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# Listeria Monocytogenes Activated Dendritic Cell Based Vaccine for Prevention of Experimental Tumor in Mice

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## ABSTRACT

**Background:** The use of dendritic cells (DCs) as a cellular adjuvant provides a promising approach in immunotherapy of cancer. It has been demonstrated that Listeria monocytogenes activated DCs pulsed ex vivo with tumor antigens trigger a systemic Th1-biased specific immune response and a single dose of this vaccine will cause a considerable anti tumor immunity. **Objective:** The present study was designed to evaluate the ability of multiple doses of tumor antigen-pulsed DCs, matured in the presence of Listeria monocytogenes components in induction of a potent anti-tumor response and the prevention of tumor formation in an experimental model. **Methods:** Bone-marrow derived DCs (BMDCs) were cultured in the presence of GM-CSF and IL-4. After 5 days, tumor lysates with/without Listeria monocytogenes lysate were added to the culture media for another 2 days. Mice received mature and tumor antigen pulsed dendritic cells subcutaneously in 3 groups. Tumor growth was monitored and two weeks after immunotherapy, cytotoxic activity of CD8+ T cells was evaluated in different groups. **Results:** According to the findings, repeated doses of vaccine did not lead to a significant increase in the activity of cytotoxic T cells and decreased tumor growth of immunized animals. **Conclusion:** The current study suggests that increased doses of vaccine do not have sufficient efficiency for prevention of tumor induction. Generation of T regulatory responses upon repeated doses of such vaccines should be considered in future investigations.

**Keywords: Dendritic Cells, Immunotherapy, Cancer, Listeria Monocytogenes**

## INTRODUCTION

Dendritic cells (DCs) efficiently induce T cell activation in the secondary lymphoid organs (1-3). Th1 arm of the immune response is very important in battle against cancer

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(4). There are evidences indicating DCs to play an important role in determining the type of immune response generated against antigens.

Several factors can influence the development of polarized immune responses such as DC lineage and its activation status. Some studies have shown that distinct DC subsets are able to promote different types of response depending on pathogen-derived signals and host-derived cytokines present in the microenvironment (5-8).

Listeria monocytogenes is a gram-positive facultative intracellular bacterium, and its virulence is due to its capacity to penetrate into mammalian cells. Dendritic cells recognize Listeria by Toll-like receptors (TLRs) (2, 9) and then mature. Matured dendritic cells secrete inflammatory cytokines, such as tumor necrosis factor (TNF), interleukin-12 (IL-12), and several chemokines, allowing the recruitment and activation of immune cells. IL-12 participates in the development of T lymphocytes expressing Th1-type cytokines such as interferon gamma (IFN-γ), TNF, and IL-2 (9-16).

Our previous study showed that Listeria monocytogenes activated DCs pulsed with tumor antigens caused a better response in lymphocyte proliferation, IL-12 production and CTL induction. A single dose of the vaccine caused significantly better outcome in tumor growth retardation and survival of animals compared to other microbial components such as LPS.

In the present study, we examined efficacy of repeated doses of Listeria monocytogenes activated DC vaccine in the prevention of mice fibrosarcoma tumor model.

## MATERIALS AND METHODS

**Animals and Cell Lines.** 6-8 weeks old female Balb/c mice were purchased from Pasteur Institute of Iran. All animal experiments were performed according to the guidelines of the local Ethical Committee. Balb/c derived fibrosarsoma (WEHI-164) and colon carcinoma (CT26) cell lines were maintained by in vitro culture in RPMI 1640 (Sigma, Steinhem, Germany) supplemented with 10% heat-inactivated fetal bovine serum (Gibco,Grand Island, USA), 2 mM L-glutamine (Sigma, Steinhem, Germany), 100 μg/ml streptomycin and 100 U/ml penicillin.

