Original Article

LMP2-DC Vaccine Elicits Specific EBV-LMP2 Response to Effectively Improve Immunotherapy in Patients with Nasopharyngeal Cancer*

ZENG Yi[1,5,&,#], SI Yong Feng[2,#], LAN Gui Ping[2], WANG Zhan[1], ZHOU Ling[1], TANG Min Zhong[3], SJ O`Brien[4], LAN Jiao[2], ZHOU Xiang Yang[2], WANG Yong Li[2], TANG Juan[2], ZHOU Zhi Xiang[5], DU Hai Jun[1,#], and LIN Hui[2,#]

1. State Key Laboratory for Infectious Disease Prevention and Control, National Institute of Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China; 2. People's Hospital of Guangxi Zhuang Autonomous Region, Nanning 530021, Guangxi, China; 3. Cancer center, Wuzhou Red Cross Hospital, Wuzhou 543002, Guangxi, China; 4. Guy Harvey Oceanographic Center, Halmos College of Natural Sciences and Oceanography, Nova Southeastern University, Florida 33004, USA; 5. College of Life Science and Bioengineering, Beijing University of Technology, Beijing 100124, China

Abstract

Objective To evaluate the safety and effectiveness of a vaccine based on latent membrane protein 2 (LMP2) modified dendritic cells (DCs) that boosts specific responses of cytotoxic T lymphocytes (CTLs) to LMP2 before and after intradermal injection in patients with nasopharyngeal carcinoma (NPC).

Methods DCs were derived from peripheral blood monocytes of patients with NPC. We prepared LMP2-DCs infected by recombinant adenovirus vector expressing LMP2 (rAd-LMP2). NPC patients were immunized with 2×10^5 LMP2-DCs by intradermal injection at week 0 and after the second and fourth weeks. Specific responses to LMP2 were detected by enzyme-linked immunospot (ELISPOT) assay at week 0 and at the fifth and eighth weeks. Local clinicians performed the follow-up and tracking of patients.

Results We demonstrated that DCs derived from monocytes displayed typical DC morphologies; the expression of LMP2 in the LMP2-DCs vaccine was confirmed by immunocytochemical assay. Twenty-nine patients with NPC were enrolled in this clinical trial. The LMP2-DCs vaccine was well tolerated in all of the patients. Boosted responses to LMP2 peptide sub-pools were observed in 18 of the 29 patients with NPC. The follow-up data of 29 immunized patients from April, 2010 to April 2015 indicated a five-year survival rate of 94.4% in responders and 45.5% in non-responders.

Conclusion In this pilot study, we demonstrated that the LMP2-DCs vaccine is safe and effective in patients with NPC. Specific CTLs responses to LMP2 play a certain role in controlling and preventing the recurrence and metastasis of NPC, which warrants further clinical testing.

Key words: LMP2- DCs; Nasopharyngeal carcinoma; Immunotherapy

Biomed Environ Sci, 2020; 33(11): 849-856 doi: 10.3967/bes2020.115 ISSN: 0895-3988

www.besjournal.com (full text) CN: 11-2816/Q

*Mega Project of Research on the Prevention and Control of HIV/AIDS, Viral Hepatitis Infectious Diseases [Grant No:2018ZX10102001]; the Key Science and Technology Program of Guangxi Zhuang Autonomous Region [Grant No. 14124003-3]; the National High Technology Research and Development Program of China [Grant No. 2007AA021107]; and the National Basic Research Program of China [973 Program, Grant No. 2011CB504800].

#Correspondence should be addressed to DU Hai Jun, E-mail: haijun70@126.com; ZENG Yi, E-mail: zengyicdc @sina.com; SI Yong Feng, E-mail: syfklxf@126.com; LIN Hui, E-mail: linhui33622@sina.com

Biographical note of the first author: ZENG Yi, male, 1929-2020, professor, academician, a distinguished virologist, majoring in oncogenic virus.

INTRODUCTION

The current standard treatment of nasopharyngeal carcinoma (NPC) is radiation therapy or a combination of radiation and chemotherapy. Although the initial rate of response and the five-year survival rate for early stage patients with NPC are promising when compared to other malignancies, recurrences and post-treatment metastases often appear in advanced-stage NPC patients. Patients with NPC recurrence and metastasis have poor prognosis, and no curative therapeutic options are available. There is substantial evidence that Epstein-Barr virus (EBV) plays an important role in NPC development[1,2]. A limited array of EBV antigens, including latent membrane proteins (LMP) 1 and 2, and EBV nuclear antigen 1 (EBNA1), are expressed in NPC cells[3]. Of these, LMP2 epitopes are the most frequently recognized by CD8+ cytotoxic T lymphocytes (CTLs)[4], and CTLs specific to LMP2 have been shown to efficiently kill NPC cells both *in vivo* and *in vitro*[5–7]. The results of these studies suggest that patients with NPC could benefit from immunotherapy.

DCs are the most potent antigen-presenting cells, are widely distributed throughout the body, and have the ability to stimulate both native and secondary immune responses to antigen(Ag)-specific T cells[8–10]. Genetic modification of DCs with genes encoding immunoregulatory molecules provides a potential approach for Ag-specific T cell-mediated immunity by selectively targeting antigen-specific T cells[11,12]. A cellular vaccine based on DCs pulsed with LMP2-antigen has previously been reported at the Global Virus Networks meeting in 2015[13]. The results showed that the vaccine induced specific CTLs were sufficient for NPC control and treatment.

