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Activity of T Cells Stimulated by Hemagglutinin-neuraminidase of Newcastle Disease Virus *in vivo*

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Abstract To investigate the stimulated activity of T cells and the anti-tumor properties of hemagglutinin-neuraminidase(HN) of Newcastle disease virus(NDV) strain Changchun(NDVcc), the expression of HN gene in hepatoma cells(human HepG-2 and mouse H22 cells) infected with the recombinant adenovirus(Ad-HN) was identified by Western blot analysis and flow cytometry. Sialidase activity of NDVcc HN expressed by Ad-HN was assayed by the periodate-resorcinol method. The *in vivo* anti-tumor effects of NDVcc HN were evaluated in the H22 solid tumor model. Regional lymph nodes of the mouse model treated with Ad-HN were removed to harvest T lymphocytes and evaluating the specific cytotoxicity of cytotoxic T lymphocyte(CTL) and natural killer(NK) cells by an *L*-lactate dehydrogenase(LDH) assay, in the mean time, the secretion of cytokines was analyzed by enzyme linked immunosorbent assays(ELISA). The results show that NDVcc HN was effectively expressed by Ad-HN in HepG-2 and H22 cells. The sialidase activity assay showed that Ad-HN significantly reduced sialic acid level of the hepatoma cells compared with the cells infected the empty adenovirus vector(Ad-mock). When treated with Ad-HN, the growth of subcutaneous H22 primary tumors in C57BL/6 mice was suppressed, and the mean mice survival increased. In addition, the treatment of Ad-HN elicited strong NK and CTL responses, and high levels of Th1 cytokines, such as IL-2 and IFN-γ. In conclusion, NDVcc HN effectively elicits T cell-mediate anti-tumor cytotoxicity *via* sialidase activity and may be a novel strategy for cancer immunotherapy.

Keywords Newcastle disease virus; Hemagglutinin-neuraminidase; Hepatoma; T Cell; Anti-tumor immunity

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1 Introduction

Newcastle disease virus(NDV) is a paramyxovirus with a negative single-stranded RNA genome which causes inflammation of the respiratory and gastrointestinal tract in a wide variety of poultry species. NDV can also infect humans and cause mild flu-like symptoms and/or laryngitis[1]. The observation that NDV replicates much more efficiently in cancer cells than in most normal cells has prompted a great interest in NDV as a potential anti-cancer agent[1]. NDV expresses two membrane viral spike proteins, hemagglutinin-neuraminidase(HN) and fusion protein. HN protein is a 74000 multifunctional membrane class II glycoprotein which protrudes spike-like from the viral envelope[2]. HN glycoprotein not only mediates the recognition of sialic acid containing receptors but also possesses neuraminidase activity(NA) which can cleave the sialic acid on those receptors[3]. The presence of both receptor recognition activity and NA on the same protein[4] is in contrast to that of the influenza virus, in which the two activities are performed by independent spike structures.

Sialic acid, which is generally found in the non-reducing terminus of most glycoproteins and glycolipids, is associated with tumor cell behavior, such as invasiveness and metastasis, *via* interfering with signal transduction and cell growth properties[5]. NDV HN protein can recognize and hydrolyze these sialic acid components. Furthermore, it is known that NDV HN protein can stimulate cells such as dendritic cells(DCs) to produce high levels of interferon-α(IFN-α) and activate macrophages, natural killer(NK) cells and T cells[5]. Additionally, the distinct structure of HN displayed on the tumor cell surface that can be recognized by the immune system strengthens the cytotoxicity[5]. All these properties render NDV HN protein an attractive candidate for tumor immunotherapy.

In this study, our main interest was to determine whether the HN gene of NDVcc has immune enhancing capabilities and therapeutic effect. We compared the effect of HN on

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established H22 tumors in C57BL/6 mice when expressed from an adenovirus vector(Ad-HN) with that of the avirulent NDVcc strain[6], which was shown to replicate selectively in the cytoplasm of tumor cells but that of normal cells[5]. Our findings indicate that the Ad-HN treatment effectively stimulated T cell immune responses and inhibited tumor growth in H22 tumor-bearing C57BL/6 mice.

