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## Tumor-peptide-pulsed dendritic cells isolated from spleen or cultured in vitro from bone marrow precursors can provide protection against tumor challenge

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**Abstract** Dendritic cells (DC) purified from murine spleen or generated in vitro from bone marrow precursors were compared for their respective abilities to stimulate T cell responses and provide tumor protection in vivo. In vitro incubation with synthetic tumor peptide conferred on both DC populations the ability to induce proliferation of tumor-peptide-specific T cells in vitro. Spleen DC were reproducibly about twofold more effective than bone-marrow-derived DC in this assay. Both DC populations could also induce cytotoxic activity in vivo. In vitro cytotoxicity assays showed that, while cytotoxic activity induced by immunization with spleen DC was clearly peptide-specific, a high non-specific cytotoxic activity was consistently observed after immunization with bone-marrow-derived DC, whether peptide-pulsed or not. Regardless of such high non-specific activity in vitro, only tumor-peptide-pulsed DC could provide protection against subsequent inoculation of tumor cells. DC not pulsed with tumor peptide were ineffective. We conclude that DC isolated from spleen or generated in vitro from bone marrow precursors are suitable reagents for use in tumor vaccination studies.

**Key words** Dendritic cell · Tumor immunotherapy · Cytotoxic T lymphocyte · Immune response · Tumor peptide

### Introduction

Cytotoxic T lymphocytes (CTL) directed against tumor cells presenting unique peptides on major histocompatibility complex (MHC) class I molecules constitute a potentially powerful effector arm of host immunity to tumors (reviewed in [2, 3]). Although the recognition of peptide-

MHC-class-I complexes is sufficient to trigger tumor cell lysis, initial priming of CTL requires a “co-stimulatory” signal probably provided by B7 molecules on the antigen-presenting cell, and CD28 on the T cell [12]. Most tumors, particularly those of non-hemopoietic origin, do not express co-stimulatory molecules. Thus induction of antitumor CTL requires the transfer of tumor antigen to “professional” antigen-presenting cells capable of co-stimulation [13], possibly dendritic cells (DC). DC are highly specialized antigen-presenting cells that serve a sentinel function, internalizing and processing antigen for presentation to naive T cells (reviewed in [23]). Inflammatory mediators can promote the “maturation” of these cells [6, 7], resulting in migration to secondary lymphoid tissue and up-regulation of co-stimulatory and adhesion molecules for T cell activation [16].

Immunizing with DC that have been loaded with MHC-class-I binding peptides in vitro has been shown to be a powerful method for inducing specific CD8<sup>+</sup> T cell immunity [21]. Mice immunized with DC cultured in vitro from bone marrow precursors (BM DC) and loaded with tumor antigen generated a strong antitumor response [4, 18, 19, 22, 24]. In contrast, tumor antigen-loaded Langerhans cells freshly isolated from skin were incapable of eliciting such a response [18]. In view of the importance of the interplay between DC and mediators within their local environments in vivo in determining maturation status, it is likely that the functional characteristics of DC isolated by different experimental procedures will vary considerably. We have previously reported that cultured BM DC have a high stimulatory capacity characteristic of “mature” DC, and a high antigen-processing function characteristic of “immature” DC, whereas DC isolated from spleen have a low antigen-processing function and a high presenting function characteristic of mature cells [10]. In this paper we address the question of whether tumor-peptide-loaded DC isolated from the spleen can, like their cultured BM counterparts, induce effective CTL-mediated protection against tumor challenge. We find that DC isolated by either procedure can provide protection.

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## Materials and methods

### In vitro culture media and reagents

Cultures were in complete medium (CM): Iscoves's modified Dulbecco minimal essential medium (IMDM), 2 mM glutamine, 1% penicillin/streptomycin, 5  $\mu$ M 2-mercaptoethanol (all Sigma Chemical Co.) and 5% fetal bovine serum (Gibco-Life Technologies, Auckland). The amino acid 33–41 peptide fragment of the lymphocytic choriomeningitis virus glycoprotein (KAVYNFATM, LCMV<sub>33–41</sub>) was prepared by in vitro chemical synthesis (Chiron Mimotopes, Clayton, Australia).

