ORIGINAL RESEARCH

Proinflammatory response induced by Newcastle disease virus in tumor and normal cells

Teridah Ernala Ginting
Jeremiah Suryatenggara
Salomo Christian
George Mathew

Division of Immunology, Mochtar Riady Institute for Nanotechnology and Medical Science Group, University of Pelita Harapan, Tangerang, Indonesia

Purpose: To investigate the specific role of immune responses induced by lentogenic Newcastle disease virus (NDV) for its antitumor effect.

Materials and methods: NDV LaSota strain was used to infect the following human cells: non-small cell lung carcinoma (A549), glioblastoma (U87MG and T98G), mammary gland adenocarcinoma (MCF7 and MDA-MB-453), hepatocellular carcinoma (Huh7), transformed embryonic kidney cells (HEK293), primary monocytes, lung fibroblast (HF19), skin fibroblast (NB1RGB) and rat astroglia (RCR-1) at 0.001 multiplicity of infection. NDV-induced cytotoxicity and expression of proinflammatory cytokines were analyzed using 3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide assay and multiplex enzyme-linked immunosorbent assay, respectively.

Results: Tumor cells (A549, U87MG, T98G, Huh7, MDA-MB-453, and MCF7) showed viability of <44%, while normal cell lines HEK293, NB1RGB, and RCR-1 showed 84%, 73%, and 69% viability at 72 hours postinfection, respectively. Proinflammatory cytokine profiling showed that NDV mainly induced the secretion of interferon (IFN)-α, IFN-β, and IFN-λ in tumor cells and only IFN-λ in normal cells. In addition, NDV infection induced the production of interleukin (IL)-6 in most cells.

Conclusion: Our findings suggest a new perspective regarding the role of IFN-λ and IL-6 in the mechanism of tumor selectivity and oncolysis of NDV.

Keywords: Newcastle disease virus, oncolytic virus, interferons, cytokines

Introduction

Every year, mortality caused by cancer is increasing globally. This calls for novel therapeutic strategies.[1] Use of oncolytic viruses represents a class of promising antitumor therapeutic options, primarily through the direct lysing of cancer cells and secondarily through the potential antitumor response induced by inflammation.[2–6]

Newcastle disease virus (NDV) is a negative-strand RNA virus from the family *Paramyxoviridae*. This virus has been reported to be an effective oncolytic agent in vitro and in vivo against many types of solid tumors such as hepatocellular carcinoma, pancreatic adenocarcinoma, melanoma, pleural mesothelioma, colorectal carcinoma, renal carcinoma, glioblastoma and many others,[5,7–13] as well as in clinical studies in patients with glioblastoma multiforme, colorectal carcinoma, non-small cell lung carcinoma, renal carcinoma, breast adenocarcinoma, mesothelioma and pancreatic adenocarcinoma.[14–18] NDV causes a disease that predominantly affects poultry, although infection in humans has been reported to cause conjunctivitis[19,20] and/or mild flu-like symptoms.[13–17,21] Based on their virulence on birds, NDV strains can be divided into

Correspondence: Teridah Ernala Ginting
Mochtar Riady Institute for Nanotechnology and Medical Science Group, University of Pelita Harapan, Jalan Boulevard Jenderal Sudirman 1688, Lippo Karawaci, Tangerang, Banten 15811, Indonesia
Tel +62 21 5421 0123
Fax +62 21 5421 0110
Email tginting@mrinstitute.org

three groups: lentogenic (low virulence), mesogenic (medium virulence) and velogenic (high virulence). According to the properties of the viral fusion protein, NDV can be divided into lytic and nonlytic strains, both of which have antitumor potential. Lytic strains are able to produce infectious progeny, while nonlytic strains mainly stimulate immune responses that result in antitumor activity.[9,12,22]

Cells respond to viral infection by the production of antiviral cytokines, mainly interferon (IFN), which is reported to have antiproliferative effects against tumors. There are three types of IFNs, ie, type I IFNs (IFN-α and IFN-β), type II IFN (IFN-γ) and type III IFNs (IFN-λ1, IFN-λ2 and IFN-λ3). Type I IFNs and type III IFNs are believed to stimulate similar IFN stimulating genes (ISGs) that have antiviral effects. The main function of type II IFN is in adaptive immune responses, although it may share some antiviral properties of type I and III IFNs.[23–27] NDV is postulated to be a potent type I IFN inducer, but due to defective IFN signaling in tumor cells NDV replicates better in tumor cells. This has been the basis of tumor selective replication of NDV.[11,18,28–31] The evidence for type I IFN production in vitro and in vivo after NDV infection has been obtained by measuring the level of IFN-α/β and the induction of ISGs. Moreover, the presence of IFN protein has been reported in cell lines after being infected with nonengineered or engineered NDV.[8,11,28] The difference in proinflammatory cytokine production between normal and tumor cells and the role in oncolysis upon NDV infection are not well understood.

