Lymphotactin Gene-Modified Bone Marrow Dendritic Cells Act as More Potent Adjuvants for Peptide Delivery to Induce Specific Antitumor Immunity

Xuetao Cao, Weiping Zhang, Long He, Zhifang Xie, Shihua Ma, Qun Tao, Yizhi Yu, Hirofumi Hamada and Jianli Wang

*J Immunol* 1998; 161:6238-6244; ;
http://www.jimmunol.org/content/161/11/6238

References
This article cites 53 articles, 31 of which you can access for free at:
http://www.jimmunol.org/content/161/11/6238.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Lymphotactin Gene-Modified Bone Marrow Dendritic Cells Act as More Potent Adjuvants for Peptide Delivery to Induce Specific Antitumor Immunity

Xuetao Cao,1,2,3* Weiping Zhang,3* Long He,* Zhifang Xie,† Shihua Ma,* Qun Tao,* Yizhi Yu,* Hirofumi Hamada,† and Jianli Wang*

Dendritic cells (DC) are regarded as attractive candidates for cancer immunotherapy. Our aim is to improve the therapeutic efficacy of DC-based tumor vaccine by augmenting DC preferential chemotaxis on T cells. Mouse bone marrow-derived DC were transduced with lymphotactin (Lptn) gene by adenovirus vector. The supernatants from Lptn gene-modified DC (Lptn-DC) were capable of attracting CD4+ and CD8+ T cells in a chemotaxis assay, whereas their mock control could not. Lptn expression of Lptn-DC was further confirmed by RT-PCR. Lptn-DC were pulsed with Mut1 peptide and used for vaccination. Immunization with the low dose (1 × 10^4) of Mut1 peptide-pulsed DC induced weak CTI activity, whereas the same amounts of Mut1 peptide-pulsed Lptn-DC could render mice resistant to a 5 × 10^5 3LL tumor cell challenge completely, but their counterpart could not. The protective immunity induced by Mut1 peptide-pulsed Lptn-DC depends on both CD4+ T cells and CD8+ T cells rather than NK cells in the induction phase and depends on CD8+ T cells rather than CD4+ T cells and NK cells in the effector phase. Moreover, the involvement of CD28/CTLA4 costimulation pathway and IFN-γ are also necessary. When 3LL tumor-bearing mice were treated with 1 × 10^4 Mut1 peptide-pulsed Lptn-DC, their pulmonary metastases were significantly reduced, whereas the same low dose of Mut1 peptide-pulsed DC had no obvious therapeutic effects. Our data suggest that Lptn-DC are more potent adjuvants for peptide delivery to induce protective and therapeutic antitumor immunity.


Departments of *Immunology and † Cellular Biology, Second Military Medical University, Shanghai, People’s Republic of China, and 3 Department of Molecular Biotherapy Research, Japanese Foundation of Cancer Research, Toshima-ku, Tokyo, Japan

Received for publication May 21, 1998. Accepted for publication August 10, 1998.

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

1 This work was supported in part by National Natural Science Foundation of China (39730420).
2 Address correspondence and reprint requests to Dr. Xuetao Cao, Department of Immunology, Second Military Medical University, 800 Xiangyin Road, Shanghai 200433, People’s Republic of China. E-mail address: caoxt@public3.sta.net.cn
3 X.C. and W.Z. contributed equally to this work.
4 Abbreviations used in this paper: DC, dendritic cell; Ad, adenovirus; GM-CSF, granulocyte-macrophage colony-stimulating factor; Lptn, lymphotactin; Lptn-DC, Lptn gene-modified DC; Lptn-DC-CK1, Lptn gene-modified DC-CD2, Lptn gene-modified DC-CD8; CTLA, cytotoxic T lymphocyte-associated protein; MIP-γ, macrophage-inflammatory protein-γ.

Copyright © 1998 by The American Association of Immunologists 0022-1767/98/$02.00

Dendritic cells (DC) are the uniquely potent APCs involved in the initiation of immune responses (1). With the development of the methods for propagating DC on a large scale from hematopoietic precursors (2–4), vaccination with tumor Ag-loaded DC represents a potentially powerful strategy to induce tumor rejection (5, 6). Up to now, most investigations about DC-based vaccines have focused on exploring feasible and effective approaches to loading tumor Ag onto DC for vaccination. Actually, various approaches have been evaluated, including pulsing DC with tumor Ag in the form of protein (7–14) or peptide (15–19), transducing cDNA encoding tumor Ag (20–28) or tumor antigen (29) into DC, and fusing tumor cells (30) with DC. It has been well documented that vaccination with tumor Ag-loaded DC is capable of eliciting protective and therapeutic antitumor immunity in animal models and clinical trials (7–18, 25–30).