2 Materials and Methods

2.1 Cell Lines, Virus and Animals

The human hepatoma cell line HepG-2 and human embryonic kidney cell line HEK-293 were obtained from the China Center for Type Culture Collection. YAC-1, an NK-sensitive lymphoma cell line of A/S(H-2a) origin was purchased from American Type Culture Collection(ATCC, Manassas, Virginia, USA). H22 murine hepatoma cells(syngeneic to C57BL/6 mice) were obtained from the Laboratory Animal Center of Jilin University(China). All the cell lines were grown in RPMI 1640 complete medium(Gibco) or Dulbecco's modified Eagle medium(DMEM) containing fetal bovine serum(FBS) plus 2.5 mmol/L *L*-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin at 37 °C/5% CO_2. The NDVcc strain[6] was a gift from Dr. JIN Kuo-shi, Academy of Military Medical Sciences of PLA, China. The adenoviral vectors, Ad-HN[7] and Ad-mock[8], were previously constructed by an improved *in vitro* ligation method. Six- to eight-week-old female C57BL/6 mice were obtained from the Experimental Animal Center of the Academy of Military Medical Sciences of PLA, China for all experiments under Chinese government guidelines.

2.2 Preparation and Titration of Virus Stocks

HEK-293 cells grown in a serum-free medium were infected with Ad-HN at a multiplicity of infection(MOI) of 0.1. After infection of 48—72 h, the medium was removed and the cells were washed with Hank's balanced salt solution(HBSS). After three freeze/thaw cycles, cell debris were scraped from the plates, centrifuged, and the supernatant was recovered. The purification of the amplified virus was performed *via* the Adeno-X Virus Purification kit(BD Bioscience Clontech) as described previously[8]. Titers of recombinant virus were determined by plaque assays and expressed as plaque-forming units(pfu) per milliliter virus suspension[5,8].

2.3 Western Blot Analysis

HepG-2 and H22 cells were infected with 10 MOI of Ad-HN for 48 h. The expression of HN was analyzed by Western blot analysis as described previously[8]. The primary antibody used for detection was anti-HN(1:1500; rabbit polyclonal, a kind gift from Dr. JIN Kuo-shi, Academy of Military Medical Sciences of PLA, China), and the secondary antibody was a horseradish peroxidase(HRP)-conjugated anti-rabbit IgG (1:2500; Abcam). The bands were visualized with Pierce enhanced chemiluminescence(ECL) Western Blotting Substrate (Pierce, Shanghai, China). Extracts of the cells infected with 10 MOI of NDVcc were used as positive control, and the extracts of cells infected with 10 MOI of Ad-mock were used as the negative control. Detection of GAPDH was used as an internal control.

2.4 Flow Cytometry Analysis

HepG-2 and H22 cells were infected with the recombinant adenoviruse(10 MOI) for 48 h, trypsinized and washed once with 1 mL of phosphate buffered saline(PBS). The cells were resuspended in 0.5 mL of 2% formaldehyde(PBS) and incubated at 37 °C for 10 min. After three times of wash with 0.5 mL of PBS, the cells(1×10^6) were resuspended in 100 μL of 0.5% bovine serum albumin(BSA) and blocked at 37 °C for 10 min. The cells were then incubated with anti-HN polyclonal antibody and fluorescein isothiocyanate(FITC) conjugated anti-rabbit IgG(1:2000; Abcam) at 37 °C for 1 h. The cell samples were resuspended in 0.5 mL of PBS and examined by flow cytometry(FACScan, Becton Dickinson, Franklin Lakes, NJ) for HN expression analysis. The cells infected with 10 MOI of NDVcc were used as positive control, and those infected with 10 MOI of Ad-mock were used as negative control.