**Tumor Challenge.** To generate tumors, Balb/c mice were challenged subcutaneously in right flanks with 0.2 ml of a cell suspension containing $10^6$ WEHI-164 cells. Tumor measurements were performed every 2 days with calipers spanning the shortest and longest surface diameters. Mice were sacrificed when the tumor diameter reached >400mm$^2$.

**Preparation of Listeria Monocytogenes Lysate.** Listeria monocytogenes (ATCC 19115) was purchased from Iranian Research Organization for Science and Technology (IROST) and grown in brain-heart infusion medium. Bacteria were sonicated in PBS and passed through a 0.2-μm pore filter. The protein concentration of the lysate was determined by Bradford method.

**Preparation of Tumor Lysate.** $5\times10^6$ WEHI-164 cells were injected S.C. in to the right flank of Balb/c mice, and tumor growth was monitored. 21 days after tumor challenge, tumors were harvested surgically from tumor-bearing mice and a single cell suspension was made. Cell lysates were generated by repeated freeze and thaw cycles. After centrifugation, the supernatant solution was collected and passed through a 0.2 μm pore filter. The protein concentration of the lysate was determined by Bradford method.

**DC Preparation and Culture**. Bone marrow (BM) derived DCs (BMDCs) were generated as described by Inaba et al. (17) with slight modifications. Briefly, murine BM

cells were harvested from femurs and tibias of sacrificed mice. Contaminating erythrocytes were lysed with distillated water and 10x phosphate buffer. Cells ($1x10^6$ cells/ml) were placed in 24-well plates in RPMI 1640 supplemented with 10% fetal calf serum, 50 μM 2-mercaptoethanol, 100 U/ml recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF) (Bender Med systems, Vienna, Austria), and 50 U/ml of recombinant murine interleukin-4 (IL-4) (Roche, Indianapolis, USA). On day 3, non adherent cells were collected and fresh media were added. On day 5, 100 μg/ml/$10^6$cell of tumor lysate was added to immature DC cultures. After 10 hours, 70 μg/ml/$10^6$cell of Listeria monocytogenes lysate were added to different wells. On day 7 mature DCs were collected and used for immunotherapy.

**Flow Cytometric Analysis**, To determine the phenotype of cultured DCs, on days 5 and 7,cells were stained with PE or FITC conjugated monoclonal antibodies against cell surface molecules CD40, CD80, CD86, CD11c and MHC-II (all purchased from BD PharMingen, San Diego, CA) and analyzed with a FACS Analysis System (Becton Dickinson). In all experiments, isotype controls were included using an appropriate mAb of the same Ig class or subclass.

**Immunization.** $10^6$ DCs /200μL PBS were injected subcutaneously at the right flank of the animals. One, two or three doses of the vaccine were administered in three different groups of mice with 10 days interval. The first dose was given 10 days before tumor challenge (-10)، (-10, 0)، (-10, 0, 10). Control group received PBS. Tumor measurements were performed every 2 days with calipers spanning the shortest and longest surface diameters. Mice were sacrificed when the tumor diameter reached >400$mm^2$.

**Cytotoxicity Assay.** 20 days after tumor challenge, splenocytes were isolated and used as effector cells. Tumor cell lines (WEHI-164, CT26) were used as target cells. Cytotoxic activity was measured by LDH cytotoxicity detection kit (Roche Applied Science). After washing the effector and target cells with the assay medium (RPMI1640 with 1% BSA), the effector cells were co-cultured with target cells at 3 different ratios ( 12.5, 25 and 50) in a 96-well round bottom plate for 6 h at 37$^o$C; then the plates were centrifuged and the supernatants were transferred to another flat-bottom ELISA plate. One hundred μl of LDH detection mixture were added to each well and incubated for 30 minutes at room temperature. Absorbance was measured by an ELISA reader at 490 nm. The percentage of cell mediated cytotoxicity was determined by the following equation:

Cytotoxicity (%) = (experimental release – spontaneous target release – spontaneous effector release) / (maximal target release – spontaneous target release) × 100%.