As adjuvants for Ag delivery, DC pick up Ags in the periphery and carry them to T cell area in lymphoid organs to prime the immune responses (31–35). The precise molecular mechanisms underlying DC in vivo migration and their interaction with T cells are not well defined. Chemokines capable of regulating the migration of immune cells may contribute to the initiation of immune responses by DC. It is evident that DC express some chemokine receptors, so chemokines participate in the migration and recruitment of DC (36–39). Moreover, DC can produce several kinds of chemokines (40–43) (e.g., macrophage-inflammatory protein-γ (MIP-γ), monocyte chemotactic protein, RANTES, MIP-1α, and DC-CX1) to actively attract T cells to initiate immune responses. Lymphotactin (Lptn) is a recently defined C chemokine that is specifically attractive to T cells (44–46). Cotransfection of Lptn and IL-2 genes into tumor tissue could induce potent antitumor immunity (47). We hypothesized that the improved preferential chemotaxis of DC on T cells by genetically modifying DC with T cell-attracting chemokine might be capable of facilitating the in vivo stimulation of T cells by DC and consequently favoring DC Ag presentation and T cell activation.

Recently, different gene transfer approaches have been explored to genetically modify DC for vaccination, and it has been found that DC genetically modified with tumor Ags or immunoregulatory cytokines are potentially advantageous in inducing antitumor immunity (12, 13, 24–27, 30). Adenovirus (Ad) vector capable of mediating gene transfer with high efficiency and acting as adjuvants to boost CTL response were demonstrated to be the potentially promising viral vector for DC genetic modification
(12, 25–28, 30). One of our previous studies showed that granulocyte-macrophage CSF (GM-CSF) gene-modified DC pulsed with tumor Ag could induce antitumor immunity more potently (12).

The primary aim of this study is to improve the efficacy of DC-based vaccines for cancer treatment by genetically modifying DC with a T cell-attracting chemokine. So in this study, replication-defective Ad vector harboring mouse Lptn was constructed and used to genetically modify mouse bone marrow DC. In the tumor model of 3LL Lewis lung carcinoma, normal mice were vaccinated with the Lptn gene-modified DC (Lptn-DC) pulsed with 3LL cell-specific Mut1 peptide (FEQNTAQP) (48), and the protective and therapeutic effects were investigated.

Materials and Methods

Cell lines and animals

293 (CRL1573; American Type Culture Collection (ATCC, Manassas, VA)) is a human embryonic kidney cell line transformed with Ad5 E1A and E1B genes and supporting propagation of E1-deleted recombinant Ads. 3LL is a murine Lewis lung carcinoma cell line derived from C57BL/6 mice by x-irradiation (42). 3LL cells were cultured in DMEM (Life Technologies, Grand Island, NY) supplemented with 10% heat-inactivated FCS (HyClone, Logan, UT), 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. The B16.F10 melanoma cell line was cultured in complete RPMI 1640. Female C57BL/6 and BALB/c mice, 5–6 wk old, were purchased from Joint Ventures Sipper BK Experimental Animal Co. (Shanghai, People’s Republic of China).

Peptides

Peptides with a purity of >95% were synthesized by an automatic solid-phase peptide synthesizer (Applied Biosystems, Foster City, CA) by Cybersyn Co. (Lenni, PA) and purified by reversed-phase HPLC. The Mut1 peptide FEQNTAQP consists of the 52–59 amino acid positions of the mutated connexin 37 protein expressed in the 3LL cell line (48). The sequence of OVA257–264 peptide is SIINFEKL (49). Peptides were dissolved in serum-free Iscove’s modified Dulbecco’s medium/50 mM 2-ME and stored at −20°C.