2.5 Sialic Acid Content Assay

The sialic acid contents on HepG-2 and H22 cells infected with 10 MOI of Ad-HN were measured every day over a 3 d period by means of 3,5-dihydroxytoluene(Sigma-Aldrich BRL, St Louis, USA) as described previously[9]. In brief, infected cells were homogenized with 500 μL of RIPA buffer[10 mmol/L Tris-HCl(pH=7.4), 150 mmol/L NaCl, 1% Triton X-100, 1% deoxycholate, 0.1% SDS and 5 mmol/L EDTA]. The samples were then acid hydrolyzed with 500 μL of 0.5 mol/L HCl at 80 °C for 1 h. After centrifugation(12000*g*), the protein contents of both the hydrolysate and the pellet were determined by virtue of GeneQuant pro(Amersham). Hydrolysate(500 μL) was added to 100 μL of 0.04 mol/L periodic acid solution. The solution was mixed thoroughly and incubated for 35 min in an ice bath. Freshly prepared resorcinol reagent(1.25 mL; 5.5 mmol/L resorcinol, 25 μmol/L $CuSO_4$ and 17% HCl) was added to the solution and then heated at 100 °C for 15 min, followed by addition of 1.25 mL of tert-butyl alcohol. To stabilize the color, the tubes were incubated for 3 min at 37 °C, and then the absorbance was measured at 630 nm on a UV-Vis Spectrophotometer(Shimadzu, Japan). The sialic acid content was quantified by comparison with known quantities of *N*-acetylneuraminic acid(Sigma-Aldrich) used as the standard. The hydrolysate of the cells infected with 10 MOI of NDV was used as a positive control, and the hydrolysates of the cells infected with 10 MOI of Ad-mock was used as a negative control.

2.6 Animal Experiments

H22 cells were harvested by trypsinization and resuspended in serum-free Hank's balanced salt solution. The number of viable cells was determined by trypan blue exclusion, and the cell concentration was adjusted to 1×10^7 cells/mL. Within 1 h of harvesting, 100 μL of the cell(1×10^6) suspension

per flank was injected subcutaneously into the right flanks of the mice. The *in vivo* anti-tumor experiments were then performed in two independent models. In the first animal experiment, H22-bearing C57BL/6 mice were divided into three experimental groups: (i) Ad-HN alone(1×10^8 pfu in 50 μL saline per mouse); (ii) Ad-mock alone(1×10^8 pfu in 50 μL saline per mouse); (iii) NDVcc alone(1×10^8 pfu in 50 μL saline per mouse) and (iv) saline(50 μL per mouse). Each group had ten mice, and all injections were carried out *via* 25-gauge needles. The injections were performed at week intervals, for up to three immunizations. Tumor sizes were measured with a caliper every 2 d. Tumor volumes were calculated as follows: [0.52×(smallest diameter of tumor)^2(largest diameter of tumor)][5,8]. 50 d after the first immunization, the animals were sacrificed by CO_2 inhalation, and the mean survival rates of the mouse groups were evaluated. In the second animal experiment, H22-bearing C57BL/6 mice were given the same injection protocol as in the first experiment. 14 d after the last immunization, the animals were sacrificed, and the spleens and sera of the mice were harvested for T cell related analyses.

2.7 Cytotoxicity Assay

CytoTox 96R Non-Radioactive Cytotoxicity Assay kits (Promega Madison, USA) were used to detect the cytotoxicity of CTL and NK cells. 14 d after the last immunization, spleens were removed and single-cell suspensions were prepared by pressing the spleen tissue against a fine nylon mesh. Erythrocytes were depleted with red blood cell(RBC) lysis buffer (Sigma), and macrophages were removed by the adherence of the splenocytes on plastic plates for 2 h. Non-adherent lymphocytes were directly used as NK effector cells. YAC-1 cells were used as target cells. Spleen lymphocytes were co-cultured with irradiated H22 cells(60 Gy) at a ratio of 25:1, with 5×10^6 lymphocytes and 2×10^5 irradiated H22 cells in 2 mL of DMEM plus 10% flow cytosorting(FCS) in each well of a 24-well plate. 5 d later, T cells were harvested and purified from the cultures by Ficoll- Paque density gradient centrifugation. These T cells were then used as CTL effector cells in an LDH-release assay against H22 target cells. 10000 target cells per well were mixed with effector cells at various effector/target cell ratios (E:T ratio) in quadrisection and were incubated for 6 h. The percentage of specific lysis(%) was calculated as: [(experimental–spontaneous)/(maximal–spontaneous)]×100%.