The primary objectives of this study were to optimize the protocol for the use of lentogenic NDV LaSota strain as a safe antitumor agent and to investigate NDV susceptibility of tumors. In this study, we were able to demonstrate the oncolytic activity of lentogenic NDV strain at low multiplicity of infection (MOI) in tumor cells compared to normal (nonmalignant) cells. This indicates for a mechanism involving soluble mediators that induce cell death. Furthermore, we found a difference in cytokine expression in tumor and normal cells, which may contribute to the basis of tumor selectivity of NDV.

Materials and methods
Cell culture
Human tumor cell lines A549 (non-small cell lung carcinoma), U87MG (glioblastoma multiforme), T98G (glioblastoma multiforme), MDA-MB-453 (breast adenocarcinoma), MCF7 (breast adenocarcinoma) and Huh7 (hepatocellular carcinoma), and normal (nonmalignant) mammalian cells HEK293 (transformed embryonic kidney), H19 (lung fibroblast), NB1RGB (skin fibroblast) and RCR-1 (transformed astroglia) were purchased from RIKEN BioResource Center – Cell Bank (Tsukuba, Japan) or American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured according to the protocol of the provider. All cells and culture conditions are listed in Table 1.

Fresh whole blood used to generate monocyte culture in this study was obtained from a healthy volunteer. Written informed consent was obtained and the ethical approval was granted from the Mochtar Riady Institute for Nanotechnology Ethics Committee. Peripheral blood mononuclear cells (PBMCs) were collected using Ficoll-paque (GE Healthcare Bio-Science, Uppsala, Sweden) according to the manufacturer's protocol. Then, PBMCs were allowed to adhere in a cell

Table 1 Conditions of normal and tumor cells

Cell name	Code	Tissue	Species	Growth medium (+10% FBS)	Culture condition
Tumor					
A549	RCB0098	Non-small cell lung carcinoma	Human	F12-K	Adherent, 37°C, 5% CO_2
U87MG	HTB14	Glioblastoma multiforme	Human	High glucose DMEM	Adherent, 37°C, 5% CO_2
T98G	RCB1954	Glioblastoma multiforme	Human	RPMI-1640	Adherent, 37°C, 5% CO_2
Huh7	RCB1942	Hepatocellular carcinoma	Human	High glucose DMEM	Adherent, 37°C, 5% CO_2
MDA-MB-453	RCB1192	Mammary gland adenocarcinoma	Human	Leibovitz-15	Adherent, 37°C, no CO_2
MCF7	RCB1904	Mammary gland adenocarcinoma	Human	MEM +0.1 mM NEAA +1 mM sodium pyruvate	Adherent, 37°C, 5% CO_2
Normal (nonmalignant)					
HEK293	RCB1637	Human embryonic kidney	Human	High glucose DMEM	Semi-adherent, 37°C, 5% CO_2
Monocytes		Adherent mononuclear cells (primary)	Human	RPMI-1640	Adherent, 37°C, 5% CO_2
NB1RGB	RCB0222	Normal skin fibroblast	Human	MEMα	Adherent, 37°C, 5% CO_2
HF19	RCB0210	Normal lung fibroblast	Human	MEMα	Adherent, 37°C, 5% CO_2
RCR-1	RCB0075	Astroglia	Rat	DMEM	Semi-adherent, 37°C, 5% CO_2

Abbreviations: DMEM, Dulbecco's Minimal Essential Medium; FBS, fetal bovine serum; F12-K, Kaighn's modification F12 nutrient medium; MEM, minimal essential medium; NEAA, nonessential amino acids; RPMI-1640, Roswell Park Memorial Institute-1640.

culture dish for 30 minutes at 37°C in 5% CO_2 atmosphere and subsequently washed to remove floating cells. Adherent mononuclear cells were detached with accutase (Biowest, Nuaillé, France) at room temperature, counted, grown in 96-well plates in Roswell Park Memorial Institute-1640 media (RPMI-1640) and treated on the next day. All media and supplements were obtained from Biowest, except for Kaighn's modification F12 nutrient medium (F12-K), Leibovitz's-15 and minimal essential medium alpha (MEMα) media that were obtained from Gibco–Life Technologies (Carlsbad, CA, USA).

Virus

Lentogenic NDV LaSota strain was cultivated and obtained from PT IPB Shigeta Animal Pharmaceuticals, Bogor, Indonesia. Virus was grown in the allantoic cavity of NDV-seronegative 10-day-old embryonated chicken eggs. Eggs were incubated at 37°C with 50-60% humidity for 5 days and subsequently incubated at 4°C overnight.[32] Allantoic fluid containing virus was collected and cleared from debris by centrifugation at 3000× g. Infectious particles were confirmed by end point dilution assay. Virus was stored in aliquots at −80°C before use.