Recombinant Ads

Recombinant Ad vector harboring LacZ reporter gene has been described previously (50). Lptn cDNA was obtained from the activated splenic T lymphocytes by RT-PCR. Briefly, mouse splenic T lymphocytes were enriched by previously (50). Lptn cDNA was obtained from the activated splenic T lymphocytes by RT-PCR. Briefly, mouse splenic T lymphocytes were enriched by positive selection using antibodies to CD3 (clone 145-2C11) and CD8 (clone 53-6.7). The purified T lymphocytes were cultured in DMEM (Life Technologies, Grand Island, NY) supplemented with 10% heat-inactivated FCS (HyClone, Logan, UT), 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. The incorporation of the expression cassette with 3′ and 5′ termini, respectively (44), and confirmed by automatic sequencer (ABI377). The recombinant Ads harboring mouse Lptn or LacZ gene were generated by homologous recombination previously described elsewhere (51). Lptn cDNA was placed under the control of CMV promoter in the modified pCI expression vector (Promega, Madison, WI). Subsequently, the expression cassette was inserted into the ClaI cloning site of cosmid vector pAdEclw (kindly provided by I. Saito, Tokyo University, Tokyo, Japan), which bears an Ad5 genome spanning 0–99.3 map units with deletions of E1A, E1B, and E3. The resultant recombinant cosmid was cotransfected into 293 cells with EcoR221-digested Ad5 DNA-terminal peptide complex by calcium phosphate precipitation, and the recombinant Ads were generated by homologous recombination (51). The incorporation of the expression cassette was confirmed by digestion with appropriate restriction enzymes. Subsequently, the recombinant viruses were propagated in 293 cells and purified on CsCl density gradient, the titers of which were determined by plaque assay on the 293 cells. The Ad solutions were stored at −80°C. The DNA structure of Lptn recombinant Ad is shown in Fig. 1.

Generation of DC from bone marrow cultures

The procedure used in this study was previously described by Porgador and Gilboa (15), with some minor modifications. Briefly, bone marrow cells from C57BL/6 mice were depleted of red blood cells with ammonium chloride and depleted of lymphocytes, granulocytes, and Ia+ cells using a mixture of mAbs and rabbit complement. The mAbs were 2.43 (anti-CD8), GK1.5 (anti-CD4), RA3-1A1/6.1 (anti-B220/CD45R), B21-2 (anti-Ia), and 2.4G2 (anti-IFN-γ) (Pharmingen, San Diego, CA), and the upper chambers of the assembly were filled with 40 μl of the appropriate cell suspension (2 × 106 cells/ml). CD4+ and CD8+ T cells were negatively selected from splenocytes of C57BL/6 mice using the mixtures (RA3–3A1/6.1 (anti-B220/CD45R), B21-2 (anti-Ia), PK136 (anti-CD4), 2.4G2 (anti-IgG1), and 2.43 (anti-CD8) or GK1.5 (anti-CD4)) and complement. Data were obtained by counting five nonoverlapping high power microscope fields from each well. Cells were considered to be chemoattracted if the chemotactic index (number of cells migrating in experimental well/number of cells migrating in medium only) was >2.

Immunization and tumor challenge

Peptide-pulsed DC (1 × 106 or 1 × 105) in 0.2 ml HBSS were injected s.c. into both thighs of normal C57BL/6 mice. One week after vaccination with peptide-pulsed DC with or without genetic modification, the mice were injected s.c. in the flank of the abdomen with a lethal dose of 5 × 103 3LL Lewis lung carcinoma cells. To evaluate the specificity of the antitumor
immunity induced by Mut1 peptide-pulsed DC or Lptn-DC, the immunized mice were also challenged with B16 tumor cells. The tumor size was monitored at regular intervals and calculated as the product of the maximal perpendicular diameters. Mice were killed when the challenged tumors reached 3 cm in diameter or severe ulceration developed. All experiments were performed three times using individual treatment groups of six mice. Data are representative of three experiments performed.

**Immunotherapy of preestablished 3LL carcinoma model**

The spontaneous metastasis model of 3LL lung carcinoma was established by inoculating 3LL tumor cells (2 × 10^5/mouse) into the footpad (53). Eighteen days later, tumor-bearing legs were amputated when the tumor size in the footpad reached 7–8 mm in diameter. Post-surgical mortality was <2%. Two days after amputation, mice were vaccinated twice s.c. with 1 × 10^5 peptide-pulsed DC at weekly intervals. Mice were killed when HBSS-treated mice died 30–35 days post-amputation. Metastatic loads were recorded with lung weights.