With these recent studies in mind, we designed and conducted a pilot clinical study to test the biological effects of LMP2-DCs in 29 patients with NPC after conventional radiotherapy and chemotherapy. We report here the promising immunological and clinical results.

MATERIALS AND METHODS

Generation and Maturation of LMP2-DCs

Autologous mononuclear cells were isolated by the Ficoll-Hypaque density gradient centrifugation (Pharmacia, 30 min, 4 °C, 800 ×*g* from peripheral blood). Peripheral blood monocytes (PBMCs) were cultured in six-well plates at 5×10^6 cells/well (37 °C, 5% CO_2) for 2 h. Supernatant PBMCs were removed, and adherent monocytes were cultured for 7 d in six-well plates in 3 mL/well with 500 U/mL IL-4 (Pepretech) and 500 U/mL GM-CSF (Pepretech) in a DC medium (Cell-GRO® DC). On day 3, half of the medium was replaced with fresh DC medium. The morphology of monocytes was observed by optical microscope.

The immature DCs were aspirated and harvested (250 ×*g*, 10 min) on day 7 and then transferred to new 6-well plates at 10^6 cells/well. DCs were then infected with rAd-LMP2 (Shen Zhen Tsinghua Yuanxing Pharmaceutical Co, Ltd, MOI100) for 2 h. DCs expressing LMP2 (LMP2-DCs) were induced to maturity by replacing cells with fresh medium containing IL-4 (500 U/mL), GM-CSF (500 U/mL), and tumor necrosis factor-alpha (TNF-α 400 U/mL Pepretech) for another two days. Mature LMP2-DCs (mLMP2-DCs) were stained with immunofluorescence antibodies CD83, CD86, and HLA-DR (BD Bioscience) and then analyzed using flow cytometry. The mLMP2-DCs were harvested by centrifugation at 250 × *g* for 10 min.

The pellets of mLMP2-DCs (250 ×*g*, 10 min) were collected and washed with PBS three times and then resuspended in freezing medium containing 10% dimethyl sulfoxide (Sigma), 20% autologous serum, and 70% DC medium 2×10^6/mL (0.5 mL/dosage). Cells were frozen at −80 °C overnight using a Cryo 1 °C Freezing Container (Nalgene) filled with isopropanol. The frozen cells were then transferred to liquid nitrogen.

Identification of Expression of LMP2 in mLMP2-DCs

Cell smears were prepared from mLMP2-DCs and fixed in cold acetone at 4 °C for 15 min. The slides were allowed to dry in air and were incubated with anti-LMP2 monoclonal antibody (Santa Cruz Biotechnology, diluted 1:100 with phosphate buffer saline, PBS) at 37 °C for 40 min. After primary antibody incubation, the slides were washed three times with PBS. Biotin-conjugated anti-human IgG antibody (diluted 1:100 with PBS) was added to the slide at 37 °C for 40 min, and the slides were washed three times with PBS. Horseradish/peroxidase-conjugated anti-biotin IgG antibody (diluted 1:100 with PBS) was added to the slides at 37 °C for 40 min. The slides were incubated with diaminobenzidine (Sigma) and H_2O_2 for 10 min and washed in distilled water. The slides were then air dried and examined under an optical microscope.

Clinical Trial Design and Subjects

We performed a pilot study in patients with NPC to determine the safety, immunogenicity, and clinical efficacy of a mLMP2-DCs vaccine. Eligible cases were histopathologically confirmed cases of NPC (stage I-IV), in which the patient's age was ≤ 70 years and the patient was at least three months post-chemotherapy and -radiotherapy and at least three months into remission with no acute diseases and normal function of liver, kidney, and marrow. Enrollment was allowed at least three months after conventional radiotherapy and chemotherapy. The demographic information regarding all patients is summarized in Supplementary Table S1 available in www.besjournal.com. PBMCs were harvested for LMP2-DCs vaccine manufacturing at the Clinical Cell and Vaccine Production Facility at the People's Hospital of Guangxi Zhuang Autonomous Region. Vaccines were cryopreserved at −196 °C, thawed and washed before administration. Patients with NPC received three doses of the vaccine (≥ 5 × 10^5 DCs/dose) intradermally every two weeks. The procedure is shown in Figure 1. Patients with NPC were evaluated every two weeks. Safety was determined using the China Food and Drug Administration Grading Standard of Adverse Events in Clinical Trials (http://www.cde.org.cn/zdyz.do?method=list). Clinical indications, including breathing, heart rate, blood pressure, and body temperature were monitored. The temperature was documented when fever occurred (body's temperature over 38 °C). Subjects underwent full examination (blood, urine, hepatorenal function, electrocardiogram) pre- and post-immunotherapy.

This study was approved by Institutional Review Board of the People's Hospital of Guangxi Zhuang Autonomous Region on September 24, 2008. All the procedures meet the ethics requirement. All patients gave written informed consent prior to initiation of any study procedures.