Virus titration

Baby hamster kidney (BHK-21) cells (RIKEN) were grown in Dulbecco's Minimal Essential Medium (DMEM; Biowest) supplemented with 10% fetal bovine serum (FBS). A 96-well plate of BHK-21 cells was prepared on the previous day to be confluent at the time of assay. Wells were washed with phosphate-buffered saline (PBS) three times. Cells were then overlaid with 25 μL of ten-fold serially diluted virus for 60 minutes at 37°C in 5% CO_2, washed with PBS three times and then incubated in maintenance medium (similar to growth medium except that FBS was replaced with 0.3% bovine serum albumin (BSA) fraction V (Gibco) and 1% l-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK)-treated trypsin (Affymetrix, Cleveland, OH, USA). The cells were incubated at 37°C in 5% CO_2 for 5 days, fixed with 10% formalin for 30 minutes and stained with crystal violet. Tissue culture infectious dose 50 value was calculated according to Reed and Muench.[33]

Virus infection for determination of cytokine production

To study the cytokine production of cells that were infected by NDV, we prepared ~5×10⁵ cells per well in triplicate in six-well plates 12–18 hours before infection. Cells were washed with PBS three times and then incubated with 200 μL of virus in PBS at 0.001 MOI, at 37°C in 5% CO_2 atmosphere for 60 minutes. Noninfected control wells were prepared by incubating cells with PBS (Gibco). At the end of the incubation, the cells were washed with PBS to remove unbound virus and overlaid with 2 mL of corresponding maintenance medium without TPCK trypsin. Culture supernatant that was collected at 48 hours after infection was used for further assay.[31]

Cytotoxicity of NDV

Triplicates of 5×10^4 cells per well in 96-well plates were prepared 12–18 hours before infection. Cells were washed three times and overlaid with 200 μL of maintenance medium without TPCK-treated trypsin containing NDV at an MOI of 0.001. Noninfected cells served as negative controls. Cytotoxicity assays were conducted as previously described[34] by adding 100 μg 3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma, St. Louis, MO, USA) reagent followed by incubation at 37°C in 5% CO_2 for 4 hours. Purple crystal formed was solubilized with dimethylsulfoxide (DMSO; Sigma), and absorbance was read at 540 nm. Data are presented as percentage of viability, which is the ratio of treated to Noninfected cells.

Multiplex proinflammatory cytokines: enzyme-linked immunosorbent assay (ELISA)

Responses of normal and tumor cells to NDV infection at 0.001 MOI were determined by measuring proinflammatory cytokine production in cell culture supernatants by using a Veriplex® 16-plex human cytokine ELISA kit (PBL Assay Science, Piscataway, NJ, USA) according to the manufacturer's protocol. Then, 50 mL supernatants from infected or noninfected cells, which were collected at 48 hours, were ultraviolet (UV) inactivated for 30 minutes for biosafety reasons and then diluted 1:1 in sample buffer followed by incubation in wells of 96-well plates that contained antibodies against 16 different proinflammatory cytokines, including IFN-α, IFN-β, IFN-γ, IFN-λ1/2/3 and cytokines released upstream and downstream of IFN signaling. Plates were incubated for 2 hours at room temperature and washed, and 50 μL of biotinylated detection antibody was added. Plates were incubated for 1 hour at room temperature and washed and 50 μL of chemiluminescent substrate was added. Chemiluminescent output was visualized and imaged using a VersaDoc 4000 imaging system (Bio-Rad, Hercules, CA, USA). Images were analyzed with Q-View® software (Quansys Bioscience, Logan, UT, USA). Data were obtained from triplicate wells, and graphs were constructed from the average pixel intensity.

Statistical analysis

Cytokine quantification data were mean values from triplicate wells. Significance of treated and Noninfected groups was analyzed by two-tailed unpaired Student's *t*-test and presented in *P*-values. Only a *P*-value of <0.001 (***P<0.001, ****P<0.0001) was discussed.

Results and discussion
### Susceptibility of normal and tumor cell lines to NDV infection

To examine whether lentogenic NDV is able to kill normal cells and tumor cells, we conducted cytotoxicity assays at low MOI (0.001) of NDV. Normal and tumor cells in this study (Table 1) were selected to represent a wide range of tissue types and to demonstrate different responses that can result from NDV infection. Cells in 96-well plates were cocultured with NDV and examined for cytotoxicity at 0, 24, 48 and 72 hours. Our study showed that normal cells such as HEK293, NB1RGB and RCR-1 were relatively resistant to NDV infection with viabilities of 84, 73 and 69% at 72 hours postinfection (PI; Figure 1A). Tumor cells A549, U87MG, T98G, Huh7, MDA-MB-453 and MCF7 cell lines were susceptible to NDV infection. As expected, almost all cells were dying at 72 hours PI with viabilities of <44% (Figure 1B). Notably, HF19, a normal human lung fibroblast cell line, was more susceptible to NDV compared to other normal cells used with a viability of 22% at 72 hours PI (Figure 1A) as has been reported by other investigators.[35] This is possibly associated with the nature of paramyxoviruses that have tropism for tissue in the respiratory tract. Human primary monocytes showed viability of 32% at 72 hours PI; however, the number was steady from 48 hours PI until the end of observation.