**Cytotoxicity assay**

One week after immunization, the immunized mice were killed, and their splenic lymphocytes (2 × 10^6 cells/ml) were restimulated in vitro with 50 μM IFN-γ-pretreated and irradiated (5000 rad) 3LL cells (2 × 10^4 cells/ml in six-well culture plates (4 ml/well). The culture medium consisted of RPMI 1640 and NCTC109 (1/1, v/v) supplemented with 10% FCS, 50 mM 2-ME, 2 mM glutamine, 10 mM HEPES (pH 7.4), 100 U/ml penicillin, and 100 μg/ml streptomycin. After 5 days of restimulation, the viable lymphocytes were collected and cultured with 2 × 10^6 [51Cr]-labeled 3LL or B16 target cells in a round-bottom 96-well microtiter plate (Nunc, Naperville, IL) in triplicate at different E:T ratios. After incubation at 37°C for 4 h, 100 μl of supernatants were harvested, and their radioactivity was measured by a gamma counter (model 1275, Wallac, Turku, Finland). The percentages of specific 51Cr release were calculated according to the following formula: % 51Cr release = 100 × [cpm spontaneous release/cpm maximum – cpm spontaneous release], where the spontaneous release was obtained from target cells cultured with medium alone, and the maximum release was obtained from target cells cultured with 0.1% NP40 instead of effector cells. The spontaneous release was <15%.

**In vivo depletion of immune cell subsets and immunoregulatory molecules**

Mice were immunized once with 1 × 10^5 Mut1 peptide-pulsed Lptn-DC and challenged with 5 × 10^5 3LL tumor cells 11 days after immunization. Four days before DC immunization or tumor challenge, the mice started to receive a total of five i.p. injections of the ascites (0.1 ml/mouse/injection) from hybridoma-bearing mice at the intervals of 3 days. The mAbs used were GK1.5 (anti-CD4), 2.43 (anti-CD8), PK136 (anti-NK; ATCC HB191), and R4-6A2 (anti-IFN-γ; ATCC HB170) mAbs. Normal rat IgG (Sigma) was given as mock control. Depletion of T cell subsets and NK cells was monitored by flow cytometry, which showed >90% specific depletion in splenocytes. To block the CD28/CTLA4 pathway of T costimulation, peptide-pulsed DC (1 × 10^6 cells/ml) were pretreated with 50 μg of CTLA-Ig in the volume of 1 ml at 4°C for 45 min before being co-implanted into mice. CTLA-Ig is a chimeric fusion protein comprising the extracellular domain of CTLA4 Ag and the Fc fragment of human IgG. CTLA-Ig was purified from its culture supernatant with a protein A affinity column (Pharmacia).

**Statistical analyses**

Data are presented as mean value ± SD. Student’s t test was used for comparison of two groups. p values of <0.05 were considered statistically significant.

**Results**

**Ad-mediated Lptn gene modification of DC**

Bone marrow DC from C57BL/6 mice were expanded in vitro for 9–10 days, followed by characterization using composite criteria of typical morphology, cell surface markers, and MLR. DC comprised >90% of the nonadherent cells present 9–10 days after bone marrow culture. Surface phenotype analysis by flow cytometry showed that bone marrow DC expressed high levels of MHC-I, MHC-II, CD40, and CD80 and moderate levels of DC-specific Ag DEC205 (data not shown). MLR revealed that bone marrow DC were potent stimulators in allogenic MLR (data not shown). The genetic modification of DC was mediated by Ad vector. The DNA structure of Lptn Ad vector (Ad.Lptn) is demonstrated in Fig. 1. The recombinant Ad vector of LacZ was previously described (50). Using LacZ as a reporter gene, we evaluated the DC gene transfer efficiency mediated by Ad vector. Twenty-four hours after infection of day 9 DC with Lptn recombinant Ads at a multiplicity of infection of 100, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside staining showed that gene transfer efficiency was >95% (data not shown), which confirms that Ad vector can efficiently mediate gene transfer into DC. Chemotaxis assay revealed that the culture supernatants from DC or LacZ-DC had no significant chemotactic activity on CD4+ or CD8+ T cells, but the 24-h culture supernatants from DC transfected with Ad.Lptn (Fig. 1) could attract both CD4+ T cells and CD8+ T cells markedly (Fig. 2). This indicates that Lptn Ad vector can mediate the expression of bioactive Lptn in DC. Lptn expression in Lptn-DC was further confirmed by RT-PCR analysis. Before PCR, the reverse transscripts were digested with RNase-free DNase I to degrade the potentially contaminated DNA templates; RNA samples without reverse transcription were directly subjected to PCR as control. As shown in Fig. 2, neither DC nor LacZ-DC expressed any detectable Lptn by RT-PCR, whereas Lptn mRNA expression was detected 4 h after gene modification of day 9 DC with Ad.Lptn. In addition, neither LacZ nor Lptn gene transfer into DC by their replication-defective Ad vectors changed their phenotype obviously.