### Mice and tumor cell lines

C57BL/6J mice (Jackson Laboratories, Bar Harbor, Maine) and "318" mice [20] transgenic for a T cell receptor specific for D<sup>b</sup> + LCMV<sub>33–41</sub>, kindly provided by Hanspeter Pircher, University Hospital, Zürich, Switzerland, were maintained in the Animal Facility of the Wellington Medical School by brother  $\times$  sister mating. The Lewis lung carcinoma LLTC (C57BL/6, H-2<sup>b</sup>) [8], kindly provided by Dr. G. Finlay, Cancer Research Laboratories, Auckland, and the murine thymoma EL4 (C57BL/6, H-2<sup>b</sup>) were both maintained by biweekly subculturing in CM. LL-LCMV, a transfectant of LLTC expressing LCMV<sub>33–41</sub>, was maintained in CM containing 0.5 mg/ml G418 (Gibco-Life Technologies, Auckland).

### Preparation of spleen DC and cultured BM DC

DC populations were isolated from murine spleen or generated in culture from bone marrow precursors in the presence of granulocyte/macrophage-colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) as described [10], with the only exception that splenic DC preparations were depleted of contaminating B cells by magnetic adherence with sheep anti-(mouse IgG)-conjugated Dynabeads (Dynal A.S., Oslo, Norway).

### Flow cytometry

Cells were stained for flow cytometry as previously described [10] using the following antibodies: N418-biotin (anti-CD11c) and fluorescein-isothiocyanate (FITC)-labeled 28-13-3S (anti-H-2K<sup>b</sup>), which were affinity purified from tissue-culture supernatants and conjugated to biotin or FITC as described [5], or anti-H-2D<sup>b</sup>-FITC (Pharmingen, San Diego, Calif.). Streptavidin-phycoerythrin was from Jackson ImmunoResearch (West Grove, Pa., USA). Cells (10 000–50 000) were analyzed on a Becton Dickinson FACSort using the CellQuest software as described [10].

### Cloning and transfection

A "minigene" encompassing amino acids 33–41 of LCMV glycoprotein was created by annealing the complementary oligonucleotides 5'-CCGGATCCACCATGAAAGCTGTTACAATTTTGCCACCTGTAAAGGATCCGG-3' and 5'-CCGGATCCTTAACAGGTGGCAAATTTAGACAGCTTTTCATGGTGGATCCGG-3' (Life Technologies, Auckland). The double-stranded DNA product was ligated into the *EcoRV* site of pcDNA3 (Invitrogen BV, Leek, The Netherlands) and then transfected into LLTC cells using Lipofectamine (Gibco-Life Technologies, Auckland). Transfectants (LL-LCMV) were selected in CM containing 1 mg/ml G418 for 3 days, followed by 9 days in CM containing 0.5 mg/ml G418. Individual clones were isolated by limiting dilution in CM containing 0.5 mg/ml G418, and then assayed for expression of the LCMV<sub>33–41</sub> epitope by a T cell proliferation assay as outlined below.

### T cell proliferation assays

The proliferative response of LCMV-specific T cells to synthetic LCMV<sub>33–41</sub>-loaded DC was determined by incubating  $5 \times 10^5$  318 spleen cells with serially diluted DC that had been pulsed with 10  $\mu$ M LCMV<sub>33–41</sub> peptide for 2 h. Proliferative responses to LLTC and LL-LCMV were determined in the presence of 10 U/ml recombinant IL-2 (Roche, Nutley, N.J., USA) by incubating 318 spleen cells with serially diluted tumor cells that had been treated with 50  $\mu$ g/ml mitomycin C (Kyowa, Tokyo, Japan) for 30 min at 37 °C and washed three times. Assays were set up in CM in 96-well microplates (Falcon, Oxnard, Calif., USA). Plates were incubated at 37 °C for 48 h, pulsed overnight with 1  $\mu$ Ci/well [<sup>3</sup>H]-thymidine (Amersham Int., UK), harvested on a Tomtec 96-well automated cell harvester and counted on a Wallac 1450 Microbeta Plus. All experiments were done in triplicate.

