Induction of Protective CTL Immunity against Peptide Transporter TAP-Deficient Tumors through Dendritic Cell Vaccination

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Abstract

A large proportion of human cancers show deficiencies in the MHC class I antigen-processing machinery. Such defects render tumors resistant to immune eradication by tumoricidal CTLs. We recently identified a unique population of CTL that selectively targets tumor immune-escape variants through recognition of MHC-presented peptides, termed TEIPP (T cell epitopes associated with impaired peptide processing), expressed on cells lacking functional TAP-peptide transporters. Previously, we showed that vaccination with TEIPP peptides mediates protection against TAP-deficient tumors. Here, we further explored the concept of TEIPP-targeted therapy using a dendritic cell (DC)-based cellular vaccine. Impairment of TAP function in DC induced the presentation of endogenous TEIPP antigens by MHC class I molecules, and immunization with these DCs protected mice against the outgrowth of TAP-deficient lymphomas and fibrosarcomas. Immune analysis of vaccinated mice revealed strong TEIPP-specific CTL responses, and a crucial role for CD8+ cells in tumor resistance. Finally, we show that TEIPP antigens could be successfully induced in wild-type DC by introducing the viral TAP inhibitor UL49.5. Our results imply that immune intervention strategies with TAP-inhibited DC could be developed for the treatment of antigen processing–deficient cancers in humans. [Cancer Res 2007;67(18):8450–5]

Introduction

The efficacy of antitumor CTL critically depends on functional processing and presentation of tumor antigens by the malignant cells. The MHC class I antigen-processing machinery involves proteolytic enzymes, transport molecules, specific chaperones, and proteins responsible for quality control (reviewed in ref. 1). A deficiency at one of the consecutive steps of this pathway impairs the display of the antigenic peptide repertoire on MHC class I molecules at the cell surface. Defects in processing molecules, e.g., proteasome subunits, TAP or tapasin, have been observed in a wide variety of human cancers (2–4). For example, TAP deficiencies have been found in up to 90% of metastatic lesions of cervical carcinoma. The mechanisms behind the failure to express these components are not completely understood, but activated oncogene action might play an important role (5–8). Defects in the antigen-processing machinery allow tumors to escape from CTL recognition and elimination (9–11). The occurrence of such defects is often associated with tumor progression, suggesting a role for CTL immunity in the selection of escape variants (12, 13). Notably, impairments in antigen processing will also undermine the efficacy of CTL-based immunotherapies that are currently under evaluation in clinical trials.

We recently reported that a subset of conventional CTL is capable of eradicating tumor immune-escape variants. These CTL recognize immunogenic peptides derived from commonly expressed self proteins that are selectively presented by MHC class I molecules on tumors with antigen-processing defects, such as TAP deficiencies (14, 15). Importantly, although the self proteins are present in normal cells, these peptides emerge on MHC class I molecules of cells with defective antigen-processing machinery. We have referred to these antigens as “T cell epitopes associated with impaired peptide processing” (TEIPP). Vaccination with a minimal peptide-epitope has been shown to prevent the outgrowth of TAP-deficient tumor cells, demonstrating that TEIPP epitopes act as true tumor rejection antigens (14). However, the effect of peptide vaccination is limited due to the current lack of additional molecularly defined TEIPP antigens. Because dendritic cells (DC) are pivotal for the initiation and orchestration of T lymphocyte responses and are prime candidates in immunotherapy for cancer (16, 17), we here aimed at the exploitation of genetically modified DC for the induction of protective TEIPP-directed CTL immunity. Importantly, the application of DC-based vaccines does not require prior identification of TEIPP antigens. Moreover, loading strategies leading to efficient antigen presentation are not needed when TEIPPs are targeted because the source proteins are commonly expressed housekeeping proteins and are most likely already present in DC. Our preclinical results set the stage for the development of new DC-based vaccines for the treatment of human malignancies frequently associated with antigen-processing defects.