**Lptn-DC pulsed with Mut1 peptide induce potent specific CTL cytotoxicity**

One of our primary aims was to determine whether vaccination of Lptn-DC pulsed with tumor peptide could induce peptide-specific CTL response more effectively. Accordingly, Lptn-DC were pulsed with peptide (Mut1 or OVA) and injected s.c. into mice at a dose of 1 × 10^5 or 1 × 10^6 cells/mouse. The CTL were determined after 5 days of in vitro restimulation with 3LL tumor cells. Mut1 is an H-2K^b-restricted Ag peptide of 3LL carcinoma cells. H-2K^b OVA peptide was used as an irrelevant peptide control in this study. Although immunization with a single dose of 1 × 10^5 Mut1 peptide-pulsed DC or Mut1 peptide-pulsed LacZ-DC induced CTL response specifically against 3LL cells, these cells were less potent CTL inducers than were Mut1 peptide-pulsed Lptn-DC (Fig. 3). Immunization with a lower dose of Mut1 peptide-pulsed DC or Mut1 peptide-pulsed LacZ-DC (1 × 10^4) induced poor CTL response, but the same low-dose Mut1 peptide-pulsed Lptn-DC did induce higher CTL activity specifically against 3LL tumor cells, which was comparable with that induced by vaccination with 10-fold peptide-pulsed DC. On the other hand, immunization of Mut1 peptide-pulsed DC or Mut1 peptide-pulsed Lptn-DC induced poor CTL activity against B16 cells (data not shown), and immunization with OVA peptide-pulsed DC or OVA peptide-pulsed Lptn-DC induced no significant CTL response against 3LL cells. This implies that Lptn-DC are more potent to deliver peptide to induce specific CTL in vivo.

**Lptn-DC pulsed with Mut1 peptide induce protective antitumor immunity**

Then, we tested whether vaccination of mice with Lptn-DC pulsed with tumor peptide was capable of inducing protective immunity against tumor challenge more potently. Seven days after a single vaccination of DC, the vaccinated mice were challenged s.c. with 5 × 10^5 3LL or B16 tumor cells. Consistent with the CTL response, vaccination with 1 × 10^5 Mut1 peptide-pulsed DC could
provide protection specifically against 3LL tumor challenge, but less effectively than the vaccination with Lptn-DC counterpart. Vaccination with a low dose ($1 \times 10^4$) of Mut1 peptide-pulsed DC could not protect the immunized mice from 3LL tumor challenge, and tumors grew in all mice. But all the mice immunized with $1 \times 10^5$ Mut1 peptide-pulsed Lptn-DC were free of tumor 90 days after 3LL tumor challenge (Fig. 4), and 83.3% of mice were free of tumor 90 days after tumor challenge in three different experiments. Immunization of Mut1 peptide-pulsed DC or Mut1 peptide-pulsed Lptn-DC induced poor protection against B16 cells (data not shown), and immunization with OVA peptide-pulsed DC or OVA peptide-pulsed Lptn-DC failed to elicit any protective immunity against 3LL tumor challenge.