Detection of Specific CTL Responses to LMP2

PBMCs were isolated from immunized patients by Ficoll-Hypaque density gradient centrifugation at week 0 and at 5 and 8 weeks 800 ×*g*, 15 min non-stop). Specific responses of CTLs to LMP2 were analyzed using human IFN-γ Elispot kit (BD Bioscience). The kit was used according to the manufacturer's protocol. PBMCs were plated at 2 × 10^5 cells/well, and six sub-peptide pools (Supplementary Table S2 available in www.besjournal.com) screened from the LMP2 pool were added to the wells at a final concentration of 5 μg/mL per peptide for overnight stimulation. Wells containing media alone were used as negative controls, and cells incubated with 1 μg/mL PhosPhomolybdic Acid (PMA) and ionomycin (Ion) (Da Kewei Co, Ltd) were used as positive controls. The chromogenic reaction was terminated by distilled water as soon as spots were ready for counting. The plate was allowed to dry in air for 10–30 min. The number of spots per well were read using Bioreader 4000 PRO, and statistic data analysis (GraphPad Prism 5) was performed. A positive response was defined as a two-fold or greater increase in the number of spot-forming cells per million PBMC in immunized wells compared to pre-immunized wells. Additionally, we tested the plasma for changes of the EBV DNA genome copies and titers of IgA antibody to VCA. If the titers of IgA were elevated more than four-fold compared to levels in pre-immunization, the difference was considered significant.

Follow Up

The benefit of LMP2-DCs to patients with NPC was investigated through follow-up and tracking of

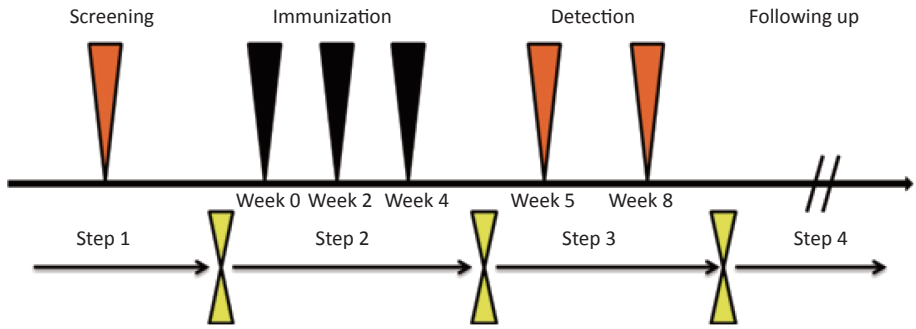

Figure 1. Steps of the study. Yellow triangle: border of different trial stage; Black triangle: time of immunotherapy; Orange triangle: time of LMP2 specific cellular immunity test.

the study subjects. Admission data was reviewed every three months and close follow-up of individual subjects was performed every half year after immunotherapy was completed. Follow-up included phone inquiry to determine physical condition and clinical monitoring of NPC progression.

RESULTS

Clinical Trials

Patients with NPC began to enroll in this study in April 2009. We completed the study in October 2010. Twenty-nine patients with NPC (age 24–66) were enrolled at the hospital for treatment with intradermal injections and agreed to participate in follow up for five years.

LMP2-DCs Can be Prepared Efficiently

Monocytes isolated from PBMCs were cultured and induced to DC differentiation *in vitro*. During differentiation, cells progressively acquired DC morphological features. Cells were loosely adherent and irregularly netted. We observed the formation of cell mass, increased cell size, folding on the cellular surface, and the formation of cytoplasmic projections (Figure 2). Immunocytochemical assay, using a LMP2 monoclonal antibody, was performed to confirm the expression of LMP2 in 100 MOI rAd-LMP2 infected DCs. An optical microscope was used to illuminate DCs expressing LMP2 (Figure 3).

LMP2-DCs Are Safe and May Offer Clinical Benefit in Subjects with NPC

We tested LMP2-pulsed matured DCs for vaccine production in a pilot study of patients with NPC after conventional radiotherapy and chemotherapy. Elutriated monocytes were obtained from the PBMCs of the study subjects and cultured in six-well plates with IL-4 and GM-CSF for 7 d. On day 8 of culturing, DCs were pulsed with rAd-LMP2 for 2 h, followed by treatment with TNF-α, IL-4, and GM-CSF for two days. mLMP2-DCs were harvested and cryopreserved before administration. At the same time, the expression of CD83, CD86, and DC-DR on DC surface was confirmed by flow cytometry. Expression of CD molecules on the surface of DCs showed considerable variation (from 10.57% to 99.87%) in 22 patients with NPC, which may be due to the different responses to LMP2 (Supplementary Table S3 available in www.besjournal.com); i.e., NPC (as indicated by CD83, CD86, and DR molecules) was not detected in seven of the patients (Patients No 4, 15, 16, 17, 18, 19, 25). mLMP2-DCs vaccines were administered through intradermal injection. Each of the study subjects received three vaccinations. In total, 87 intradermal vaccinations were performed. All vaccines were well tolerated, with only local rigor and swelling at the injection site, which subsided the following day without treatment. Weight, blood pressure, heart rate, respiratory signs, and other clinical monitoring data were within the normal range before and after injection, and no serious side effects were observed.

mLMP2-DCs Vaccine Elicits Tangible Specific CTL Responses

To analyze the effects of vaccination, specific CTL responses to LMP2 were examined pre- and post-injection for each of the three injections. CTL responses to LMP2 increased in 18 of 29 immunized NPC patients (62.1%), compared with pre-immunized subjects (Figure 4A, B). The size of responses varied from 2.0 to 8.4-fold (Figure 5A). Among these 18 patients, two (2/3, 66.7%) were in stage I of the disease, nine (9/11, 81.8%) in stage II, five (5/10, 50.0%) in stage III, and two (2/5, 40.0%) in stage IV (Figure 5B). There was a higher incidence of

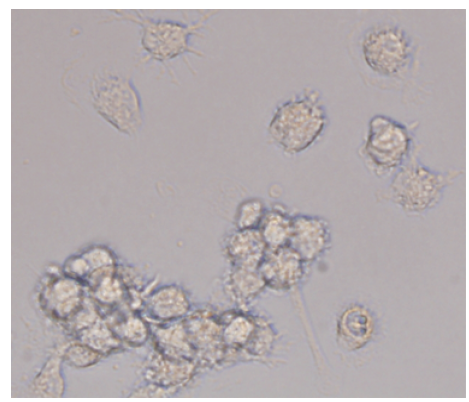

Figure 2. Morphology of mature DCs expressing LMP2. Magnification, 400×.