Materials and Methods

Cell lines and mice. Lymphomas RMA, RMA-S (TAP2-deficient), EC7.1 (TAP2- and class I–deficient), C4.4-25 (β2m-deficient), and TAP1-deficient MCA fibrosarcoma (clone MTAP1A) have been previously described (14). D1 cells are growth factor–dependant immature DCs (18). The mouse TAPI gene and the bovine herpes virus 1–derived gene UL49.5 were introduced using retroviral vectors as previously described (14). TEIPP-specific CTL clones used in this study all display similar specificity. Control CTL c17 recognizes the tumor-specific peptide NKGENAQAI (14). CTL were restimulated weekly with irradiated tumor cells (RMA-SB7 or RMA), 10 CU/mL of human rIL-2 (Cetus), and irradiated splenocytes. Natural killer (NK) cells were driven from the adherent fraction of rIL-2 (800 CU/mL)-treated splenocytes on
day 4. All cell lines were cultured in Iscove's modified Dulbecco's medium (Bio Whittaker Europe), supplemented with 8% heat-inactivated fetal calf serum (Life Technologies), 2 mmol/L of l-glutamine (ICN Biomedicals, Inc.), 100 IU/ml of penicillin (Yamanouchi Pharma), and 30 μmol/L of 2-mercapto-ethanol (Merck) at 37°C in humidified air with 5% CO2.

All cell lines were derived from mice on a C57BL/6 (B6) background bred under specific pathogen-free conditions in the in-house facility of the Department of Microbiology, Tumor and Cell Biology of the Karolinska Institutet, Stockholm, Sweden. Experiments were approved by the local animal ethical committee in Stockholm.

CTL and NK assays and flow cytometry. Measurements of IFN-γ release and cytotoxic capacity against 51Cr-labeled targets were described previously (14). Presentation of OVA peptide SIINFEKL by DC was measured in a CPRG assay using B3Z T-cell hybridoma, as described previously (18). OVA-immunocomplexes (1 μg/mL) were loaded onto 2 × 10^5 D1 cells and B3Z reactivity was examined 48 h later. For NK cell assays, cells were stained with PKH26 membrane dye (Sigma) and mixed in a 1:1 ratio with nonlabeled cells and NK cells for 18 h. Efficiency of lysis was measured by flow cytometry. Cell surface molecules were determined with flow cytometry using a FACSCalibur (Becton Dickinson). The following antibodies used were from BD: anti-Db (clone 28-14-8), anti-CD40 (clone 3/23), and anti-MHC II (M5/114).

Preparation and immunization of DC. Bone marrow–derived DCs were prepared as previously described (19). Briefly, bone marrow was cultured for 6 days in medium supplemented with murine recombinant granulocyte macrophage colony-stimulating factor (Peprotech). The cells were harvested and incubated overnight in medium containing 1% normal mouse serum and 1,000 IU/mL of IFN-γ. All DC cultures contained at least 75% CD11c+ and MHC class II+ cells and expressed high levels of CD40, CD80, and CD86. DC were injected s.c. at a dose of 1 × 10^6 cells/mouse. Spleens of immunized mice were cultured with RMA-S.B7 stimulator cells and tested for cytolytic activity against tumor cells. For tumor protection experiments, immunized mice were challenged s.c. 14 days after the last immunization either with RMA-S tumor cells that were passed in vivo (1.5 × 10^6 in nondepleted and 3 × 10^6 in NK-depleted animals) or with MCA tumor cells that were incubated in vitro in normal mouse serum (1 × 10^6). Tumors were measured twice a week and mice were euthanized when tumors reached a volume of 1,000 mm^3. NK cells were depleted by repeated injections of anti-NK1.1 antibody (clone PK136), 200 μg/mouse/wk. CD8 cells were depleted by injections with anti-CD8 antibody (clone YTS169), 500 μg/mouse/wk.

Results

TAP-deficient DC induce TEIPP-specific CTL. TEIPP peptides are derived from widely expressed "self" proteins, but only emerge in MHC class I molecules on cells with impaired processing function, regardless of malignant transformation. In view of the crucial role of DC in the initiation of T cell responses and their application in anticancer vaccines, we assessed the display of TEIPP peptides by DC with impaired TAP function.

![Figure 1](https://example.com/figure1.png)
Bone marrow–derived DC from TAP1−/− mice, but not from the wild-type mice, were efficiently recognized by previously established TEIPP-specific CTL (Fig. 1A). Control CTL directed against RMA lymphoma cells, however, were not stimulated by wild-type nor TAP1−/− DC populations. These findings prompted us to test the capacity of these autologous DC to induce TEIPP-specific CTL responses in vivo. Mature DC from TAP1−/− mice were injected into syngeneic mice, and cytolytic activity in the spleens of recipients was tested against two prototypic tumor cells: TAP-deficient RMA-S cells, which do present TEIPP, and β2m-negative C4.4-25 cells, which do not present TEIPP (Fig. 2). All CTL cultures preferentially killed RMA-S cells, whereas reactivity against C4.4-25 cells was generally low, indicating that the immunization strategy resulted in the induction of TEIPP-specific CTL responses. NK cells, which are known to kill MHC class I low RMA-S and C4.4-25 targets, were depleted during vaccination to exclude potential confounding reactivity. Together, these results indicate that TAP-deficient DC are capable of generating TEIPP-specific CTL responses in vivo.