### CTL induction in vivo and cytotoxicity assays

DC ( $1 \times 10^6$  cells/ml) were loaded with peptide by incubation in CM containing 10  $\mu$ M LCMV<sub>33–41</sub> peptide for 2 h and then washed. C57BL/6J mice were injected s.c. into the right flank with  $10^5$  peptide-loaded or control DC in IMDM. After 7 days, mice were sacrificed and spleen cell suspensions prepared and cultured at  $4 \times 10^6$ /well in CM containing 0.01  $\mu$ M LCMV<sub>33–41</sub> peptide in 24-well plates (Falcon) for 5 days. At the end of 5 days, viable cells were washed, counted and assayed for CTL activity by the JAM test [17] on 5000 EL4 target cells that had been pre-incubated with 1  $\mu$ M LCMV<sub>33–41</sub> peptide or CM for 1 h at 37 °C. After 3 h at 37 °C, cells were harvested and counted on a Microbeta Plus  $\beta$ -counter. Cytotoxicity was expressed as the percentage specific lysis calculated as follows:

$$\text{Specific lysis \%} = \frac{S - E}{S} \times 100$$

where S = [<sup>3</sup>H]-thymidine incorporation in the absence of killers, and E = [<sup>3</sup>H]-thymidine incorporation in the presence of killers.

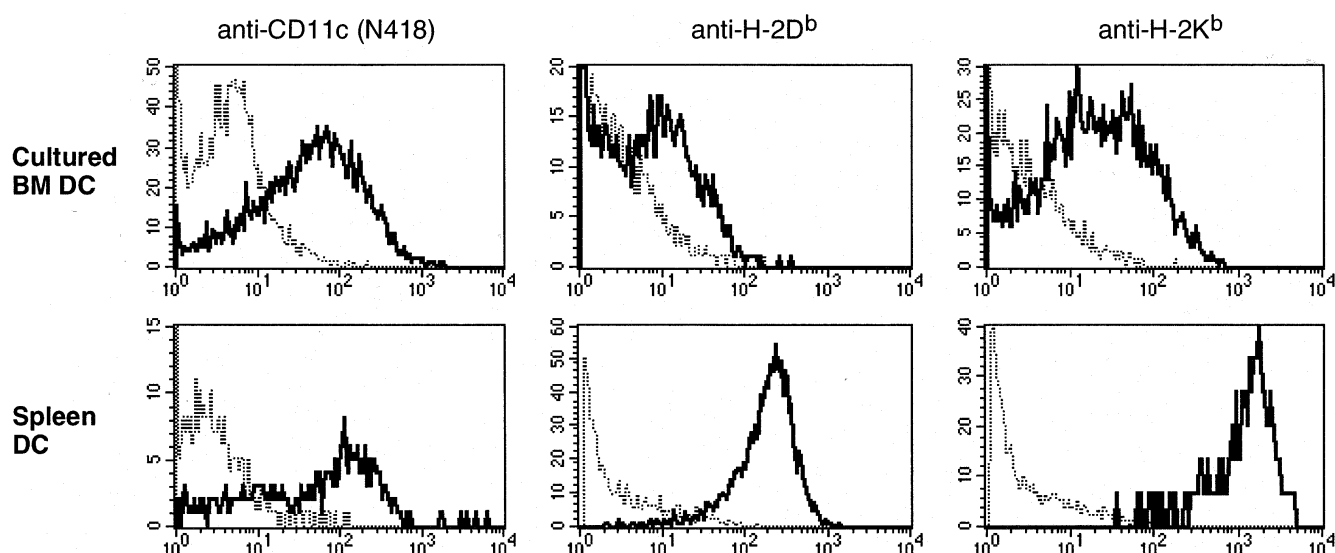
### In vivo tumor protection

Groups of C57BL/6J mice ( $n = 8$ ) were immunized s.c. on day 0 and day 7 with  $10^5$  peptide-loaded DC or control DC as described above. In other experiments mice ( $n = 5$ ) were immunized once with either  $10^7$  spleen cells that had been loaded with peptide as described for DC, or 100  $\mu$ g LCMV<sub>33–41</sub> peptide in 200  $\mu$ l complete or incomplete Freund's adjuvant (Sigma Immunochemicals). After 7 days from the last immunization, all animals were challenged with  $1 \times 10^6$  LL-LCMV tumor cells, injected s.c. into the opposite flank. Mice were monitored every 3–4 days, and mean tumor size for each group was calculated as the mean of the products of bisecting tumor diameters. Tumor growth was recorded until the first animal in each group reached a threshold tumor size of 200 mm<sup>2</sup>. All experiments were performed following procedures approved by the Wellington School of Medicine Animal Ethics Committee, according to the guidelines of the Council for International Organizations of Medical Sciences.