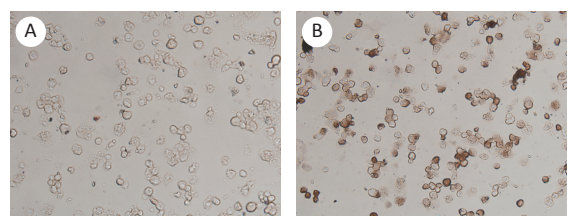

Figure 3. LMP2-expression in monocyte-derived DC infected by rAd-LMP2 with 100 MOI. (A) Negative control; (B) LMP2-DCs. Magnification, 200×.

increased specific responses median 4.3 and 3.2-fold) in subjects at an early stage of NPC (than in advanced stage subjects (median 3.1 and 2.9-fold) (Supplementary Table S4 available in www.besjournal.com). However, 11 of the 29 patients did not show any difference in CTL responses to LMP2 (Figure 6). CTL responses to LMP2 increased (1.8-fold) in Patient No. 24 but were still below two-fold and the case was therefore defined as a non-responder. Of the 11 non-responder patients, one was in stage I, two in stage II, five in stage III, and three in stage IV. The expression of CD83, CD86, and DC-DR on the surface of DCs was lower in non-responders than in responders, which caused no CTL responses to LMP2. The titer of IgA antibody to VCA did not show obvious variation (≥ 4-fold titer changes defined as positive responses) (Supplementary Table S5 available in www.

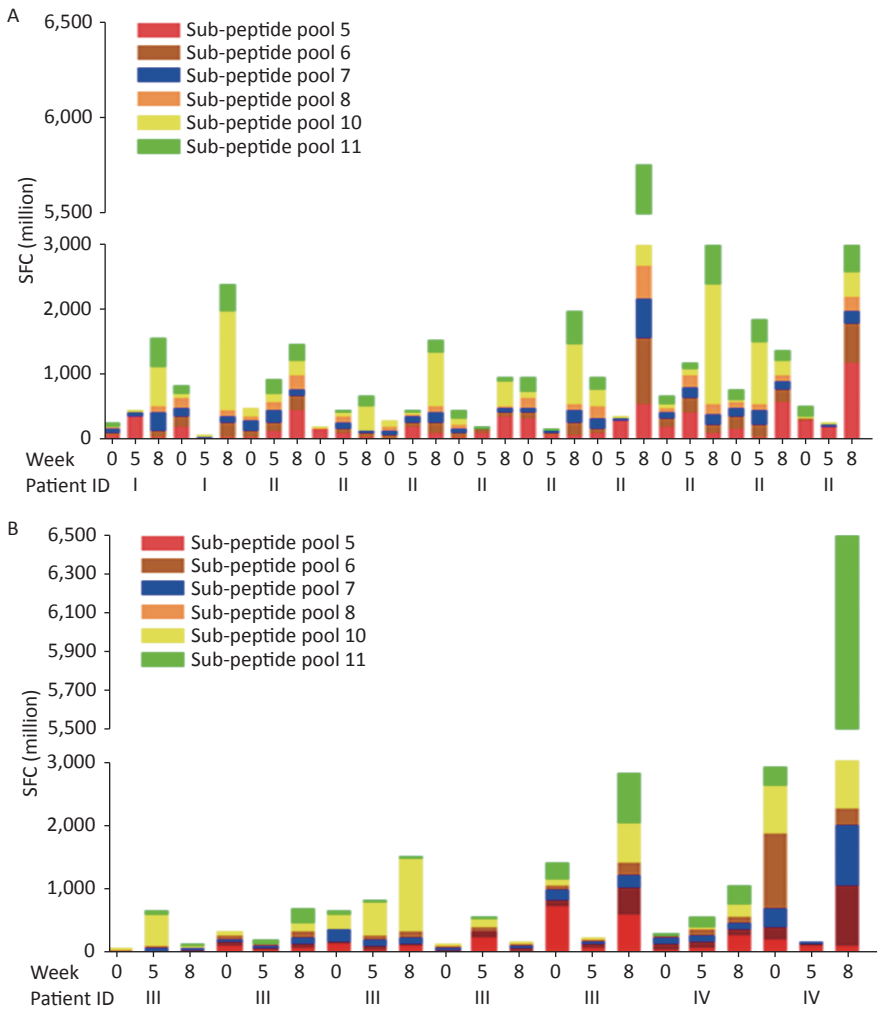

Figure 4. Boosted CTLs responses to LMP2 peptide sub-pools before and after immunization. (A) CTLs responses specific to LMP2 peptide sub-pools before and after immunization in early-stage NPC patients. Boosted T-cell responses to LMP2 in 11 of 14 NPC stage I and II patients. 18 of 29 NPC patients (62.1%) had boosted CTL responses to LMP2, as shown in Figures 4–5. However, 11 of 19 patients did not show positive CTL responses to LMP2. Of 18 patients, 2 (2/3, 66.7%) were in stage I, 9 (9/11, 81.8%) were in stage II, 5 (5/10, 50.0%) were in stage III, and 2 (2/5, 40.0%) were in stage IV. Specific CTL responses were augmented 2.0 to 8.4-fold compared with pre-immunization. Increased size of specific responses is higher in early stage (median 4.3- and 3.2-fold) than at advanced stage (median 3.1- and 2.9-fold). (B) specific responses of CTLs to LMP2 peptide sub-pool before and after immunization in advanced patients with NPC. T-cell responses to LMP2 were boosted in 7 of 15 stage III and IV patients. CTL, cytotoxic T lymphocyte; SFC, spot-forming cell.