In vivo depletion analysis

To investigate the potential roles of T cell subpopulations and NK cells in the induction of protective immunity by Lptn-DC pulsed with MHC-I-restricted peptide, mice were depleted of CD4+ or CD8+ T cells or NK cells during immunization or during challenge. As shown in Fig. 5, depletion of CD8+ T cells during immunization or during challenge abrogated the protective immunity induced by peptide-pulsed Lptn-DC. Mice depleted of CD4+ T cells during immunization failed to reject tumor challenge. However, the mice could reject tumor challenge when CD4+ T cells were depleted during challenge. These results are consistent with the report by Porgador and Gilboa (15), in which they found that CD8+ T cells were the predominant effector cells in the MHC-I-restricted peptide-induced antitumor immunity and that CD4+ T cells were required for the induction of CD8+–dependent T cell immunity but were unnecessary in the effector phase. Although Lptn was recently reported to be capable of attracting NK cells besides T cells (25), mice depleted of NK cells during immunization or during challenge were protected from tumor challenge, suggesting that interaction with T cells and subsequent activation of T cells are responsible for the antitumor immunity induced by peptide-pulsed Lptn-DC. To evaluate the potential role of T cell co-stimulation in peptide-pulsed Lptn-DC-induced immune response,
model of 3LL was established by injection of 2 immunogenic and highly metastatic. The spontaneous metastasis model was further evaluated in the treatment of mice with the preestablished 3LL Lewis lung carcinoma cells. Tumor size was monitored at regular intervals and calculated as the product of maximal perpendicular diameters. Tumor measurements were made 20 days after tumor challenge. Columns represent mean tumor diameters, and dots represent individual tumor diameters (six mice per group). Data are representative of three experiments performed.

the CD28/CTLA4 costimulation pathway was functionally blocked by the chimeric fusion protein CTLA-Ig. The results showed that blockade of the CD28/CTLA4 pathway during immunization abrogated the protective immunity induced by Mut1 peptide-pulsed Lptn-DC. Mice were immunized once with 1 \times 10^5 Mut1 peptide-pulsed Lptn-DC and challenged with 5 \times 10^5 3LL tumor cells 11 days after immunization. Four days before DC immunization (A) or tumor challenge (B), the mice started to receive a total of five i.p. injections of 0.1 ml/mouse/injection ascites from hybridoma-bearing mice at the intervals of 3 days. The Abs used were GK1.5 (anti-CD4), 2.43 (anti-CD8), PK136 (anti-NK), and R4-6A2 (anti-IFN-\(\gamma\)) mAbs. Normal rat IgG was given as control Ab. Depletion of T cell subsets and NK cells was monitored by flow cytometry, which showed >90% specific depletion in splenocytes. To block the CD28/CTLA4 pathway of T costimulation, 50 \(\mu\)g of CTLA-Ig was admixed with peptide-pulsed DC and coinjected into mice. Tumor measurements were made 20 days after tumor challenge. Columns represent mean tumor diameters, and dots represent individual tumor diameters (six mice per group). Data are representative of three different experiments performed.

**FIGURE 4.** Immunization with 1 \times 10^5 (A) or 1 \times 10^4 (B) Mut1 peptide-pulsed Lptn-DC induces potent protective immunity to lethal 3LL tumor challenge. Normal C57BL/6 mice were immunized s.c. with HBSS, Mut1 peptide-pulsed DC, LacZ-DC pulsed with Mut1 peptide, Lptn-DC pulsed with OVA peptide. DC were administered at a single dose of 1 \times 10^5 (A) or 1 \times 10^4 (B). One week after immunization, the mice were injected s.c. with a lethal dose of 5 \times 10^5 3LL Lewis lung carcinoma cells. Tumor size was monitored at regular intervals and calculated as the product of maximal perpendicular diameters. Tumor measurements were made 20 days after tumor challenge. Columns represent mean tumor diameters, and dots represent individual tumor diameters (six mice per group). Data are representative of three experiments performed. Mice were killed when challenge tumors reached 3 cm in diameter or severe ulceration developed.