## Results

### T-cell-stimulatory capacity of spleen DC and cultured BM DC

Murine DC can be identified by the expression of the surface marker CD11c. We used expression of this marker,

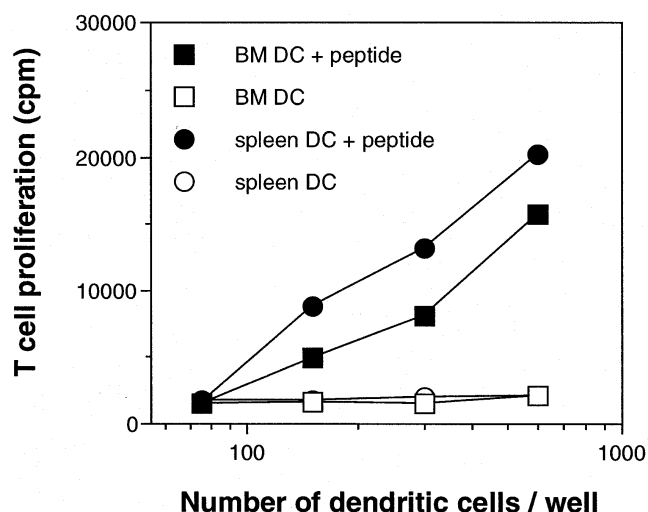


as measured by flow cytometry with the mAb N418, as an indicator of the purity of DC populations isolated from the spleen, or cultured in vitro from BM precursors. Typical N418 profiles are presented in Fig. 1. The levels of expression of MHC class I molecules were determined for N418-positive cells from the two different sources. Spleen DC express higher levels of both H-2D<sup>b</sup> and H-2K<sup>b</sup> than do their cultured BM counterparts (Fig. 1).

To determine whether DC isolated from spleen or cultured from BM have a similar capacity to stimulate MHC class-I-restricted T cells, we compared the ability of the two DC populations to induce proliferation of T cell receptor transgenic T cells in vitro. Splenocytes from 318 mice, expressing a transgenic T cell receptor specific for an

**Fig. 1** Dendritic cell (DC) isolated from spleen express higher levels of MHC class I molecules than DC from bone marrow (BM) cultures. Flow cytometry of cultured BM DC (top row) and spleen DC (bottom row) showing expression of CD11c (N418) on total populations, and H-2D<sup>b</sup> and H-2K<sup>b</sup> on N418-positive gated cells. Bone marrow cultures were harvested on day 8

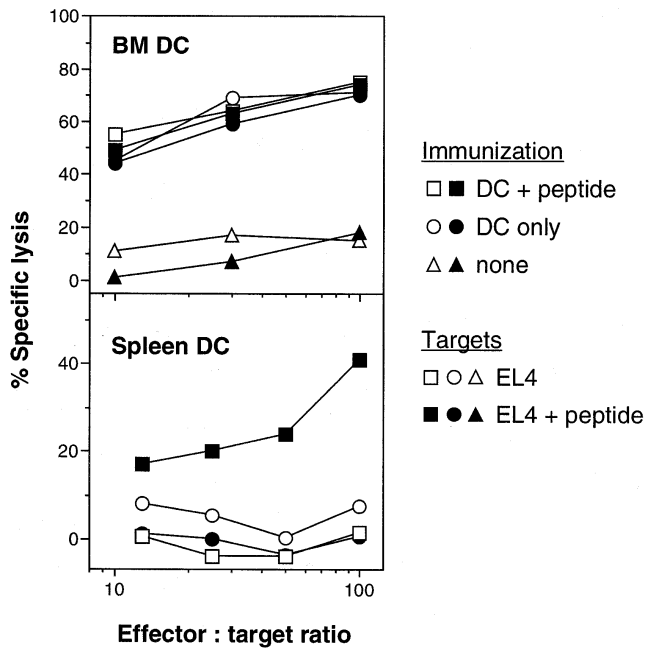
H-2D<sup>b</sup>-restricted epitope of LCMV glycoprotein (LCMV<sub>33-41</sub>), were co-incubated with DC alone, or with DC that had been loaded with synthetic LCMV<sub>33-41</sub> peptide (10 μM, an amount found to be limiting in these conditions). Significant T cell proliferation was observed with peptide-loaded DC from either source, with as few as 100 DC being required to induce a response (Fig. 2). However, splenic DC consistently induced a twofold stronger response than cultured BM DC.