besjournal.com). A test for EBV DNA proved to be negative for both pre- and post-immunization of DC-LMP2 in all 29 patients. All the patients were clinically disease-free after treatment with chemotherapy and radiotherapy (Supplementary Table S6 available in www.besjournal.com). However, EBV DNA was detected in the plasma of one patient (No. 22) and increased along with the development of disease (578 copies/mL in 2012 and 4.6×10^4 copies/mL in 2013) (Supplementary Table S6). The size of LMP2-specific T cell responses gradually decreased with development of cancer in patients with NPC (Figure 6).

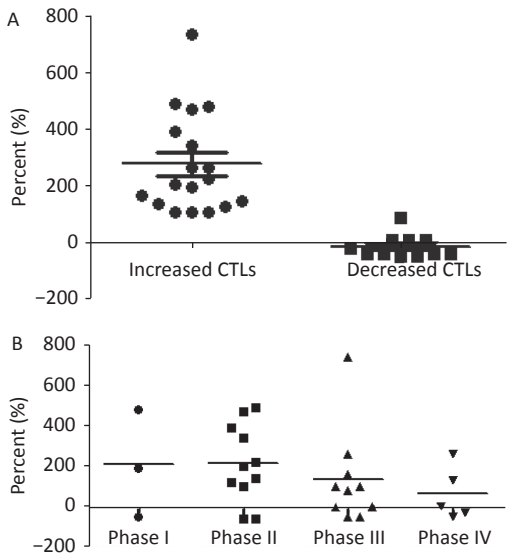

Figure 5. The size of responses to DC vaccine in patients with NPC. (A) size (percent increase) of LMP2-specific T cell responses stimulated by LMP2-DCs vaccine. (B) size (percent increase) of the responses in patients at different stages of NPC. CTL, cytotoxic T lymphocyte.

LMP2-specific Response of CTLs Plays an Important Role in Controlling and Preventing NPC Recurrence and Metastasis

To evaluate the long-term therapeutic efficacy after vaccination, we followed up with the study subjects for five years (from October 2009 to December 2015). Seven patients died, of whom one (1/11) had stage II NPC, four (4/10) had stage III NPC, and two (2/5) had stage IV NPC. Only one of the deceased subjects was a responder, the other six of the seven deceased subjects were non-responders (Supplementary Table S7 available in www.besjournal.com). The five-year survival rate, from immunotherapy completion to the end of 2015, was 94.4% in responders and 45.5% in non-responders (Table 1). The survival durations were 1–4 years in the seven patients who died within five years, with durations in most patients being 2–3 years (Supplementary Table S8 available in www.besjournal.com). This suggests that CTLs responses that are specific to LMP2 play a role in controlling and preventing NPC recurrence and metastasis.

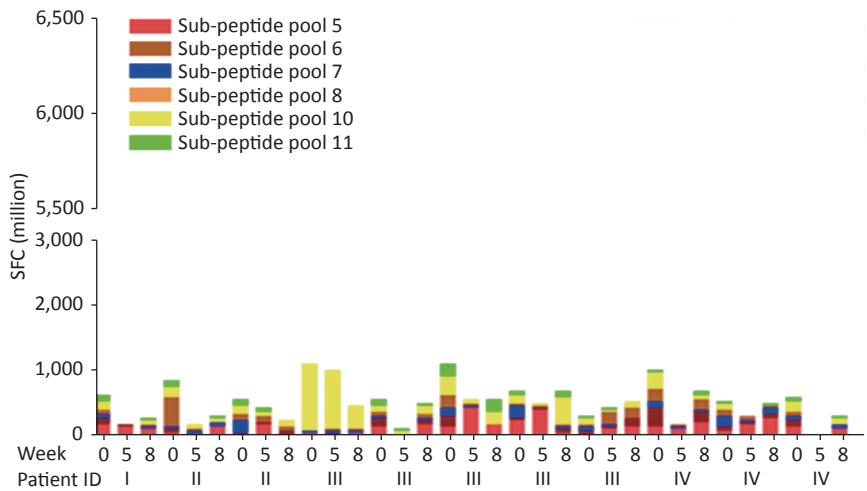

Figure 6. CTL responses to LMP2 peptide sub-pool before and after immunization. In 11 of 29 patients, there were no positive CTL responses to LMP2; rather, CTL levels were slightly elevated, unchanged, or decreased. 72.7% of the patients were in later stages of NPC (stage III and stage IV). Patient characteristics are presented in Supplementary Table S1. CTL, cytotoxic T lymphocyte; SFC, spot-forming cell.