A difference in IFN response in tumor cells when compared to normal cells after NDV infection

To investigate whether the immune response induced by NDV is associated with the cytotoxicity, we infected normal and tumor cells with NDV at 0.001 MOI and examined proinflammatory cytokines produced by cells after 48 hours of infection. This time point was chosen as most cells showed a remarkable reduction in viability (Figure 1), and this might be the consequence of cytokine expression.

We observed that NDV infection predominantly induced the production of both type I (IFN-α and IFN-β) and type III IFNs (IFN-λ) in tumor cells as was observed in A549, U87MG, T98G, MDA-MB-453 and MCF7; while NDV induced IFN-λ production mainly in normal cells, ie, HEK293, HF19 and NB1RGB (Figure 2). Compared to IFN-α and -β, IFN-λ production was very high in normal cells (Table S1) even when lower MOI was used. It is our contention that IFN-α and IFN-β are not the major antiviral responses in normal cells. This suggests that NDV is a potent type III IFN inducer in normal and tumor cells. Higher induction of type III IFN was observed in all tumor cells except for Huh7. However, because some IFNs exhibited signals over the detection limit, it is unclear if each type of IFN was expressed differently in each tumor cell line used in this study.
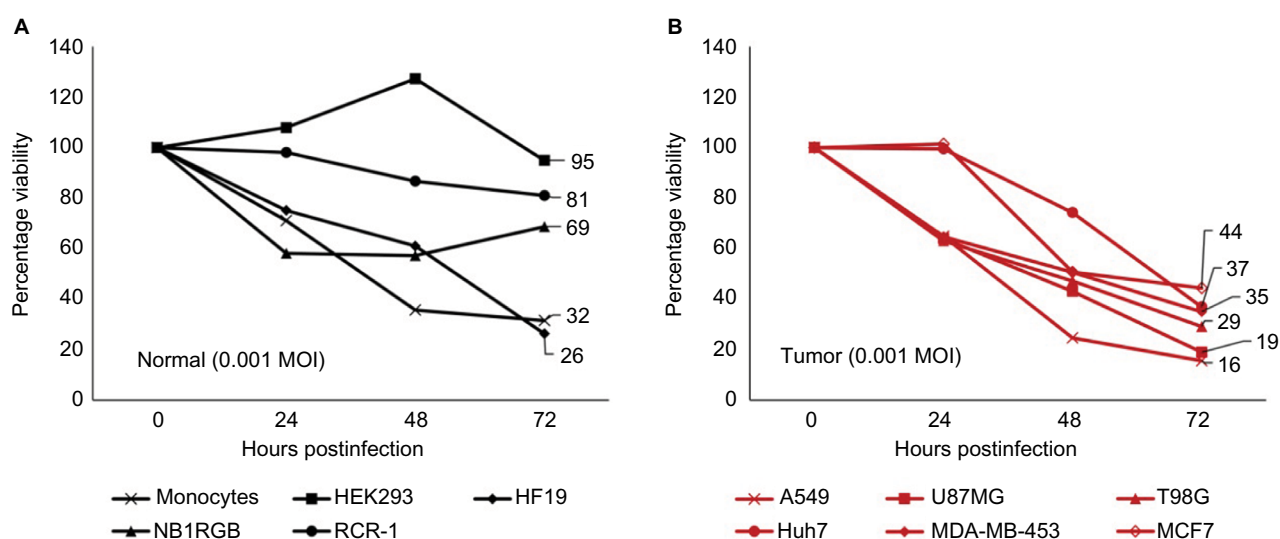
Figure 1 Cytotoxicity of NDV.
Notes: Cytotoxicity was evaluated every 24 hours by using MTT assay on normal cells (**A**) and tumor cells (**B**) at 0.001 MOI. Percentage of viability was obtained from the ratio of treated cells with noninfected cells (not shown), where untreated cells were considered as 100% viable. Data were taken from triplicate wells.
Abbreviations: MOI, multiplicity of infection; MTT, 3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide; NDV, Newcastle disease virus.