2.8 Quantitation of Cytokine Secretion

Fourteen days after the last immunization, regional lymph nodes were removed for harvesting T lymphocytes. These T cells were co-cultured with lyzed H22 cells(60 Gy) at a ratio of 2:1. The supernatants were harvested at day 3 for IL-2, IFN-γ, IL-4, and IL-10 quantification. Secreted cytokines were quantified with Mouse Th1/Th2 ELISA kits(eBioscience). Briefly, a 96-well ELISA plate was coated with 100 μL/well of capture antibody and sealed and incubated overnight at 4 °C, followed by block with 200 μL/well of the assay diluent for 1 h at room temperature after washing 5 times with wash buffer. Samples were added to the wells and incubated at room temperature for 2 h. Following 5 times of wash, 100 μL/well of detection antibody was added into the wells, and the plate was incubated at room temperature for 1 h. Then 100 μL/well of avidin-horseradish peroxidase(HRP) was added into the corresponding wells, and the plate was incubated at room temperature for 30 min. Following incubation with substrate solution(100 μL/well) for 15 min at room temperature, 50 μL/well of stop solution was added into each well. The plate was read at 450 nm, and the data were analyzed.

2.9 Statistical Analysis

Differences in values were evaluated for statistical significance using the Student's *t* test. Kaplan-Meier tests were used for survival analysis. Findings were considered significant when two-tailed $P<0.05$.

3 Results

3.1 Transgene Expression of Recombinant Adenoviruses

The hepatoma cell lines(HepG-2 and H22) infected with Ad-HN were analyzed by flow cytometry to evaluate the expression of the HN protein. Cells infected with NDVcc or Ad-mock were used as positive or negative controls, respectively. As shown in Fig.1(A), 92% of the Ad-HN infected HepG-2 cells and 84% of NDVcc infected HepG-2 cells exhibited positive staining, as compared to only 12% of Ad-mock infected cells displaying background positive staining. As for the H22 cell line[Fig.1(B)], the positive staining of Ad-HN infected cells(83%) was comparable to that of the NDVcc infected cells(84%); meanwhile, only 7% of Ad-HN infected cells were positively stained. The expressions of Ad-HN mediated HN in HepG-2 and H22 cells were also evaluated *via* Western blot analysis as described in Section Materials and Methods. As shown in Fig.1, Ad-HN infected HepG-2 [Fig.1(C)] and H22[Fig.1(D)] cells produced significant levels of HN proteins, whereas no HN proteins were detected after

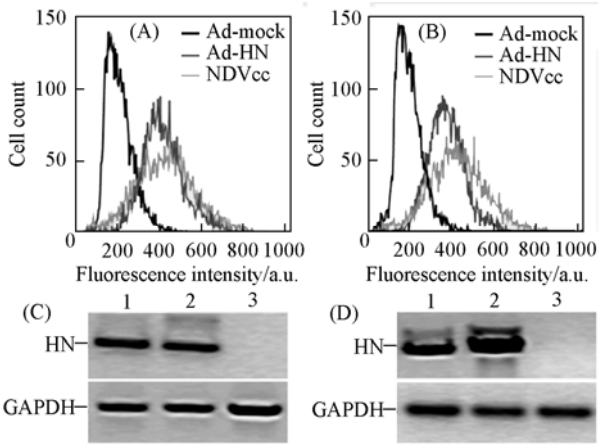

Fig.1 Adenovirus-mediated transgene expression

(A) HN expressing assay in HepG-2 cells by flow cytometry; (B) HN expressing assay in H22 cells by flow cytometry; (C) HN expressing assay in HepG-2 cells by Western blot analysis; (D) HN expressing assay in H22 cells by Western blot analysis. Lane 1: Ad-HN; lane 2: NDVcc; lane 3: Ad-mock.

Ad-mock infection. Therefore, we confirmed that the HN transgene was successfully expressed in Ad-HN infected HepG-2 and H22 cells.