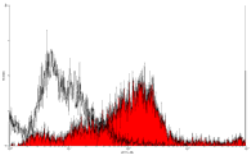
**Intracellular Cytokine Assay.** Intracellular IFN-γ staining of splenocytes was performed as previously described (36). Briefly, $10^6$ splenocytes were stimulated in 96-well flat bottom plates with 100 μg tumor lysate or 5 μg Con A for 24 h at 37°C in 5% CO2 , and brefeldin A (Sigma, Steinhem, Germany) was added for the last 4 h at 37°C in 5% CO2. Cells were washed with staining buffer (PBS with 3% FBS and 0.09% sodium azide), and then stained with anti-CD8 PE, (BD Pharmingen, CA) on ice for 20–30 min. Cells were then permeabilized, fixed and stained for intracellular IFN-γ with anti-IFN- γ FITC or a FITC-labeled isotype control mAb.

**Statistical Analysis.** The results are expressed as mean ± standard error. Statistical analysis was performed using a Student's t test, with the exception of the survival data, which was analyzed using the Kaplan and Meier test. $p < 0.05$ were considered significant.

## RESULTS

**DC Maturation with Listeria Monocytogenes Components.** 5 and 7 day DCs stained with mAb against CD40, CD80, CD86, CD11c and MHC-II. BMDC exposed to Listeria and tumor lysate for 48 h up-regulated the surface expression of CD40, CD80 (B7.1), CD86 (B7.2), and MHC class II to a similar extent (Figure 1).

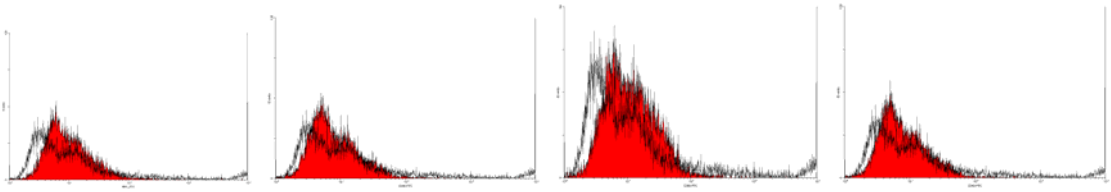
(a)



CD11c

(b)