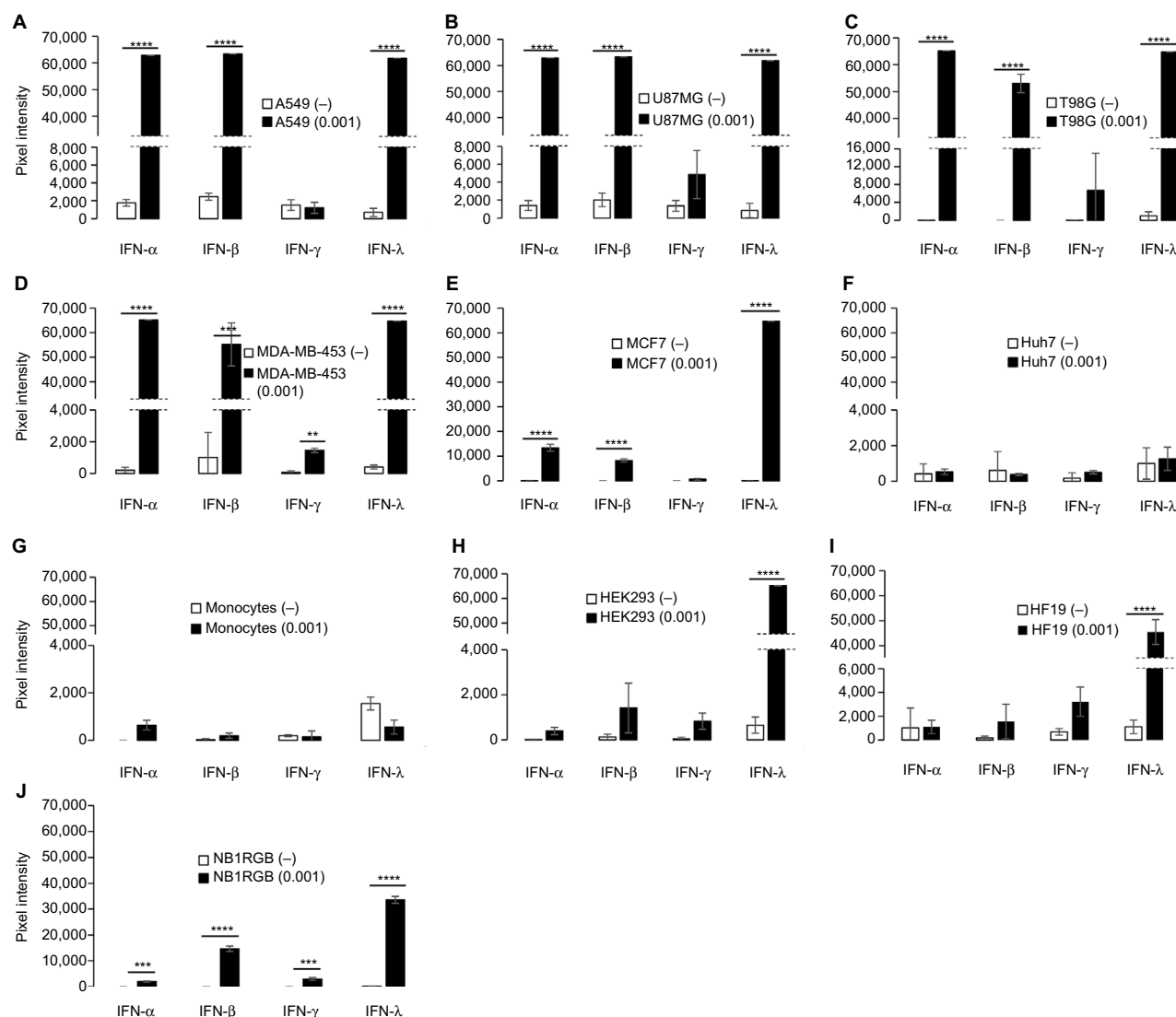
Figure 2 Type I, II and III IFNs production in tumor and normal cells.
Notes: (**A**) A549; (**B**) U87MG; (**C**) T98G; (**D**) MDA-MB0453; (**E**), MCF7; (**F**) Huh7; (**G**) monocytes (**H**) HEK293; (**I**) HF19; and (**J**) NB1RGB. IFNs in cell culture supernatant 48 hours after NDV infection at 0.001 MOI (black bars). Supernatant from noninfected cells (–) are shown as white bars. Results are luminescence intensity that was taken from the average of triplicate wells and photographed by using VersaDoc 4000 imaging system. Pixel intensity was analyzed by Q-View software. Data represent means from triplicate wells. Standard deviation of mean is shown. ***P<0.001, ****P<0.0001.
Abbreviations: IFN, interferon; MOI, multiplicity of infection; NDV, Newcastle disease virus.

Hepatocellular carcinoma (Huh7) cells did not show any IFN expression above the lower limit of quantification, at least 48 hours after infection. Given the low percentage of viability observed in the cytotoxicity assay, Huh7 susceptibility to NDV was presumably not associated with direct IFN effects. Although type II IFN (IFN-γ) secretion was detected in U87MG and T98G, it was not at appreciable levels.

A previous study demonstrated the presence of type I IFNs in pancreatic adenocarcinoma cell lines upon NDV infection by measuring ISG expression.[8] Another study also demonstrated that genetically engineered NDV induced IFN-α and IFN-β in normal cells but only IFN-β in most tumor cells;[28] in addition, IFN-α was shown to be expressed in mouse bronchoalveolar lavage (BAL) and mouse/human

monocytes.[29] Yet, there have been no reports demonstrating that IFN-λ is expressed higher than IFN-α/β in normal human cells after NDV infection.

