts with the β Chain of the Interleukin-2 Receptor

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A cytokine was identified that stimulated the proliferation of T lymphocytes, and a complementary DNA clone encoding this new T cell growth factor was isolated. The cytokine, designated interleukin-15 (IL-15), is produced by a wide variety of cells and tissues and shares many biological properties with IL-2. Monoclonal antibodies to the β chain of the IL-2 receptor inhibited the biological activity of IL-15, and IL-15 competed for binding with IL-2, indicating that IL-15 uses components of the IL-2 receptor.

The proliferation and differentiation of T lymphocytes is regulated by cytokines that act in combination with signals induced by the engagement of the T cell antigen receptor. A principal cytokine used by T cells during immune responses is IL-2 (*1*), itself a product of activated T cells. IL-2 also stimulates a number of other cell types, including B cells, monocytes, lymphokine-activated killer cells, natural killer cells, and glioma cells (*2*). IL-2 interacts with a specific cell surface receptor (IL-2R) that contains at least three subunits, α, β, and γ (*3*). A number of other cytokines also stimulate the proliferation of T cells, and recent evidence has suggested that the receptors for several of these cytokines include the γ chain of IL-2R (*4*). We describe a cytokine whose biological activity resembles that of IL-2 and which also uses components of IL-2R.

In the course of testing supernatants from a simian kidney epithelial cell line, CV-1/EBNA (*5*), for cytokine activity, it was discovered that these cells produced a soluble factor capable of supporting proliferation of the IL-2–dependent cell line, CTLL (*6*). The protein responsible for this biological activity was purified from serum-free supernatants of CV-1/EBNA cells by a combination of hydrophobic interaction and anion-exchange chromatography, high-pressure liquid chromatography (HPLC), and SDS–polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1A, bottom). Analysis of the biological activity in the final HPLC fractions (Fig. 1A, top) and in horizontal slices of the SDS-PAGE gel (Fig. 1B) indicated that the activity coincided with a band of approximately 14 to 15 kD. This protein was blotted to a polyvinylidene difluoride (PVDF) membrane, and the NH_2-terminal residues were directly sequenced. We used degenerate oligonucleotide primers on the basis of this amino acid sequence and the polymerase chain reaction (PCR) to clone a 92–base pair (bp) complementary DNA (cDNA) fragment from mRNA of CV-1/EBNA cells. This cloned cDNA fragment was used to probe a plasmid library containing cDNA inserts prepared from mRNA of CV-1/EBNA cells. A full-length cDNA clone was obtained that encodes a 162–amino acid precursor polypeptide containing an unusually long 48–amino acid leader sequence that is cleaved at the experimentally determined NH_2-terminus to form the mature protein. The amino acid sequence (Fig. 1C) exhibits no similarity to any protein in GenBank or EMBL databases. However, IL-15 and IL-2 sequences were compared to determine if there might be structural similarities. The three-dimensional (3D) structure of IL-2 (*7*) consists of a four-helix bundle, and IL-2 belongs to the helical cytokine family (*8*). Although the members of this family show no sequence similarity, they show many structural similarities, and IL-15 is no exception. The secondary struc-

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