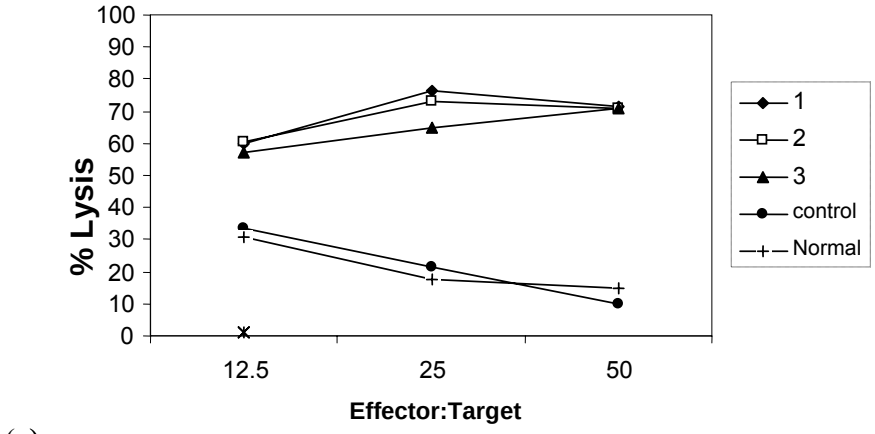
**Listeria+Tumor Lysate**



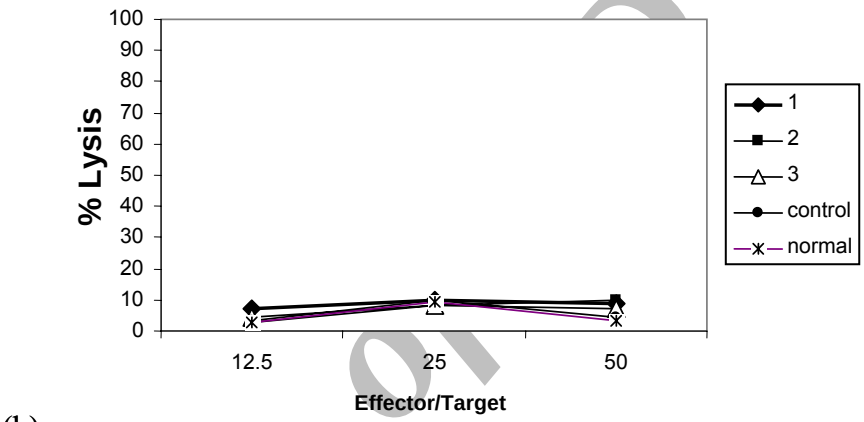
CD40 MHC-II CD86 CD80

**Figure 1.** (a) The histograms showing the expression density of CD11c of immature DCs (full histograms) and isotype control (open histograms). (b) CD80, CD86 and CD40 expression of immature DCs (open histograms) and mature DCs (full histograms).

**Tumor-Specific Cytotoxic T Cell Immune Response.** To determine whether repeated vaccination could stimulate a stronger CTL response, splenocytes were obtained 20 days after tumor challenge. Splenocytes were co cultured with WEHI164 tumor cells as target or CT26 cells as control for 6-8 hours and cytotoxicity was determined using a LDH release assay. As shown in Figure 2a, immunotherapy with repeated (2 and 3) doses of vaccine did not cause any considerable augmentation in specific cytotoxicity. The CTL activity was significantly ($p=0.0044$) higher than the control group. Furthermore we demonstrated that the cytotoxicity was specific for WEHI-164 tumor cells because there was little cytotoxicity effect on CT26 tumor cells (Figure 2b).




(a)




(b)

**Figure 2.** Tumor-specific CTL response to fibrosarcoma cells in different groups of mice immunized with 1, 2 and 3 doses of DC vaccine. Targets included WEHI 164 and CT26. (a).The specific CTL activity in test groups was significantly higher than controls. (b). Specificity of the cytotoxic effect on WEHI164 tumor cells due to the lack of cytotoxic effect on CT26 tumor cells.

**Intracellular IFN-γ Staining.** For intracellular IFN-γ staining, splenocytes of three mice in each group were co-cultured with tumor lysate. Splenocytes from normal mice were used as controls. With the increasing doses of the vaccine, percent of IFN-γ positive CD8 T cells did not significantly increase. Comparing to the no treatment group, number of IFN-γ positive cells were higher but the difference was not significant. However, compared to the normal mice, the difference was found to be significant (p=0.0055) (Figure 3).

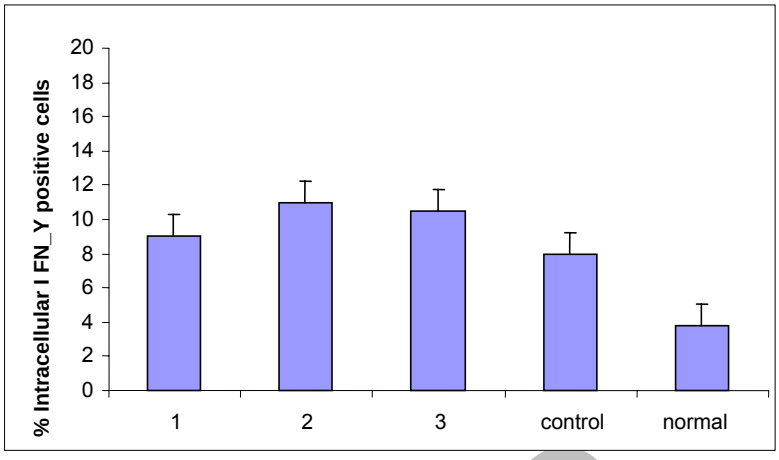



**Figure 3.** Percentage of intracellular IFN-γ Positive CD8 T cells after in vitro stimulation with tumor lysate in vaccinated animals (1, 2, and 3 doses), non vaccinated control and normal mice without tumor challenge or vaccination.