3.2 HN Decreases Sialic Acid Content *in vitro*

We assayed the total sialic acid content using the periodate-resorcinol method and compared the mean values of Ad-HN, Ad-mock or NDVcc infected cells with those of untreated cells. As expected, with longer infection time, sialic acid contents of HepG-2 or H22 cells infected with Ad-HN and NDVcc decreased. As shown in Fig.2, at 24 h after infection of HepG-2 and H22 cells, we found no significant differences in the content of total sialic acid among Ad-HN(300—390 pmol/mg protein), Ad-mock(300—420 pmol/mg protein), NDVcc (270—390 pmol/mg protein) treatment and control(310—430 pmol/mg protein) groups(P>0.05). At a later time point(48 h), in HepG-2 and H22 cells, the mean values of the total sialic acid contents of Ad-mock infected(440—530 pmol/mg protein) and control(460—550 pmol/mg protein) groups were similar, but Ad-HN(230—320 pmol/mg protein) and NDVcc(120—270 pmol/mg protein) treated cells contained significantly lower levels of sialic acid(compared with Ad-mock infected cells and control groups, P<0.01). When the treatment time was prolonged to 72 h in both HepG-2 and H22 cells, the concentrations of sialic acid of Ad-mock infected(540—610 pmol/mg protein) and the control(550—640 pmol/mg protein) groups increased, which was accompanied by cell proliferation; however, the total sialic acid content of Ad-HN infected cells decreased to 200—310 pmol/mg protein(compared with Ad-mock infected cells and control groups, P<0.01). It is notable that in HepG-2 and H22 cells, the sialic acid components of the

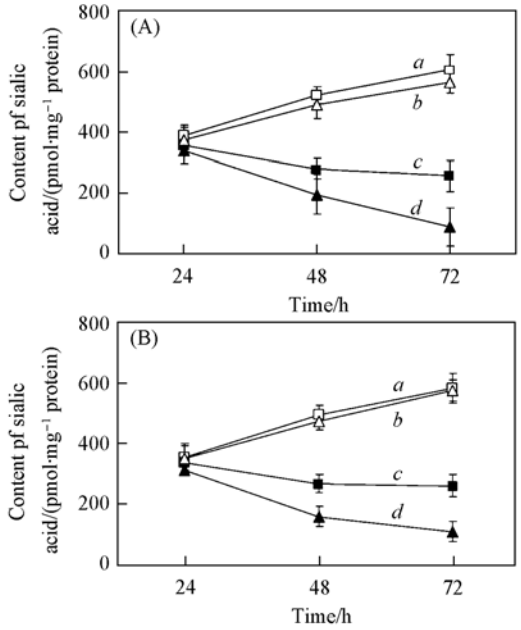

Fig.2 Mean amounts of sialic acid in HepG-2 and H22 cells after Ad-HN, NDV or Ad-mock treatment

(A) Ad-HN mediated decrease of sialic acid in HepG-2 cells; (B) Ad-HN mediated decrease of sialic acid in H22 cells. Values were given in pmol in relation to mg of total protein. Data were mean±SD. *a.* Cell; *b.* Ad-mock; *c*. Ad-HN; *d*. NDVcc.

NDVcc treatment groups(20—150 pmol/mg protein) were almost eliminated. These results suggest that the NDVcc HN protein could effectively degrade sialic acid on HepG-2 and H22 cells, and perhaps, as an oncolytic virus, NDVcc may be used to induce strong anti-tumoral activity in hepatoma cells.

3.3 Ad-HN Prolonged Mean Survival of Tumor Bearing Mice

We examined the anti-tumor potential of Ad-HN in the H22 tumor model. The growth kinetics of the tumors following treatment is shown in Fig.3(A). Although immunization with Ad-HN did not lead to a complete regression of the established tumors, the growth was inhibited compared with that in control mice treated with Ad-mock or saline(P<0.05 for all comparisons from 12 d to 50 d). However, there was no statistical difference between the Ad-mock and saline groups(P>0.05 for all comparisons from 0 d to 50 d). Consistent with the tumor growth kinetics results, saline-treated and Ad-mock-infected groups had the worst survival, while infections by Ad-HN and NDVcc significantly improved the mean survival of model mouse[Fig.3(B)]. The mean survival time was 38.2 d for saline-treated mice, 39.1 d for Ad-mock-infected mice. However, tumor bearing mice infected with Ad-HN and NDVcc survived much longer than those in the other groups and the mean survivals were 45.6 and 46.7 d, respectively. This observation demonstrated that Ad-HN could suppress the syngeneic graft and effectively improve the mean survival.