DISCUSSION

DC-based immunotherapy shows great potential for the treatment of cancer[14,15]. DC immunotherapy is conducted mostly through tumor antigen peptides or tumor gene-loaded DCs. However, few tumor-specific or associated antigens have been identified. EBV infection is closely associated with NPC and is recognized as an etiological agent. LMP2 encoded by EBV is detected in most NPC biopsies. This protein contains CD8 T cell epitopes and is considered one of the ideal target antigens for immunotherapy[5]. Therefore, LMP2 was selected as the target antigen for NPC immunotherapy in our study. Although DC vaccine could be administered by venous transfusion, lymph node, and intra-tumoral injection; fewer DCs were required for intra-tumoral injection to stimulate an efficient Th1/Th2 response[16–18]. Thus, it is more practical to administer DC vaccination to tumor patients *via* intradermal injection. Our group has previously reported nine NPC patients who received ^{60}Co irradiated LMP2-DCs through intradermal injection, and the CTL responses specific to LMP2 were boosted in five of these nine NPC patients[19]. rAd-LMP2 is a replication-defective adenovirus which has a good safety and may affect the function of LMP2-DCs that were irradiated by ^{60}Co. In the present study, LMP2-DCs were directly immunized through intradermal injection instead of ^{60}Co irradiation. Our results suggest that CTL responses specific to LMP2 in the early stage of NPC are much better than that in the advanced stage of the disease (Table 2). Therefore, we consider intradermal injection more suitable for patients with early-stage NPC than those in the advanced stage. Among the 29 patients, 11 had been for 3 months after radiotherapy or/and chemotherapy, and specific CTL responses improved in nine of these eleven patients after injection of LMP2-DCs, which indicates that immunity levels were back to normal for most of the patients three months after radiation and chemotherapy. The LMP2-specific responses of CTL in some responsive NPC patients are reduced very quickly. One of our main concerns is how to maintain LMP2-specific CTL responses at a certain level. We are currently testing if we can maintain LMP2-specific CTL responses by increasing injections and using multi-vector forms of immunity. We did not detect changes in IgA antibody titers to EBV-VCA, since our experimental design was to activate LMP2

Table 1. Specific CTLs responses in patients with NPC after immunization with LMP2-DC

Stage	Early stage		Advanced stage		5-year survival rate (%) (*n* = 29)
	I (*n* = 3)	II (*n* = 11)	III (*n* = 10)	IV (*n* = 5)	
Positive reaction	2	9	5	2	18/29 (62.1)
Death	0	0	1	0	1/18 (5.6)
Survival	2	9	4	2	17/18 (94.4)
5-years survival rate (%)	100.0		85.7		
Negative reaction	1	2	5	3	11/29 (37.9)
Death	0	1	3	2	6/11 (54.5)
Survival	1	1	2	1	5/11 (45.5)
5-years survival rate (%)	66.7		37.5		
Total death rate (%)	0.0	9.1	40.0	40.0	7/29 (24.1)
Total survival rate (%)	100.0	90.9	60.0	60.0	22/29 (75.9)

Table 2. Relation between the stage of NPC and LMP2 specific-CTLs

Stage	Cases	LMP2 CTLs POS (%)	LMP2 CTLs NEG (%)	Total (%)
I	3	2 (66.7)	1 (33.3)	100.0
II	8	7 (87.5)	1 (12.5)	100.0
III	5	3 (60.0)	2 (40.0)	100.0
IV	3	1 (33.3)	2 (66.7)	100.0
Total	19	13 (68.4)	6 (31.6)	100.0

specific cellular immune responses, not humoral immune responses. In addition, two months was not sufficient to detect the changes in IgA antibody levels.

In previous studies it has been reported that EBV genome copies could serve as an index of treatment efficacy for early diagnosis and prognosis of NPC[20,21]. We therefore examined plasma EBV genome copies of NPC patients before and after DC immunotherapy, but the plasma data results were negative in all NPC patients, which suggests that clinical post-radiotherapy and post-chemotherapy may inhibit the replication of the EB virus. However, EBV DNA was detected in the plasma of one patient (No. 22) and increased along with the development of the disease (578 copies/mL in 2012 and 4.6×10^4 copies/mL in 2013).

Each subject received three consecutive injections of LMP2-DCs: at week 0 and after at thesecond and fourth weeks. LMP2-specific CTL responses were examined pre-injection, at week 0, and at the fifth and eighth weeks. To further confirm the therapeutic efficacy of LMP2-DCs vaccines in treating NPC, future studies should include follow-up for long-term therapeutic effects, the launch of large multicenter clinical trials, and so on. How to best combine laboratory data with efficacious clinical treatment is a topic for future research.

AUTHORS CONTRIBUTION AND ACKNOWLEDGMENT

Zeng Y designed the experiments and supervised the project. Si YF, Du HJ, and Lin H supervised the project. SJ O'Brien, Lan GP, Wang Z, Zhou L, Tang MZ, Lan J, Zhou XY, Wang YL, and Tang J designed and conducted the experiments and analyzed results. Du HJ and Zhou ZX wrote the manuscript. Zeng Y gratefully acknowledges the founding support. The authors declare no competing financial interests