**In Vivo Anti-Tumor Response.** Anti-tumor immunity induced by DC vaccine was evaluated in mice immunized with different doses of the vaccine. Compared to the non-treated group, tumor growth rate was significantly decreased in the treated animals (p=0.0049 ) . Repeated doses of vaccine did not show any significant effect on the tumor growth (Figure 4a).

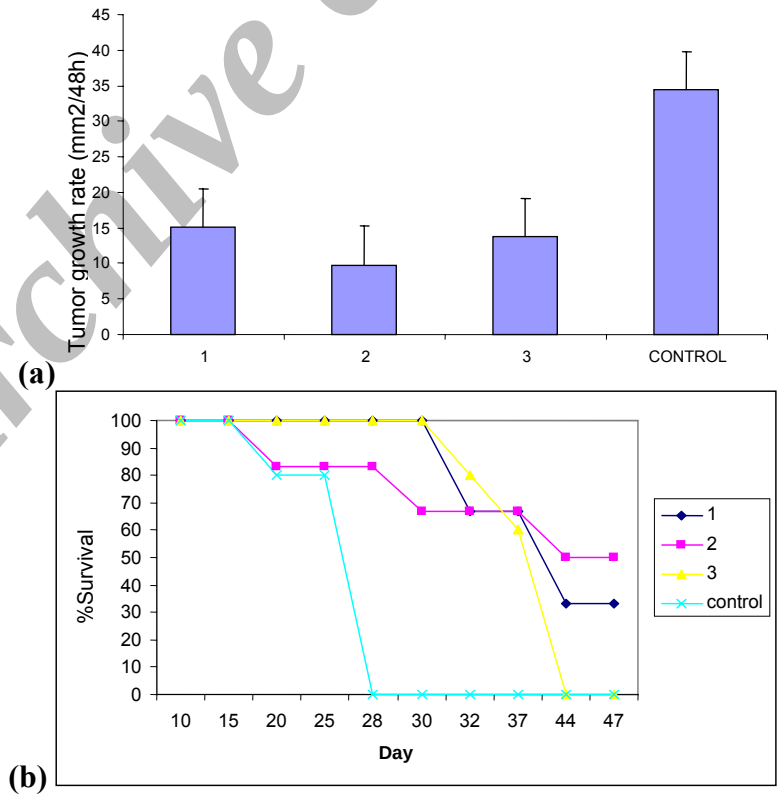



**Figure 4.** The effect of different doses of DC vaccine (1, 2 and 3) in a subcutaneous WEHI 164 tumor model. (a) The tumor growth rate and (b) survival of each group of mice monitored for 50 days. Each group consisted of five mice.

As depicted in Figure 4b, 28 days after tumor challenge, when there was no live mouse in the control group, the survival rate in the mice receiving 1, 2 and 3 doses of the vaccine was 100%, 83% and 100%, respectively.

## DISCUSSION

Previous studies have revealed that DCs exhibit different activation responses to different classes of pathogens and there is a notable correlation between the activation status of DCs and the type of their inducible Th cell response (16). DCs are used extensively for antigen-specific immunotherapy of cancer because they are the most efficient APCs can inducing primary immune response (18, 19).

Knowing the role of Listeria monocytogenes in induction of Th1 (cellular arm) immune responses and the importance of this arm in anti-cancer immunity (9, 20, 21), the present study was conducted to investigate the potency of Listeria monocytogenes activated DC based vaccine for eliciting anti tumor immune response and prevention of tumor induction in mice.