**Fig. 2** Induction of antigen-specific T cell proliferative responses in vitro by DC. DC from spleen or bone marrow cultures were loaded for 2 h with 10 μM LCMV<sub>33-41</sub> peptide, washed extensively, and then incubated at the indicated cell numbers with splenocytes from LCMV<sub>33-41</sub>-specific T cell receptor transgenic mice ( $5 \times 10^5$  cells/well) for 48 h. Each data point represents the mean  $\pm$  SE from triplicate wells

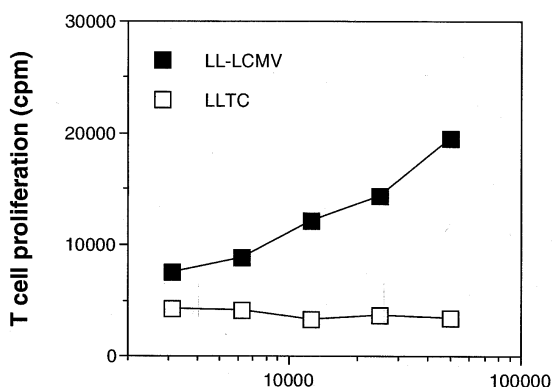
Capacity of peptide-loaded spleen DC or cultured BM DC to prime CTL in vivo

To determine whether a CTL response could be primed in vivo with peptide-loaded spleen or BM DC, we immunized C57BL/6J mice with LCMV<sub>33-41</sub>-peptide-loaded DC from either source. Mice were immunized with a single dose of  $10^5$  peptide-loaded DC injected s.c. Control animals were injected with untreated DC. The in vivo priming of LCMV<sub>33-41</sub>-specific cells was assayed by culturing spleen cells in vitro for 5–6 days in the presence of specific peptide, and testing CTL activity by the JAM test on peptide-loaded or non-loaded EL4 targets (Fig. 3). LCMV<sub>33-41</sub>-specific CTL were primed in mice injected with peptide-loaded spleen DC, whereas animals immunized with untreated spleen DC did not generate a cytotoxic response. In contrast, animals immunized with cultured BM DC generated a strong but non-specific cytotoxic response regardless of whether the DC had been loaded with peptide or not. This cytotoxic response was also directed against H-2<sup>d</sup> targets (P815, not shown), and was also observed when BM DC were cultured in normal mouse serum, or when



**Fig. 3** Peptide-loaded spleen DC induce antigen-specific lytic activity in vivo, whereas peptide-loaded DC from bone marrow cultures induce a non-specific response. C57BL/6J mice were injected s.c. with  $10^5$  peptide-loaded DC, or  $10^5$  DC only. Control animals were not immunized. After 7 days, splenocytes were restimulated in secondary culture with  $0.01 \mu\text{M}$  LCMV<sub>33-41</sub> peptide for 5 days, and cytotoxic activity was measured against EL4 tumor cells, or EL4 loaded with LCMV<sub>33-41</sub> peptide. Percentage specific lysis was calculated from the means of triplicate wells as described in Materials and Methods

MHC class-II-defective BM DC were used (data not shown), indicating that the response was not due to carry-over of bovine serum components in the medium. Depletion of adherent cells from the spleens of BM DC immunized mice before in vitro culture [21] also failed to reduce the nonspecific cytotoxic activity significantly (data not shown).