Received: December 4, 2019;
Accepted: July 2, 2020

REFERENCES

1. Laboratory of tumor viruses of cancer institute, laboratory of tumor viruses of institute of epidemiology, department of radiotherapy of cancer institute, laboratory of cell biology of cancer institute. Establishment of an epitheloid cell line and a fusiform cell line from a patient with nasopharyngeal carcinoma. Sci Sin, 1978; 21, 127−34.
2. Chua MLK, Wee JTS, Hui EP, et al. Nasopharyngeal carcinoma. Lancet, 2016; 387, 1012−24.
3. Young LS, Dawson CW, Clark D, et al. Epstein-Barr virus gene expression in nasopharyngeal carcinoma. J Gen Virol, 1988; 69, 1051−65.
4. Lee SP, Tierney RJ, Thomas WA, et al. Conserved CTL epitopes within EBV latent membrane protein 2: a potential target for CTL-based tumor therapy. J Immunol, 1997; 158, 3325−34.
5. Pan Y, Zhang J, Zhou L, et al. *In vitro* anti-tumor immune response induced by dendritic cells transfected with EBV-LMP2 recombinant adenovirus. Biochem Biophys Res Commun, 2006; 347, 551−7.
6. Wang Z, Yang S, Zhou L, et al. Specific cellular immune responses in mice immunized with DNA, adeno-associated virus and adenoviral vaccines of Epstein-Barr virus-LMP2 alone or in combination. Sci China Life Sci, 2011; 54, 263−6.
7. Mo WN, Tang AZ, Zhou L, et al. Analysis of Epstein-Barr viral DNA load, EBV-LMP2 specific cytotoxic T-lymphocytes and levels of CD4+CD25+ T cells in patients with nasopharyngeal carcinomas positive for IgA antibody to EBV viral capsid antigen. Chin Med J (Engl), 2009; 122, 1173−8.
8. Hart DN. Dendritic cells: unique leukocyte populations which control the primary immune response. Blood, 1997; 90, 3245−87.
9. Knight SC, Stagg AJ. Antigen-presenting cell types. Curr Opin Immunol, 1993; 5, 374−82.
10. Steinman RM. The dendritic cell system and its role in immunogenicity. Annu Rev Immunol, 1991; 271−96.
11. Meixlsperger S, Leung CS, Rämer PC, et al. CD141+ dendritic cells produce prominent amounts of IFN-α after dsRNA recognition and can be targeted via DEC-205 in humanized mice. Blood, 2013; 121, 5034−44.
12. Schlitzer A, McGovern N, Teo P, et al. IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. Immunity, 2013; 38, 970−83.
13. Zeng Y. Serological massive surveys and vaccines for controlling nasopharygeal carcinoma. 7th Annual Global Virus Networks Meeting 2015, May, 17. www.gvn.org/events/gvn-meetings/.
14. Sabado RL, Balan S, Bhardwaj N. Dendritic cell-based immunotherapy. Cell Res, 2017; 27, 74−95.
15. Zhou ZX, Li D, Guan SS, et al. Immunotherapeutic effects of dendritic cells pulsed with a coden-optimized HPV 16 E6 and E7 fusion gene *in vivo* and *in vitro*. Asian Pac J Cancer Prev, 2015; 16, 3843−7.
16. Palucka K, Banchereau J. Cancer immunotherapy *via* dendritic cells. Nat Rev Cancer, 2012; 12, 265−77.
17. Verdijk P, Aarntzen EH, Lesterhuis WJ, et al. Limited amounts of dendritic cells migrate into the T-cell area of lymph nodes but have high immune activating potential in melanoma patients. Clin Cancer Res, 2009; 15, 2531−40.
18. Prince HM, Wall DM, Ritchie D, et al. In vivo tracking of dendritic cells in patients with multiple myeloma. J Immunother, 2008; 31, 166−79.
19. Zuo JM, Zhou L, Chen ZJ, et al. Induction of cytotoxic T lymphocyte responses *in vivo* after immunotherapy with dendritic cells in patients with nasopharyngeal carcinoma. J.Microbiol immunol, 2006; 4, 41−8.
20. Lin JC, Wang WY, Chen KY, et al. Quantification of plasma Epstein-Barr virus DNA in patients with advanced nasopharyngeal carcinoma. N Engl J Med, 2004; 350, 2461−70.
21. Lo YM, Chan AT, Chan LY, et al. Molecular prognostication of nasopharyngeal carcinoma by quantitative analysis of circulating Epstein-Barr virus DNA. Cancer Res, 2000; 60, 6878−81.

Supplementary Table S1. Characteristics of NPC patients in this study

Patient ID	Sex	Age	Stage at enrollment	Time after radiotherapy and chemotherapy (months)
1	M	37	I	9
2	F	39	I	3
3	M	37	I	9
4	M	49	II	6
5	F	61	II	3
6	F	37	II	3
7	M	34	II	6
8	F	34	II	7
9	F	53	II	3
10	F	34	II	5
11	F	61	II	3
12	F	30	II	8
13	F	51	II	3
14	M	44	II	3
15	M	66	III	8
16	M	36	III	4
17	M	24	III	9
18	F	35	III	6
19	M	39	III	3
20	M	36	III	6
21	F	37	III	5
22	M	55	III	3
23	F	60	III	9
24	F	33	III	4
25	M	28	IV	7
26	M	33	IV	3
27	M	36	IV	9
28	F	50	IV	8
29	M	30	IV	3

Supplementary Table S2. Peptide mixes from LMP2

Sub-peptide pool	Sequences of amino acids in LMP2	Purity (%)
5	MGSLEMVPM	95
5	LPVIVAPYL	95
5	PYLFWLAAI	95
5	FTASVSTVV	95
5	IEDPPFNSL	95
5	RRRWRRLTV	95
5	RRWRRLTVC	95
5	RRLTVCGGIMF	95
5	TVCGGIMFL	95
5	SSCSSCPLSKI	95
5	ILLARLFLY	95
5	TYGPVFMCL	95
5	LTAGFLIFL	95
5	LIVDAVLQL	95
6	LFWLAAIAASCF	75
6	AAIAASCFTASV	75
6	STVVTATGLALS	75
6	LALSLLLLAAVA	75
7	LTAVVTFFAICL	75
7	FNSLLFALLAAA	75
7	LFALLAAAGGLQ	75
7	CGGIMFLACVLV	75
7	MFLACVLVLIVD	75
8	CVLVLIVDAVLQ	75
8	TVVSMTLLLLAF	75
8	MTLLLLAFVLWL	75
8	LLTLAAALALLA	75
8	LTTMFLLMLLWT	75
10	AGILFILAILTE	75
10	GLLTMVAGAVWL	75
10	MVAGAVWLTVMS	75
10	SAWILTAGFLIF	75
10	FLIFLIGFALFG	75
11	IYVLVMLVL	95
11	LLWTLVVLL	95
11	WTLVVLLI	95
11	FLYALALLL	95
11	CLGGLLTMV	95
11	VMSNTLLSAW	95
11	LLSAWILTA	95