In microbial infections, certain molecular patterns of microbial components directly stimulate immature DCs in the periphery to differentiate them into mature DCs by binding to pattern recognition receptors such as Toll-like receptors (TLRs) that play a critical role in the innate immunity. Stimulation of TLR signaling in DCs causes an increase in surface expression of MHC-peptide for T cell recognition, up regulation of co-stimulatory molecules important for T cell clonal expansion and secretion of immunomodulatory cytokines, which direct T lymphocyte differentiation into effector cells (12, 22, 23).

According to our previous study, Listeria monocytogenes activated and tumor lysate pulsed DC immunotherapy resulted in considerable levels of specific cytotoxicity against tumor, diminution of tumor growth rate and improvement of survival compared to other microbial components and the control group (15). In that study, a single dose of the vaccine was injected after tumor establishment as a therapeutic vaccine. In the present study, the effect of multiple doses of the vaccine before tumor challenge was evaluated as a prophylactic measure.

Immunotherapy in all vaccinated animals (1, 2 or three times vaccinated) with 10 days interval between second and third immunization caused significant anti tumor response compared to the controls, although complete rejection of the tumor was not seen in all of them. Repeated doses of the vaccine did not lead to its long term effect.

For improving the efficacy of anti tumor vaccines, various vaccination protocols, such as DC dose, vaccination schedule (best time for vaccine injection and time interval between first and booster immunizations), number of vaccinations and antigenic components need to be optimized (24).

Recent studies suggest that most of the migrating DCs die after their arrival at lymphoid tissues, and the short life span of DCs in vivo must be extended to improve an effective response (25). When BM derived or splenic DCs were transferred into tumor bearing mice, only a small proportion (0.1–1%) of the implanted DCs were found in the draining lymph nodes. Increasing the number of injected DCs or tissue conditioning through inflammatory cytokines can improve the number and function of DCs. Such modifications can result to even 40 folds increase in antigen specific CD4 T cells in draining lymph node (26). According to Dasilva et al, balance between tumor burden and the

number of tumor-specific T cells determines whether a therapeutic vaccine will be efficacious and result in long-term therapy (27).
Cytotoxic T cell response evaluation in this study demonstrated that in spite of a significant rise in anti tumor cytotoxicity after single dose vaccination, there was no significant rise in this response upon expanding the vaccine dose.
There are many different ways for tumor antigen delivery to DCs. Most of the initial DC vaccination protocols used DC loaded with tumor-associated peptides (28). Disadvantages associated with peptide loaded DC vaccines include the limitation of this strategy to patients with a specific HLA type, a few defined epitopes among most tumors, potential generation of tumor Ag escape variants when immunizing against a single Ag, and the presence of a high proportion of non-tumor-specific peptides when using whole tumor cell eluates. Furthermore, the weak immunogenicity of many tumors also represents a barrier to the effective induction of antitumor immunity (29). Tumor lysate containing collection of tumor antigenic epitopes with least mentioned limitations is another option that we used in the current study.
Since tumor cells have self origin and potentially can induce immunologic tolerance, the ultimate success might depend on the balance between the capacity of the immunogenic stimulus to "break tolerance" and on the inherent physiologic limitation against activation of "self"-reactive T cells (30).
Recent evidence indicates that DCs can induce the activation and proliferation of CD4+CD25+ regulatory T cells (Treg) in vitro and in vivo (31). In turn, the ability of DCs to activate CD4+and CD8+ T cells is substantially increased by depletion of Treg (32).
Several reports have documented that depletion of Treg can enhance spontaneous immune responses to tumors, and increase the antitumor effect of different immunotherapeutic procedures (32, 33). Also depletion of Treg in mice treated with DC considerably increased the potency of the DC vaccine, and induced long-term tumor immunity (34).
Therefore, induction of regulatory responses after repeated doses of the vaccine should be considered in future vaccine design. Using tumor lysate as tumor antigen source for DC loading is another subject which should be considered because it contains concoction of tumor and self antigens, and is prone to elicit regulatory responses.