Immunization with TAP-deficient DC protects mice against TAP-negative tumors. On the basis of these data, we tested whether vaccination with TAP-deficient DC could protect mice from the outgrowth of processing-deficient tumors. Two different tumor types were chosen for this examination, the TAP2-deficient RMA-S lymphoma and a TAP1-deficient MCA fibrosarcoma. Both tumors were strongly recognized by previously established TEIPP-specific CTL clones, whereas the reactivity against TAP-expressing counterpart tumors was less efficient (Fig. 1B). The observation that tumors originating from different tissues are recognized by the same TEIPP CTL clones illustrates that their cognate peptide epitopes are derived from widely distributed self proteins, like the ceramide regulator Trh4 (14).

B6 wild-type mice were immunized with syngeneic bone marrow–derived DC from TAP1−/− or wild-type mice and challenged with a lethal dose of TAP-deficient RMA-S (Fig. 3A). All mice that received TAP-deficient DC were protected, whereas the majority of control mice developed progressively growing tumors (Fig. 3A). To determine that this protection was not exclusively mediated by NK reactivity, which is known to readily lyse TAP-deficient tumors, we repeated these experiments in NK-depleted mice (Fig. 3, left and right, RMA-S and MCA, respectively). On average, 50% of the mice were completely protected against a lethal tumor dose and most other mice displayed delayed tumor outgrowth. To further substantiate these findings, we examined the tumor protection capacity in CD8-depleted mice (Fig. 3C, left and right, RMA-S and MCA, respectively). CD8+ T cells indeed accounted for the prevention of the growth of TAP-deficient tumors. In conclusion, our data indicate that TAP-deficient DC could mediate protection against processing-deficient tumors through the activation of TEIPP CTL responses in vivo.

Viral inhibitors of TAP function induce the presentation of TEIPP. Thus far, we have employed DC with a genetic loss of TAP1 in our studies. Application of this concept in the clinic would, however, involve autologous DC that are rendered TAP-deficient.
To examine the feasibility of such an approach, we made use of an immune evasion protein that could inhibit TAP function in human as well as mouse cells (15, 20). Introduction of this UL49.5 gene resulted in a 40% to 50% reduction of surface MHC class I display (Fig. 4A, top), indicating that the inhibitor, at least partly, impaired TAP-mediated transport of peptides. No alterations were observed in the surface display of other molecules, such as CD40 (Fig. 4A, bottom). Importantly, TEIPP-specific CTL clones responded selectively against UL49.5-expressing DC (Fig. 4B), indicating that impairment of TAP in DC indeed induced the presentation of TEIPP peptides. UL49.5-DC were still capable of processing and presenting a TAP-dependent peptide from the OVA model antigen, confirming that TAP function was only partially inhibited (Fig. 4C).

Furthermore, due to this partial effect of UL49.5, the DC were relatively protected against NK-mediated lysis (Fig. 4D), which would prolong the survival and CTL-inducing activity of the DC in vivo (19). Together with other known TAP-inhibiting proteins, ICP47 and US6, the UL49.5 protein constitutes a formidable tool for the arsenal of a simultaneous CTL response towards TEIPP as well as conventional tumor antigens to protect against human tumors and their immune-escape variants.

Discussion

A major limitation of T cell–based immunotherapy for cancer is the frequent escape of tumor variants from protective immunity through loss of antigen presentation by MHC class I. Our work presented here provides a potential answer for this problem through the exploitation of TEIPP antigens. These antigens are peptide-epitopes presented by tumors with antigen-processing defects, and in that sense, constitute a novel category of tumor-associated antigens. Importantly, TEIPP-specific CTL do not inflict damage to healthy tissues (14), implying that these peptides are not, or are rarely, presented by normal cells. In this study, we show that upon blockade of the peptide transporter TAP, the presentation of TEIPP peptides could be induced on the surface of DC, which are potent initiators of T cell responses. This was anticipated because TEIPP epitopes are derived from household proteins, but are competed away from MHC class I presentation by the large flow of TAP-transported peptides in normal cells. Vaccination with these modulated DC resulted in a CD8+ T cell–dependent protection against tumor immune-escape variants of lymphoma and fibrosarcoma. The broad tissue distribution of TEIPP is a clear advantage for the application of this concept in the clinic, aiming at the prevention of selective outgrowth of processing-deficient tumor variants from a wide range of different tissues.