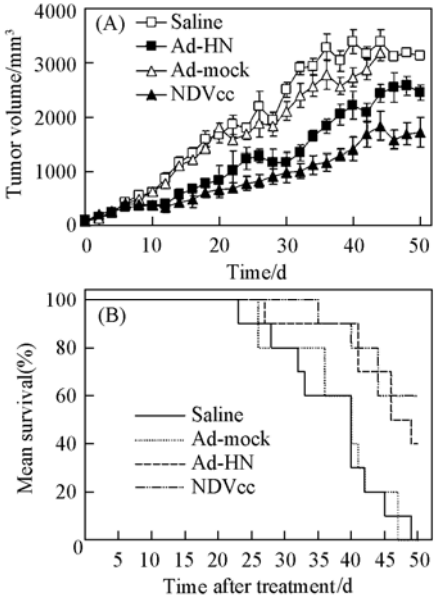

Fig.3 Ad-HN suppressed solid hepatoma and prolonged mean survival of C57BL/6 mice model

(A) Tumor growth kinetics of mice that received intratumorally injections, data were represented as mean±SD; (B) survival curve of mice treated intratumorally. The day that the first injection performed was considered as starting day, day 0.

3.4 Enhanced NK and CTL Responses Induced by Ad-HN Plasmids

Nonspecific and specific cytotoxicity induced by Ad-HN were evaluated in a LDH release assay. As shown in Fig.4(A),

when effector/target cells ratio(E:T) is above 50:1, the splenic NK activity in mice immunized with Ad-HN plasmid is markedly increased compared to those in mice treated with Ad-mock and saline(P<0.05). Furthermore, to evaluate the specific killing activity of H22-reactive T cells, a CTL cytotoxicity assay was performed. As shown in Fig.4(B), when E:T cells ratio is above 50:1, H22-specific CTL activity in mice vaccinated with Ad-HN and NDVcc increases significantly compared to the CTL activity in mice treated with Ad-mock and saline(P<0.05). These results demonstrate that infection by Ad-HN markedly increases the NK and tumor-specific CTL activity.

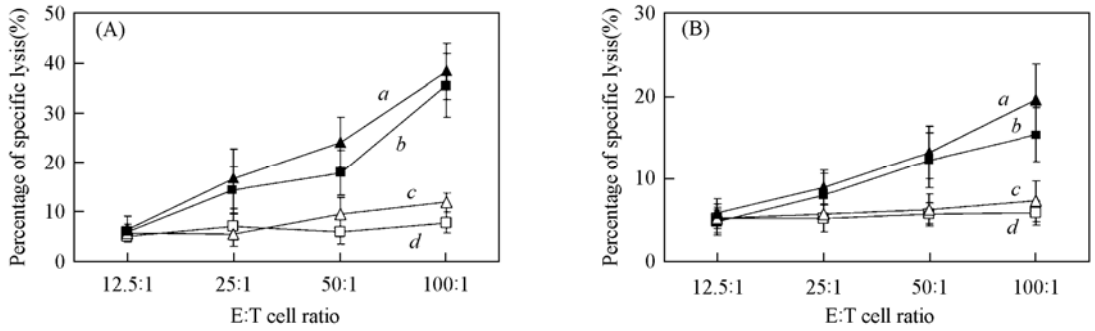

Fig.4 Cytotoxicity assays for NK(A) and CTL(B)

Values represent the mean of 3 separated tests from two experiments. Data were mean±SD. *a*. NDVcc; *b*. Ad-HN; *c*. Ad-mock; *d*. cell.

3.5 Increased Th1 Cytokine Secretion After Immunization with pVHN

We employed an ELISA assay of IL-2, IFN-γ, IL-4 and IL-10 cytokines to evaluate the phenotype of T cells after the injection of Ad-HN. As shown in Fig.5, T cells from the mice treated with Ad-HN secreted a significantly higher level of IL-2(P<0.01) and IFN-γ(P<0.01) than T cells from mice treated with Ad-mock and saline. In comparison to the secretion of Th1 cytokines, slightly low IL-4 and IL-10 levels were found in the T cells from Ad-HN-treated mice. This profile of cytokine secretion suggests that Ad-HN enhances the induction of immune responses *via* a Th1-dominant response.