Characterization of other proinflammatory cytokines upon NDV infection

Apart from the IFN response, we also evaluated the expression of a number of proinflammatory cytokines using a multiplex ELISA kit (Figure 3). In general, interleukin (IL)-8 was expressed in all cells in this study, and the level was increased after NDV infection; therefore, this cytokine is presumably expressed by cultured cells due to factors such as serum components contained in the media. We also observed that IL-6 was strongly expressed in most tumor cells, ie, A549,
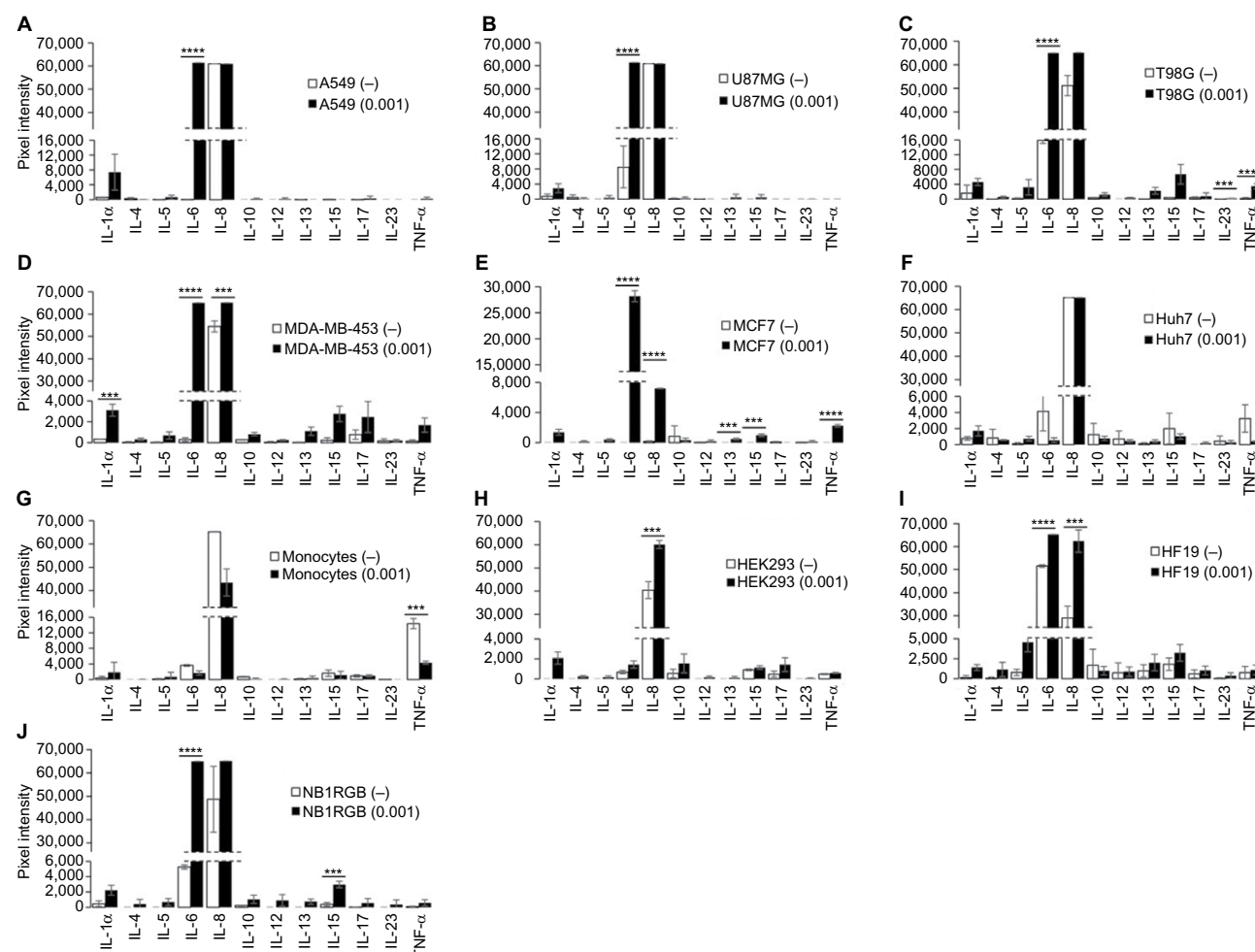
Figure 3 Production of proinflammatory cytokines other than IFNs.
Notes: (**A**) A549; (**B**) U87MG; (**C**) T98G; (**D**) MDA-MB0453; (**E**), MCF7; (**F**) Huh7; (**G**) monocytes (**H**) HEK293; (**I**) HF19; and (**J**) NB1RGB. Other proinflammatory cytokines in cell culture supernatant 48 hours after NDV infection at 0.001 MOI (black bars). Supernatants from noninfected (–) cells are shown as white bars. Results are expressed as luminescence intensity that was recorded by using VersaDoc 4000 imaging system and analyzed by Q-View software. Data represent means from triplicate wells. Standard deviation of mean is shown. ***P<0.001, ****P<0.0001.
Abbreviations: IL, interleukin; MOI, multiplicity of infection; NDV, Newcastle disease virus.