**Fig. 4** Induction of antigen-specific T cell proliferative responses in vitro by tumor cells expressing the LCMV<sub>33-41</sub> epitope. The indicated numbers of mitomycin-C-treated tumor cells were cultured with splenocytes from LCMV<sub>33-41</sub>-specific T cell receptor transgenic mice ( $5 \times 10^5$  cells/well) for 3 days in the presence of interleukin-2. Each data point represents the mean  $\pm$  SE from triplicate wells

Generation of a tumor cell line expressing the LCMV<sub>33-41</sub> epitope

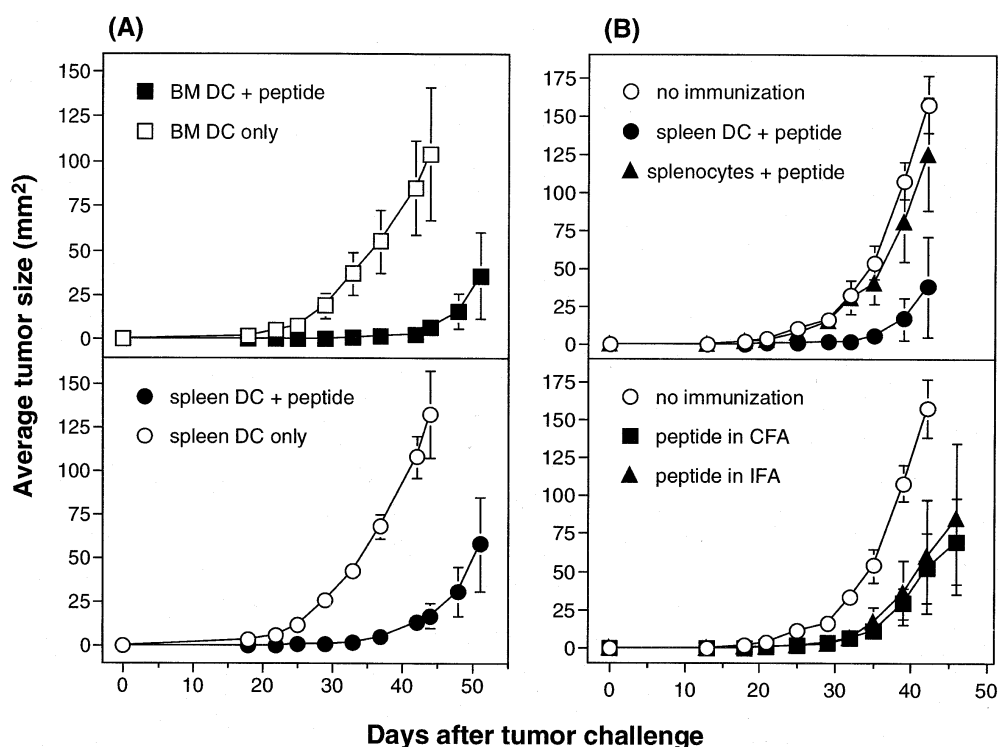
We wished to establish a tumor cell line expressing a known MHC-class-I-restricted epitope that could be used as a tumor-specific antigen to evaluate the effectiveness of the different DC populations at inducing MHC-class-I-restricted antitumor immune responses. The Lewis lung carcinoma cell line (LLTC) was transfected with a "mini-gene" plasmid construct encoding the peptide sequence LCMV<sub>33-41</sub> flanked by start and stop codons, and placed under the control of the cytomegalovirus promoter. To confirm that the peptide was expressed and could associate with MHC class I molecules on the transfected tumor cells, we determined the ability of the stably transfected cell line (LL-LCMV) to induce proliferation of transgenic LCMV<sub>33-41</sub>-specific T cells. As shown in Fig. 4, LL-LCMV induces a significant proliferation of LCMV<sub>33-41</sub>-specific T cells relative to the parental cell line, indicating that the peptide is expressed and can be recognized by T cells.

Peptide-loaded DC from spleen or cultured bone marrow can induce protective responses against a tumor expressing the LCMV epitope

We next tested the capacity of both spleen DC and cultured BM DC to induce a protective response against tumors. LCMV<sub>33-41</sub>-peptide-loaded DC were used to vaccinate mice against subsequent challenge with LL-LCMV tumor cells. Groups of mice ( $n = 8$ ) were injected twice with  $1 \times 10^5$  peptide-loaded DC isolated from either spleen or bone marrow cultures. The cells were injected s.c. into the right flank. Groups of control mice were injected at the same time with DC that had not been loaded with peptide. Seven days after the second DC injection, all animals were challenged with  $1 \times 10^6$  LL-LCMV tumor cells injected s.c. into the left flank.