**FIGURE 5.** Involvement of T cell subsets, NK cells, CD28/CTLA4 costimulation, and IFN-\(\gamma\) in the induction phase (A) or effector phase (B) of the protective immunity induced by Mut1 peptide-pulsed Lptn-DC. C57BL/6 mice were depleted of CD4^+ T cells, CD8^+ T cells, or NK cells or blockage with an IFN-\(\gamma\) or CD28/CTLA4 costimulation pathway during immunization. Mice were immunized once with 1 \times 10^5 Mut1 peptide-pulsed Lptn-DC and challenged with 5 \times 10^5 3LL tumor cells 11 days after immunization. Four days before DC immunization (A) or tumor challenge (B), the mice started to receive a total of five i.p. injections of 0.1 ml/mouse/injection ascites from hybridoma-bearing mice at the intervals of 3 days. The Abs used were GK1.5 (anti-CD4), 2.43 (anti-CD8), PK136 (anti-NK), and R4-6A2 (anti-IFN-\(\gamma\)) mAbs. Normal rat IgG was given as control Ab. Depletion of T cell subsets and NK cells was monitored by flow cytometry, which showed >90% specific depletion in splenocytes. To block the CD28/CTLA4 pathway of T costimulation, 50 \(\mu\)g of CTLA-Ig was admixed with peptide-pulsed DC and coinjected into mice. Tumor measurements were made 20 days after tumor challenge. Columns represent mean tumor diameters, and dots represent individual tumor diameters (six mice per group). Data are representative of three different experiments performed.

**Lymphotactin Gene-Modified DC-Based Vaccine**

Lptn-DC pulsed with Mut1 peptide induce therapeutic antitumor immunity

To be more stringent and clinically relevant, the therapeutic effects of vaccination with Lptn-DC pulsed with tumor-associated peptide were further evaluated in the treatment of mice with the preestablished 3LL metastasis model. The 3LL tumor cell line is poorly immunogenic and highly metastatic. The spontaneous metastasis model of 3LL was established by injection of 2 \times 10^3 3LL tumor cells into the footpad. The average lung weight of a normal mouse was 190–210 mg. Thirty to thirty-five days after amputation, the mean lung weight of the HBSS-treated control group was about 600 mg. As shown in Fig. 6, vaccination with 1 \times 10^5 Mut1 peptide-pulsed DC or Mut1 peptide-pulsed LacZ-DC could slightly reduce lung metastases (with the mean lung weights of 436 and 402 mg, respectively), but less effectively than the Lptn-DC counterpart (256 mg in mean lung weight). The therapeutic effects of Mut1 peptide-pulsed DC or Mut1 peptide-pulsed LacZ-DC disappeared when the DC dose was reduced to 1 \times 10^4, but the same dose of Mut1 peptide-pulsed Lptn-DC could still inhibit pulmonary metastases markedly, yielding a mean lung weight of 312 mg. The survival time of the mice treated with Mut1 peptide-pulsed Lptn-DC was also greatly extended, much more effectively than with the mock control (data not shown), whereas vaccination with Mut1 peptide-pulsed DC or Mut1 peptide-pulsed Lptn-DC could not inhibit B16 pulmonary metastasis (data not shown), and OVA peptide-pulsed DC or OVA peptide-pulsed DC Lptn-DC did not exhibit any therapeutic effects on 3LL pulmonary metastases. These findings suggest that peptide-pulsed Lptn-DC can induce specific therapeutic antitumor immunity more potently.
with 1 bearing mice. Two days after amputation, mice were vaccinated twice s.c. to reach 7–8 mm in diameter, amputation was performed on the tumor-bearing mice. Two days after amputation, mice were vaccinated twice s.c. with 1 × 10^5 (open column) or 1 × 10^3 (solid column) peptide-pulsed DC at weekly intervals. The control mice were not immunized. Mice were killed when control groups died 30–35 days after amputation, and metastatic loads were recorded with lung weights. Columns represent mean lung weight, and dots represent individual lung weights (six mice per group). The results are representative of three different experiments.

Discussion

Vaccination with DC pulsed with tumor Ag is well documented to be capable of inducing Ag-specific CTL response, protecting immunized animals against tumor challenge, and resulting in regression of preestablished tumors, which has led to related clinical trials (7–19). Tumor Ag recognized by tumor-specific CTL are being defined for several human tumors (54, 55). Vaccination with tumor Ag-pulsed DC seems to be an attractive approach for cancer immunotherapy (5, 6). In the present study, we demonstrate that Lptn-DC pulsed with Mut1 tumor peptide are more potent to induce specific antitumor immunity, since they can induce specific CTL more potently than their counterparts of untransfected or LacZ-DC pulsed with Lptn-DC. 3LL tumor cells were inoculated into the footpads of normal mice (2 × 10^5 cells/mouse). When local tumors in the footpads reached 7–8 mm in diameter, amputation was performed on the tumor-bearing mice. Two days after amputation, mice were vaccinated twice s.c. with 1 × 10^5 (open column) or 1 × 10^3 (solid column) peptide-pulsed DC at weekly intervals. The control mice were not immunized. Mice were killed when control groups died 30–35 days after amputation, and metastatic loads were recorded with lung weights. Columns represent mean lung weight, and dots represent individual lung weights (six mice per group). The results are representative of three different experiments.