U87MG, T98G, MDA-MB-453 and MCF7, and some normal cells, ie, HF19 and NB1RGB. IL-6 has been reported as being involved in the inflammatory response to viral infection in some cell lines, and its production is triggered through NFκB activation.[36] However, whether IL-6 contributes to tumor specificity and oncolysis needs to be explored further. In addition, monocytes secreted TNF-α either naturally or after NDV infection. High cytotoxicity observed in monocyte culture after NDV infection is presumably because of apoptosis induction through the activation of TNF-α receptor-associated death domain (TRADD) that leads to apoptosis cascade.[37] In tumor cell environment, the effects of TNF-α would be direct oncolysis, and a potential signal to produce IFNs in surrounding cells, thus indirectly killing tumor cells.[29]

Conclusion

The efficacy, safety and tumor selectivity have been the rationales of using NDV as antitumor agent.[3,12,13,21,28,31] In

this study, to demonstrate the cytotoxicity and tumor selectivity of NDV, we utilized a lentogenic LaSota strain, which has been long used as a vaccine strain worldwide, at low MOI. We examined IFN levels in cell culture supernatant after NDV infection and compared them to the cytotoxicity scores. Despite the hallmark of NDV as a potent type I IFN inducer, our results showed that NDV strongly induced type I IFNs only in tumor cells, while the induction was minor in normal cells. Indeed, we found that NDV is a very strong inducer of IFN-λ in both normal and tumor cell types, even at a very low MOI.

The possible cause of the efficient killing rate of NDV in tumor cells (A549, U87MG, T98G, MDA-MB-453 and MCF7) when compared with normal cells (HEK293 and NB1RGB) could be because of the cumulative effects of type I IFNs (IFN-α and IFN-β) and type III IFN (IFN-λ), or the consequence of high expression of type I IFNs (IFN-α and IFN-β), which is peculiar to tumor cells. Both type I and

III IFNs have antiproliferative effects, potentially stimulating the same genes, but they bind to different cell surface receptors.[24–26,38] This finding introduces a new concept of NDV oncolysis: first, lentogenic nonlytic NDV is a very potent inducer of type I and III IFNs in human tumor cells, and second, the cumulative effect of IFNs induced by NDV results in better oncolysis rates in most tumor cells. Of all tumor cells used, hepatocellular carcinoma (Huh7) did not produce any IFNs although they showed cytotoxicity similar to other tumor cells. This suggests that the cytotoxicity of Huh7 in this study was driven by other mechanisms such as direct lysis due to virus replication.

Measurement of other cytokines showed that IL-6 was highly produced in most cell types after NDV infection, but because IL-6 has both pro- and anti-inflammatory properties,[39] further study is needed to conclude whether its expression leads to cytotoxicity or cell survival in NDV infection. IL-8, which is a chemotactic agent, functions to attract neutrophils during inflammation, also associated with malignancy and cell survival, and its production can be stimulated by many extracellular signals.[40–43] High IL-8 production in all cells even in the Noninfected cells in this study was probably caused by the stimulation of other factors contained in the media. We tested each medium for the presence of proinflammatory cytokines but none were detected, thus confirming that there was no cytokine contamination.

Proinflammatory responses induced by NDV infection have been shown to have favorable effects on killing of tumor cells selectively.[8,29,44] In this study, we conclude that lentogenic NDV LaSota strain has oncolysis ability at low MOI, presumably due to the effect of soluble mediator. We have shown that NDV-infected cells exhibit inflammatory response through the rise of IFNs and IL-6 in culture supernatant. Moreover, our study is the first to demonstrate different secretion of IFNs in normal and tumor cells and the presence of IFN-λ in human cell lines upon NDV infection. However, exact mechanisms of cell death and tumor specificity of NDV-infected cells need to be determined further.

Given the important functions of IL-6 in both proinflammatory and anti-inflammatory responses, and of type III IFNs to activate hundreds of IFN-stimulated genes that have effects such as antiviral, antiproliferation and inducing apoptosis,[9,23–25,28,29,35] in this study we provide a strong rationale for further study of IL-6 and the balance of IFN-α, IFN-β and IFN-λ in oncolysis and tumor specificity of NDV.

Acknowledgments

Disclosure

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Supplementary material

Table S1 Cytokine quantification (pg/mL) from supernatant of cells after NDV infection at 0.001 MOI.