We previously showed that, after administration, TAP-deficient DCs are vulnerable to attack by NK cells, resulting in reduced vaccination efficacy (19). Importantly, we show here that the TAPI−/− DC were capable of inducing tumor protection in the presence of NK cells (Fig. 3A). These data suggest that enough DC survived in vivo to prime TEIPP-specific CTL. In addition, a partial block in TAP function by viral evasion molecules was efficient enough to induce TEIPP presentation, and at the same time, limited the sensitivity for NK-mediated lysis (see Fig. 4B and D). Moreover, in the search for mechanisms that may further protect DC against elimination by NK cells in vivo, we observed that stabilizing peptides

Figure 3. Immunization with TAPI−/− DC protects mice from the outgrowth of TAP-deficient tumor variants. A and B, B6 mice were injected with syngeneic DC from wild-type mice (DC wt), DC from mice with TAPI−/− genetic background (DC TAPI−/−), or saline solution (naive). These mice were challenged with TAP2-deficient RMA-S lymphoma cells (left) or TAP1-deficient MCA fibrosarcoma cells (right). Tumor cells gave rise to progressive tumor outgrowth and mice were sacrificed when tumors reached a volume of 1,000 mm3. NK cells were depleted in vivo with antibodies in (B) to prevent NK-mediated killing of administered TAP-negative tumor cells. The survival curves represent the combined results from two experiments with 10 mice in each group. The differences between the wild-type DC and TAPI−/− DC are statistically significant (log-rank analysis for (A), P < 0.0001; log-rank analyses for (B) P = 0.0044 (left) and P < 0.0001 (right)). C, CD8+ cells are responsible for the protection against TAP-deficient tumors. B6 mice were treated with syngeneic DC from wild-type mice (DC wt) or from TAPI−/− mice (DC TAPI−/−). In another group of mice, CD8+ cells were depleted by injection of specific monoclonal antibodies just before tumor challenge (DC TAPI−/− CD8 depleted). NK cells were depleted in all groups throughout the experiment. Survival curves differ with statistical significance from each other [log-rank analyses, P = 0.020, n = 10 (left) and P = 0.001, n = 5 (right)].
binding to the nonclassical MHC molecule Qa-1β (the mouse HLA-E homologue) strongly enhanced the life span of TAP-deficient DC through inhibiting signals via the CD94/NKG2A receptor on NK cells. In conclusion, our results show that vaccination with modulated DC could induce protection against the outgrowth of tumor escape variants. We speculate that similar DC-based vaccines can be used for eliciting TEIPP-specific CTL immunity in humans.

Figure 4. A viral TAP inhibitor induces the presentation of TEIPP antigens in DCs. A, DC (D1 cells) were retrovirally transduced with the UL49.5 gene (thin lines) or empty vector (thick lines). Cell surface expression of MHC class I (top) and CD40 molecules (bottom) was detected using specific antibodies. Filled histograms, background staining without antibodies. Mean fluorescence for each histogram is indicated. B, TEIPP-specific CTL clones recognize UL49.5-expressing DC (DC-UL49.5; D1 cells). IFN-γ release of the two independent TEIPP CTL clones upon cocultivation with TAP-deficient RMA-S cells was comparable for both CTL clones (13 and 17 ng/mL, respectively), indicating that the TEIPP peptide recognized by the CTL (right) was presented less efficiently in DC than in lymphoma cells. Points, mean results from one out of three comparable experiments done in triplicate; bars, SD. C, UL49.5-expressing DCs display residual TAP function and could still process and present the TAP-dependent OVA peptide. DC (DC-vector and DC-UL49.5) were loaded with OVA protein and presentation of the Kβ-binding SIINFEKL peptide was measured with the T cell hybridoma B3Z. Columns, mean results from one out of two comparable experiments done in triplicate; bars, SD. D, UL49.5-positive DCs are relatively resistant to lysis by NK cells. DC-UL49.5 cells (left) and NK-sensitive EC7.1 cells (right) were stained with a red dye and mixed with unstained DC vector cells. IL-2–activated NK cells were added (bottom) and percentage of lysis was calculated as compared with DC vector cells. This experiment was repeated twice with comparable outcomes.

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