## ACKNOWLEDGEMENTS

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## REFERENCES

1 Banchereau J, Steinman R M. Dendritic cells and the control of Immunity. Nature. 1998; 392:245-52.
2 Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, et al. Immunobiology of dendritic cells. Annu Rev Immunol. 2000; 18:767-811.
3 Mellman I, Steinman RM. Dendritic cells: specialized and regulated antigen processing machines. Cell .2001; 106: 255-8.
4 Ikeda H, Chamoto K, Tsuji T, Suzuki Y, Wakita D, Takeshima T et al. The critical role of type-1 innate and acquired immunity in tumor immunotherapy. Cancer Sci. 2004; 95:697-703
5 Vieira PL, de Jong E, Wierenga E, Kapsenberg M, Kalinski P. Development of Th1-inducing capacity in. myeloid dendritic cells requires Environmental instruction. J Immunol. 2000; 164:4507-12.
6 De Smedt T, Butz E, Smith J, Maldonado-Lopez R, Pajak B, Moser M, et al. CD8alpha(-) and CD8alpha(+) subclasses of dendritic cells undergo phenotypic and functional maturation in vitro and in vivo. J Leukokoc Biol .2001; 69:951-8.
7 MacDonald AS, Straw AD, Bauman B, Pearce EJ. CD8- dendritic cell activation status plays an integral role in influencing. Th2 response development. J Immunol. 2001;167:1982-8.