It is evident that chemokine can regulate the migration of T cells as well as DC; thus, chemokine may play potentially regulatory roles in the priming of T cell immunity by DC. Recent investigations demonstrated that DC could express chemokine receptors (e.g., CXCR4) and respond to chemokines to migrate directionally (36–39). In a more active manner, DC can attract T cells by secreting chemotactic activity on T cells, which implies that the autocrine of chemokine by DC may be very low and under the threshold of chemotaxis assay. Nevertheless, the supernatants from Lptn-DC can attract CD4+ and CD8+ T cells markedly, which indicates that Lptn gene modification of DC can improve their preferential chemotaxis on T cells and consequently may optimize the microenvironment of Ag presentation and favor DC Ag presenting to T cells. Although Lptn is also capable of attracting NK cells (46), in vivo depletion of NK cells during immunization did not abrogate the protective immunity induced by peptide-pulsed Lptn-DC.

Consistent with previous reports about peptide-pulsed DC (15), both CD4+ and CD8+ T cells are necessary for the induction of MHC-I-restricted peptide immunity by Lptn-DC, and only CD8+ T cells are required in the effector phase. In addition, T cell costimulation and IFN-γ are also found to be required for the induction of T cell immunity by peptide-pulsed Lptn-DC. The above data supported our hypothesis that Lptn autocrine by DC in the local microenvironment enables them to preferentially attract T cells more efficiently, favors DC Ag presentation, and, hence, improves the efficacy of DC-based vaccines. Whether Lptn autocrine by Lptn-DC affects their in vivo migration and tissue localization is under further investigation.

Genetic modification of DC is receiving much attention in DC-based vaccines. Different viral vectors, including retroviral vector (20–24), Ad vector (25–28), and vaccinia vector (26), have been evaluated to genetically modify DC. By coculture with retrovirus-producing packaging cells or repeated rounds of infection, mouse and human DC could be transduced with reporter genes (e.g., β-galactosidase, CD2, and LNGFR) and human tumor-associated Ag genes (e.g., mucin and MART-1), but the gene transfer efficiency is relatively low (20–75%) compared with that mediated by Ad vector. In addition, the former protocol is not compatible with the current guideline about the clinical use of retroviral vector. Ad vector could mediate gene transfer into DC with high efficiency (>95%). In animal models, a single vaccination with 1–3 × 10^5 DC genetically modified with Ad vector harboring cDNA for model Ags (e.g., β-galactosidase and OVA) have been documented to be capable of inducing protective and therapeutic antitumor immunity (25, 26). In our previous studies (12), mouse DC were transduced with GM-CSF Ad vector, and it was found that GM-CSF gene-modified DC acquired more potent costimulatory activity and could induce protective and therapeutic antitumor immunity against B16 melanoma after they were pulsed with tumor Ag. Although Ad vector is highly immunogenic, it is reported that repeated injections of Ad-infected DC induced only low titer of neutralizing Abs, and that the presence of neutralizing Abs specific for Ad did not affect the usefulness of infected DC to boost CTL response by repeated applications (26). Thus, Ad vector seems to be a promising tool to be used to genetically modify DC for vaccination. Besides viral vectors, nonviral methods were used to transduce DC, including lipofectin, calcium phosphate precipitation, and electroporation, but none of them yielded efficient gene transfer compared with Ad vector (27).

Our finding that a low dose of Lptn-DC (1 × 10^3) pulsed with tumor Ag peptide can exhibit a marked therapeutic effect on a preestablished tumor reduces the conventional dose of DC at least 10 times. Moreover, immunization with low-dose DC facilitates a reduction of the risk of autoimmune diseases related with DC-based vaccines. To our knowledge, this is the first demonstration that the improved chemotaxis of DC on T cells via genetic modification of DC with chemokine can increase the efficacy of DC-based vaccines. This study also provides an applicable approach to utilizing chemokines in the immunologic intervention.

References


LYMPTHACTIN GENE-MODIFIED DC-BASED VACCINE