Supplementary Table S3. Detection of CD83, CD86, and DR molecules on the cell surface in LMP2-DCs

Patients ID	DC-CD83 (%)	DC-CD86 (%)	DC-DR (%)
1	99.32	99.76	99.61
2	67.24	47.54	66.86
3	64.07	44.24	57.27
4	Missed	Missed	Missed
5	84.45	94.92	93.74
6	99.39	84.88	88.09
7	60.65	52.13	61.61
8	17.02	52.67	37.26
9	55.17	42.34	53.40
10	76.77	77.48	92.56
11	49.53	51.08	81.82
12	74.76	65.75	78.67
13	69.80	71.86	67.83
14	99.86	99.87	99.52
15	Missed	Missed	Missed
16	Missed	Missed	Missed
17	Missed	Missed	Missed
18	Missed	Missed	Missed
19	Missed	Missed	Missed
20	91.52	97.53	97.45
21	53.10	59.51	87.67
22	68.51	57.63	72.53
23	54.99	38.25	52.29
24	98.91	86.72	96.93
25	Missed	Missed	Missed
26	83.28	94.22	92.25
27	10.57	60.12	47.38
28	29.13	36.80	20.92
29	80.31	72.40	80.28

Note. The positive rates of CD83, CD86, and DC-DR on DC surface were also detected by flow cytometry. Expression of CD molecules on the surfaces of DCs varied widely in patients with NPC.

Supplementary Table S4. Specific-CTLs responses induced by LMP2-DCs in NPC patients

Patient ID	Stage at enrollment	Pre-vaccination (Spot-forming cells/million PBMC)	Post-vaccination (Spot-forming cells/million PBMC)	Percent increase (%)
1	I	270	1,576	484
3	I	840	2,395	185
4	II	500	1,478	196
5	II	212	670	216
6	II	310	1,530	394
9	II	450	977	117
10	II	975	1,980	103
11	II	980	5,760	488
12	II	680	2,995	340
13	II	770	1,850	140
14	II	534	3,023	466
17	III	80	673	741
18	III	350	685	96
19	III	675	1,775	163
20	III	160	580	263
23	III	1,420	2,805	98
26	IV	293	1,055	260
29	IV	2,910	6,590	126

Supplementary Table S5. IgA /VCA antibody titer in plasma

Patient ID	Stage at enrollment	Week 0	Week 5	Week 8
1	I	320	160	160
2	I	80	80	80
3	I	40	40	40
4	II	160	80	160
5	II	20	40	20
6	II	160	160	160
7	II	40	40	40
8	II	80	80	80
9	II	40	40	40
10	II	80	40	80
11	II	160	160	160
12	II	20	20	20
13	II	80	80	80
14	II	80	80	80
15	III	20	20	20
16	III	40	40	40
17	III	40	80	40
18	III	40	40	40

Continued

Patient ID	Stage at enrollment	Week 0	Week 5	Week 8
19	III	40	40	40
20	III	320	320	160
21	III	40	40	40
22	III	80	160	80
23	III	40	40	40
24	III	20	20	20
25	IV	40	40	40
26	IV	80	40	80
27	IV	80	80	80
28	IV	320	320	320
29	IV	80	80	80

Supplementary Table S6. EBV-DNA concentration in plasma

Patient ID	Stage at enrollment	Year (2011)	Year (2012)	Year (2013)	Year (2014)	Year (2015)
1	I	–	–	–	–	Neg
2	I	Neg	–	Neg	–	Neg
3	I	Neg	Neg	–	Neg	Neg
4	II	Neg	Neg	Neg	Neg	Neg
5	II	Neg	Neg	Neg	–	Neg
6	II	–	–	–	–	–
7	II	Neg	Neg	Neg	Neg	Neg
8	II	–	–	Neg	–	–
9	II	Neg	Neg	Neg	–	–
10	II	Neg	–	–	–	–
11	II	–	–	–	–	–
12	II	–	–	Neg	–	–
13	II	Neg	1.6×10^3	–	–	–
14	II	Neg	Neg	Neg	Neg	–
15	III	Neg	–	Neg	Neg	
16	III	Neg	–	–	–	Neg
17	III	Neg	Neg	Neg	Neg	–
18	III	–	–	–	–	–
19	III	Neg	–	Neg	Neg	–
20	III	Neg	Neg	Neg	–	–
21	III	Neg	–	–	–	–
22	III	Neg	578	4.6×10^4		
23	III	Neg	Neg	Neg	Neg	–
24	III	–	–	–	–	–
25	IV	–	Neg	–	–	–
26	IV	Neg	Neg	Neg	Neg	Neg
27	IV	–	–	–	–	–
28	IV	–	–	–	–	–
29	IV	Neg	Neg	Neg	Neg	3×10^3