8 Maldonado-Lopez R, Maliszewski C, Urbain J, Moser M. Cytokines regulate the capacity of CD8alpha (+) and CD8alpha (-) dendritic cells to prime Th1/Th2 cells in vivo. J Immunol. 2001;167:4345-50.
9 Pamer EG. Immune responses to Listeria monocytogenes. Nat Rev Immunol. 2004; 4: 812–23.
10 Kopp E, Medzhitov R. Recognition of microbial infection by Toll-like receptors. Curr Opin Immunol. 2003; 15:396-401
11 Kadowaki N, Ho S, Antonenko S, Malefyt RW, Kastelein RA, Bazan F et al. Subsets of human dendritic cell precursors express different Toll-like receptors and respond to different microbial antigens. J Exp Med. 2001; 194:863–9.
12 Michelsen KS, Aicher A, Mohaupt M, Hartung T, Dimmeler S, Kirschning CJ et al. The role of toll-like receptors (TLRs) in bacteria-induced maturation of murine dendritic cells (DCS): peptidoglycan and lipoteichoic acid are inducers of DC maturation and require TLR2. J Biol Chem. 2001; 276: 25680–6.
13 Edelson BT, Unanue ER. Immunity to Listeria infection. Curr Opin Immunol. 2000; 12: 425–31.
14 Cousens LP, Wing EJ. Innate defenses in the liver during Listeria infection. Immunol. Rev. 2000; 174:150–9
15 Motamedi M, Khansari N, Moazzeni SM, Vodjgani M, Keihani A, Gheflati Z et al. Cancer Immunotherapy Using Dendritic Cells Matured With Bacterial Components. Yakhteh .2006; 252-7
16 Kikuchi T. Genetically modified dendritic cells for therapeutic immunity. Tohoku J Exp Med. 2006; 208:1-8
17 Inaba K, Inaba M, Romani N, Aya H, Deguchi M, Ikehara S et al. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/ macrophage colony-stimulating factor. J Exp Med. 1992;176:1693–702
18 Chagnon F, Tanguay S, Ozdal OL, Guan M, Ozen ZZ, Ripeau JS et al. Potentiation of a Dendritic Cell Vaccine for Murine Renal Cell Carcinoma by CpG Oligonucleotides. Clin Cancer Res. 2005; 11:1302–11
19 Correale P, Cusi MG, Del Vecchio MT, Aquino A, Prete SP, Tsang KY et al. Dendritic cell-mediated cross-presentation of antigens derived from colon carcinoma cells exposed to a highly cytotoxic multidrug regimen with gemcitabine, oxaliplatin, 5-fluorouracil, and leucovorin, elicits a powerful human antigen-specific CTL response with antitumor activity in vitro. J Immunol. 2005;175:820-8
20 de Vries IJ, Lesterhuis WJ, Scharenborg NM, Engelen LP, Ruiter DJ, Gerritsen MJ et al. Maturation of dendritic cells is a prerequisite for inducing immune responses in advanced melanoma patients. Clin Cancer Res, 2003;9:5091-100
21 Mun H, Aosai F, Norose K, Chen M, Piao LX, Takeuchi O et al. TLR2 as an essential molecule for protective immunity against Toxoplasma gondii infection . Int Immunol. 2003;15:1081-9
22 De Vries IJ, Krooshoop DJ, Scharenborg NM, Lesterhuis WJ, Diepstra JH, Van Muijen GN et al. Effective migration of antigen-pulsed dendritic cells to lymph nodes in melanoma patients is determined by their maturation state. Cancer Res, 2003; 63:12-7.
23 Akira S, Hemmi H. Recognition of pathogen-associated molecular patterns by TLR family .Immunol Lett. 2003; 85:85-95
24 Park MY, Kim CH, Sohn HJ, Oh ST, Kim SG, Kim TG. The optimal interval for dendritic cell vaccination following adoptive T cell transfer is important for boosting potent anti-tumor immunity. Vaccine. 2007; 25:7322-30.
25 Kim JH, Chen J, Majumder N, Lin H, Falo LD Jr, You Z. 'Survival gene' Bcl-xl potentiates DNA-raised antitumor immunity. Gene Ther. 2005;12:1517–25.
26 MartIn-Fontecha A, Sebastiani S, Hopken UE, Uguccioni M, Lipp M, Lanzavecchia A et al. Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and priming. J Exp Med. 2003; 198:615–21.
27 Da Silva DM, Schiller JT, Kast WM. Heterologous boosting increases immunogenicity of chimeric papillomavirus virus-like particle vaccines. Vaccine. 2003; 21:3219-27
28 Mayordomo JI, Zorina T, Storkus WJ, Zitvogel L, Celluzzi C, Falo LD et al. Bone marrow derived dendritic cells pulsed with synthetic tumour peptides elicit protective and therapeutic antitumour immunity. Nat Med. 1995; 1:1297-302
29 Thurner B, Haendle I, Röder C, Dieckmann D, Keikavoussi P, Jonuleit H et al. Vaccination with mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. J Exp Med. 1999; 190:1669-78
30 Chakraborty NG, Chattopadhyay S, Mehrotra S, Chhabra A, Mukherji B. Regulatory T-cell response and tumor vaccine-induced cytotoxic T lymphocytes in human melanoma. Hum Immunol. 2004 ;65:794-802
31 Yamazaki S, Iyoda T, Tarbell K, Olson K, Velinzon K, Inaba K. Direct expansion of functional CD25_CD4_ regulatory T cells by antigen-processing dendritic cells. J Exp Med. 2003; 198:235-47
32 Prasad SJ, Farrand KJ, Matthews SA, Chang JH, McHugh RS, Ronchese F. Dendritic cells loaded with stressed tumor cells elicit long-lasting protective tumor immunity in mice depleted of CD4+CD25+ regulatory T cells. J Immunol. 2005; 174:90-8.
33 Woo EY, Yeh H, Chu CS, Schlienger K, Carroll RG, Riley JL et al. Cutting edge: regulatory T cells from lung cancer patients directly inhibit autologous T cell proliferation. J Immunol.2002;168:4272–6
34 Oldenhove G, De Heusch M, Urbain-Vansanten G, Urbain J, Maliszewski C, Leo O et al. CD4+ CD25+ regulatory T cells control T helper cell type 1 responses to foreign antigens induced by mature dendritic cells in vivo. J Exp Med. 2003; 198:259-66.