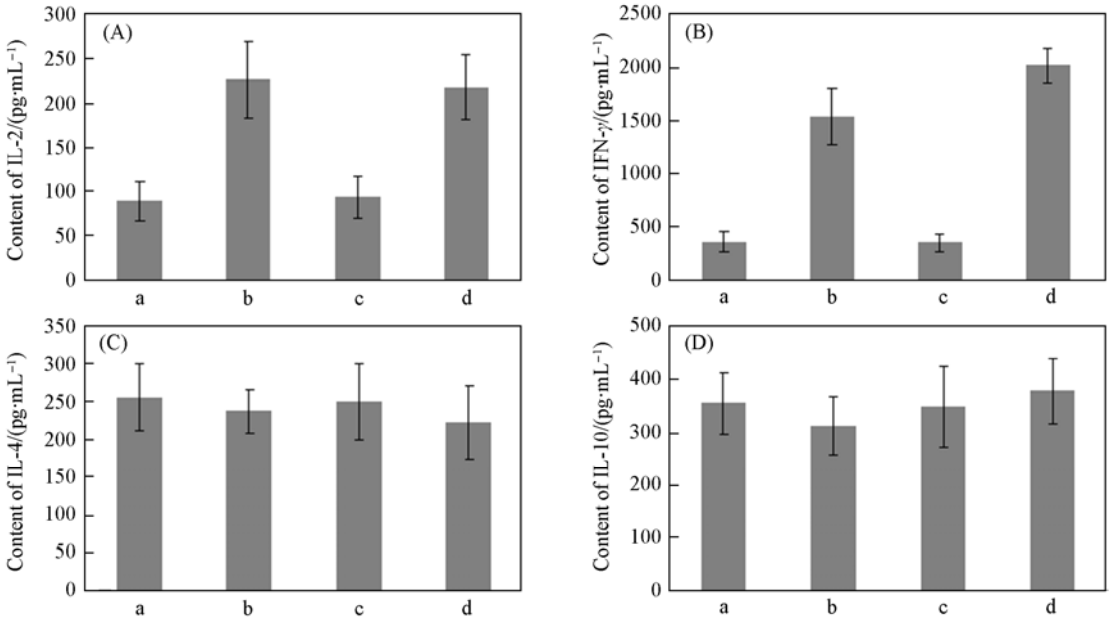

Fig.5 Effects of Ad-HN on cytokine secretion of T cells from regional lymph nodes

Values represent the mean±standard deviation(SD)(pg/mL)/0.5×10^6 cells of triplicate samples. a. Cell; b. Ad-HN; c. Ad-mock; d. NDVcc. (A) Content of IL-2; (B) content of IFN-γ; (C) content of IL-4; (D) content of IL-10.

4 Discussion

NDV targeted vaccines have reached economical significance in veterinary medicine where they are used to protect poultry against respiratory diseases caused by this virus. It is known that this avian virus has anti-tumor properties[1]. Cassel[5], who already in the 1960s described the anti-tumor properties of NDV, developed the oncolytic substrain NDV 73T which was then used to produce melanoma oncolysates as vaccines for clinical applications[5]. In 1996, the avirulent NDVcc was isolated in Changchun, China, by Jin *et al.*[6]. In previous studies, we showed that NDVcc had the features of an oncolytic virus and that the NDVcc HN gene played an important role in anti-tumor activities of NDVcc[10]. *In vitro* studies show that the NDV HN protein which connects with the outer virionic membrane through a hydrophobic region of the N-terminus is one of the major structural proteins of NDV[11].

The HN protein regulates infection and replication of the virus by interacting with sialic acid components on the surface of the target cells[5,12].