A representative experiment is shown in Fig. 5A. Animals from both control groups developed progressive tumors that appeared, on average, on day 22 after challenge (Table 1). By day 42, the first animals in each of the control groups had developed tumors in excess of  $200 \text{ mm}^2$ , and were culled. In contrast, animals injected with peptide-loaded DC from both spleen and bone marrow cultures were significantly protected against tumor challenge. On average, 57% of the animals immunized with cultured BM DC + peptide, and 34% of the animals immunized with spleen DC + peptide were completely protected from tumor growth. In contrast, an average of only 13% of animals immunized with non-treated BM DC were free from tumors, and 7% of animals immunized with non-treated spleen DC were free from tumors. Another noticeable effect of immunization with peptide-loaded DC was a delay in the initial appearance of tumors in those animals that were not completely protected. This delay was typically a period of 10 days relative to tumor appearance in the control group. The growth of these later tumors was otherwise unhindered.

**Fig. 5A, B** Peptide-loaded DC isolated from spleen or bone marrow cultures have a similar capacity to induce protection against tumor challenge, while total spleen cells are ineffective. **A** Groups of C57BL/6J ( $n = 8$ ) mice were injected twice s.c. with  $10^5$  peptide-loaded DC, or  $10^5$  DC only, at 1-week intervals. **B** Groups of C57BL/6J ( $n = 5$ ) mice were either left untreated, or injected once s.c. with  $10^5$  spleen DC or  $10^7$  spleen cells pulsed with peptide, or 100  $\mu$ g peptide in complete or incomplete Freund's adjuvant. Seven days after the last immunization all animals were challenged with  $10^6$  LL-LCMV tumor cells. Measurements for each group were terminated when the first animal developed a tumor in excess of 200 mm<sup>2</sup>



**Table 1** In vivo tumor protection experiments. Data are combined from two experiments. "Tumor-free" animals remained without tumor for more than 80 days before being culled. *BM* bone marrow, *DC* dendritic cells

Immunization regime	Mean time of onset of tumor after challenge (days)	Tumor-free animals at completion of experiment (%)
BM DC only	22 ± 2	13 ± 13
BM DC + peptide	32 ± 3	57 ± 7
Spleen DC only	21 ± 1	7 ± 7
Spleen DC + peptide	31 ± 1	34 ± 5

To assess their relative efficiencies, we also compared the tumor-protective effect of spleen DC with that of total spleen cell populations and of LCMV<sub>33-41</sub> peptide in adjuvant. Spleen DC ( $10^5$ ) and total spleen cells ( $10^7$ ) were loaded with LCMV<sub>33-41</sub> in vitro and injected once s.c. into C57BL/6J recipients. LCMV<sub>33-41</sub> peptide (100  $\mu$ g) in 200  $\mu$ l complete or incomplete Freund's adjuvant was administered s.c. once. After 7 days all mice were given a  $1 \times 10^6$  LL-LCMV tumor cell challenge. As shown in Fig. 5B, peptide-pulsed spleen cells did not induce a tumor protective response. In contrast, injection of LCMV<sub>33-41</sub> peptide in incomplete or complete Freund's adjuvant induced significant tumor protection, which is consistent with the reported ability of these adjuvants to support CTL priming in vivo [1, 14]. Spleen DC were at least as effective as LCMV<sub>33-41</sub> peptide in adjuvant at providing tumor protection.

## Discussion

The potential for the use of DC in tumor immunotherapy has long been recognized [11, 15]. However, the lack of defined tumor-specific antigens has delayed the progress of studies in this direction. The isolation of tumor-specific, MHC-class-I- and class-II-restricted epitopes has now made these studies feasible [2], and a number of groups have recently reported the successful use of DC in tumor vaccination [9, 18, 19, 22].

In studies where DC were used to induce tumor-protective, MHC-class-I-restricted T cell responses, the DC populations utilized were either transformed DC [19], or DC prepared by culturing bone marrow precursors in vitro in the presence of appropriate lymphokines [18, 19, 22]. However, one of these studies also reported that freshly isolated Langerhans cells are ineffective at inducing tumor protection, and that BM DC cultured in lymphokines other than GM-CSF and IL-4 are also ineffective [18]. The ability to induce in vivo responses is likely to depend on a number of different properties of the antigen-presenting cells, such

as lifespan *in vivo*, ability to migrate to secondary lymphoid organs, and T-cell-stimulatory ability. Because these properties may vary during the physiological differentiation of DC, we wished to establish whether DC isolated from murine spleen, which represent a more "mature" DC population, are also able to provide tumor protection like their BM-derived counterparts. We have previously shown [10] that spleen DC and cultured BM DC have comparable abilities to stimulate CD4<sup>+</sup> T cells *in vitro*. In the present study we extend those findings to CD8<sup>+</sup> T cells and *in vivo* immune responses, and show that spleen DC are indeed able to induce tumor protection. This is perhaps surprising considering that BM DC appear to express lower levels of MHC class I molecules than do spleen DC, and are also somewhat less effective at stimulating proliferation of CD8<sup>+</sup> T cells *in vitro*. It is possible that other properties of the BM DC, such as for example migration *in vivo*, may be compensating for their relatively weaker T-cell-stimulatory capacity. Alternatively, subtle quantitative differences may exist between the tumour-protective capacities of the two DC types, which are not revealed in our experiments. It was, however, clear from other experiments (Fig. 5B) that DC immunization was at least as effective or superior to either immunization with peptide in incomplete or complete Freund's adjuvant in providing tumor protection.

Tumor protection induced by vaccination with DC + MHC-class-I-binding peptides has been shown to be dependent on CD8<sup>+</sup> T cells [4]. We show here that the DC populations used for *in vivo* immunization were also very effective at inducing proliferation of unprimed CD8<sup>+</sup> T cells *in vitro*. In addition, splenocytes from mice immunized with DC + peptide exhibited significant cytotoxic activity against tumor cells after secondary *in vitro* stimulation. This cytotoxic activity was completely specific to the LCMV<sub>33-41</sub> peptide in the case of mice receiving spleen DC + peptide. In contrast, a high nonspecific cytotoxic activity was consistently observed in spleen cultures from mice immunized with BM DC, whether peptide-loaded or not. This high nonspecific cytotoxicity was only marginally diminished by depletion of splenic adherent cells before secondary *in vitro* restimulation [21], and could not be eliminated by culturing BM DC in normal mouse serum before *in vivo* injection. It is not clear whether this nonspecific *in vitro* tumoricidal activity was mediated by CD8<sup>+</sup> T cells or by other populations of effector cells, or whether it could in any way contribute to the observed *in vivo* tumor-protective response. We do not think this latter possibility is very likely, as mice immunized with peptide-loaded spleen DC or cultured BM DC were protected from tumor growth, while mice immunized with DC only, whether from spleen or BM cultures, were susceptible. This observation suggests that the immune responses induced by cultured BM DC were indeed peptide-specific *in vivo*.

The tumor protection observed in our vaccination experiments was not absolute, and a proportion of mice that had received peptide-loaded DC later developed progressing tumors (Fig. 5A). However, these tumors appeared later than in control mice receiving DC only, and in a smaller proportion of animals (Table 1). We do not think

that the partial protection was due to the *in vivo* selection of epitope-negative tumor variants, as we could show that tumor cells *ex vivo* were able to stimulate the proliferation of transgenic LCMV<sub>33-41</sub>-specific T cells *in vitro* (not shown). Transient *in vivo* down-modulation of the tumor epitope is another possibility that cannot be completely ruled out by these *in vitro* experiments. Instead, it appears more likely that the partial effect of vaccination may be due to the relatively large numbers of tumor cells used in our experiments (10<sup>6</sup>/mouse, when only 3 × 10<sup>4</sup>–10<sup>5</sup> tumor cells were sufficient to induce tumors). Alternatively, repeated injections of peptide-loaded DC may be required to achieve a long-lasting tumor protection; this possibility may be especially important in the case of slowly progressing tumors like the ones used in our experiments. Indeed, complete protection could be observed in separate experiments where the same immunization protocol but a different tumor cell line was used (data not shown).

In conclusion, tumor peptide-loaded DC, whether cultured *in vitro* from bone marrow precursors, or isolated *ex vivo* from murine spleen, appear able to provide *in vivo* tumor protection, and are suitable for use in immunotherapy studies. The choice of one or the other DC source can then be dictated by the availability of appropriate techniques for DC growth and isolation, as well as by the specific requirements of protocols that may prove effective for the treatment of already-established tumors.

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