Sialic acid, which is generally found in the non-reducing terminus of most glycoproteins and glycolipids, is associated with tumor cell behavior, such as invasiveness and metastasis, *via* interfering with signal transduction and cell growth properties. In the present study, we investigated the effects of NDVcc HN on sialic acid contents in hepatoma cell lines. When the HepG-2 or H22 cells were treated with Ad-HN and NDVcc *in vitro*, the elimination of sialic acid was evident within 48 h; by contrast, the sialic acid loss was not observed after treatment with Ad-mock at any time(Fig.2). These findings indicate that the NDVcc HN is associated with significant sialic acid decrease in NDVcc infected hepatoma cell lines. Here, we also observed the anti-tumor activity of NDVcc HN *in vivo*. Although the injection of Ad-HN did not lead to complete elimination of the tumors, effective inhibition was observed in an established primary tumor model[Fig.3(A)]. Ad-mock treatment induced some tumor-inhibit activity, but the effects were very limited[Fig.3(A)]. The *in vivo* experiments also demonstrate that the administration of Ad-HN renders significant survival benefits in the mouse model. All the animals in the saline- or Ad-mock-treatment groups died when the experiment was terminated(day 50). While 40% of mice in the Ad-HN treated group survived. The results described here indicate that NDVcc HN could suppress the growth of the solid tumors and prolong the life span of the tumor-bearing mice.

In contrast to the influenza virus which has independent structures performing the initial cell attachment and sialic cleavage functions[5], a number of studies show that the NDV HN glycoprotein mediates both the activities. As an adjuvant for inducing anti-tumor immune responses, the HN protein can activate DCs, monocytes, NK cells and stabilize activated T cells and activates cells to produce the β-chain of the IL-12 receptor[12]. Moreover, the HN protein can activate DCs to generate large amounts of IFN-α and up-regulate a series of factors associated with antigen recognition, cell interaction, cell adhesion and cytotoxicity[12]. Previous reports have demonstrated that CTLs and NK cells play important roles in anti-tumor immune responses[5]. In current study, we evaluated the anti-tumor immune responses after NDVcc HN gene transfer into C57BL/6 mice bearing H22 hepatomas. The results show that compared with those of control and Ad-mock treated groups, NK and tumor-specific CTL activities were markedly increased in mice immunized with Ad-HN in the H22 model. Furthermore, our results indicate that T cells from the lymph nodes of mice vaccinated with Ad-HN secreted high levels of the Th1 cytokines IL-2 and IFN-γ, demonstrating that the regression of tumor cells was related to a Th1-type dominant immune response. These findings are consistent with the results of other immune response studies with NDV HN[5].

In conclusion, we demonstrated a synergistic effect of IL-2 and IFN-γ that could activate T cells, induce CTL predominance, and cell-mediated Th1 type anti-tumoral immune responses by application of NDVcc HN.

References

[1] Lech P. J., Russell S. J., *Expert. Rev. Vaccines*, **2010**, *9*(11), 1275

[2] Fournier P., Aigner M., Schirrmacher V., *Int. J. Oncol.*, **2010**, *37*(5), 1203

[3] Scheid A., Choppin P. W., *J. Virol.*, **1973**, *11*(2), 263

[4] Liu C., Air G. M., *Virology*, **1993**, *194*(1), 403

[5] Li X., Jin N., Lian H., Guan G., Sun L., Li X., Zheng H., *Chinese Sci. Bull.*, **2006**, *51*(22), 2724

[6] Jin K., Jin N., Wang X., Ding Z., Guo Z., Wang H., Chang G., Yin Z., *Chin. J. Prev. Vet. Med.*, **2000**, *22*(2), 143

[7] Chen L., Jin N., Li X., Liu L., Jia P., Liu Y., Gao P., Lu Y., Li M., Chi B., *Chin. J. Immunol.*, **2009**, *24*(2), 132

[8] Li X., Liu Y., Wen Z., Li C., Lu H., Tian M., Jin K., Sun L., Gao P., Yang E., Xu X., Kan S., Wang Z., Wang Y., Jin N., *Mol. Cancer*, **2010**, *20*(9), 10

[9] Glogarova K., Buckiova D., *Birth. Defects. Res. A. Clin. Mol. Teratol.* , **2004**, *70*(3), 142

[10] Guan G., Jin N., Mi Z., Li X., Lian H., Jin C., Sun L., Wen L., *Chinese Journal of Otorhinolaryngology Head and Neck Surgery*, **2005**, *40*(8), 566

[11] Connaris H., Takimoto T., Russell R., Crennell S., Moustafa I., Portner A., Taylor G., *J. Virol.*, **2002**, *76*(4), 1816

[12] Nia J., Schirrmachera V., Fourniera P., *Vaccine*, **2010**, *28*, 6891