	IFN-α	IFN-β	IFN-γ	IFN-λ	IL-1α	IL-4	IL-5	IL-6	IL-8	IL-10	IL-12	IL-13	IL-15	IL-17	IL-23	TNF-α
(A)																
ULOQ	4001.54	21000.08	11273.76	11500.16	10058.7	3726.34	5000.45	6204.35	2836.87	2508.1	2998.7	4299.97	4693.35	4704.24	46069.67	3700.03
LLOQ	57.73	83.82	41.19	153.63	109.57	14.7	18.36	221.52	9.81	9.31	12.43	17.75	66.26	18.22	214.32	14.95
Huh7 (−)	<57.73	<83.82	43.66667	205.13333	<109.57	<14.7	19.46333	<221.52	811.44	10.05667	13.38333	<17.75	<66.26	<18.22	243.0833	53.83333
Huh7 (0.001)	<57.73	88.73667	<41.19	<153.63	<109.57	<14.7	20.79333	<221.52	835.2433	<9.31	<12.43	<17.75	<66.26	<18.22	253.6467	19.19333
MCF7 (−)	<57.73	<83.82	<41.19	<153.63	<109.57	<14.7	21.1	<221.52	<9.81	<9.31	<12.43	<17.75	<66.26	<18.22	<214.32	<14.95
MCF7 (0.001)	**154.9933**	**597.9633**	<41.19	**>11500.16**	<109.57	<14.7	20.28667	**410.35**	**32.63**	<9.31	<12.43	<17.75	<66.26	<18.22	<214.32	**22.3**
MDA-MB-453 (−)	<57.73	<83.82	<41.19	<153.63	<109.57	<14.7	<18.36	<221.52	230.5033	<9.31	<12.43	<17.75	<66.26	<18.22	<214.32	<14.95
MDA-MB-453 (0.001)	**3985.06**	4283.353	<41.19	**>11500.16**	<109.57	<14.7	<18.36	**1972.603**	**2712.69**	<9.31	<12.43	<17.75	<66.26	31.09	<214.32	18.56
NB1RGB (−)	<57.73	<83.82	<41.19	<153.63	<109.57	<14.7	<18.36	<221.52	218.05	<9.31	<12.43	<17.75	<66.26	<18.22	221.65	<14.95
NB1RGB (0.001)	<57.73	**1056.05**	<41.19	**1380.1433**	<109.57	<14.7	21.05	**5190.457**	>2836.87	<9.31	<12.43	<17.75	<66.26	20.55333	<214.32	15.84333
T98G (−)	<57.73	<83.82	<41.19	155.81667	<109.57	<14.7	<18.36	260.1367	214.2233	<9.31	<12.43	<17.75	<66.26	<18.22	<214.32	<14.95
T98G (0.001)	**3838.603**	**3860.257**	113.5933	**>11500.16**	<109.57	<14.7	33.87	**>6204.35**	1750.367	<9.31	<12.43	19.62667	<66.26	<18.22	<214.32	54.06333
(B)																
ULOQ	4025.55	18971.79	3666.67	11500	10500	3700	5247.43	713.15	2800	92.59	3000	4316.3	1533.33	Incalculable	Incalculable	3700
LLOQ	156.47	237.8	135.8	141.98	388.89	3700	1458.6	229.47	311.12	92.59	333.33	1472.42	170.37	Incalculable	Incalculable	1233.33
A549 (−)	173.97	690.83	1649.6	1043.56	294.69	1413.46	0	139.89	>2800	11.88	>3000	190.18	56.42	No Fit	No Fit	0
A549 (0.001)	**>4025.55**	**>18971.79**	1474.07	**>11500**	399.36	>3700	187.94	**1096.97**	>2800	30.59	175.09	25.47	0	No Fit	No Fit	247.18
U87MG (−)	156.56	601.49	1454.79	1087.47	324	2719.26	132.64	253.27	>2800	43.06	>3000	>4316.3	66.95	No Fit	No Fit	380.91
U87MG (0.001)	**>4025.55**	**>18971.79**	**>3666.67**	**>11500**	349.04	2361.85	183.46	**1096.97**	>2800	60.3	165.17	182.61	125.17	No Fit	No Fit	380.91
(C)																
ULOQ	3698.15	18834.96	11005.25	11552.05	3980.73	3699.03	536.87	2223.34	980.47	920.1	326.69	3451.86	1764.62	4699.47	46160.26	1245.99
LLOQ	161.07	2247.56	460.92	123.32	400.4	143.53	61.42	80.59	301.5	33.14	10.57	179.54	58.32	180.45	6000.85	150.58
HEK293 (−)	<161.07	<2247.56	<460.92	235.9	<400.4	<143.53	<61.42	<80.59	857.4067	<33.14	12.45	<179.54	<58.32	<180.45	<6000.85	<150.58
HEK293 (0.001)	344.8467	<2247.56	<460.92	**>11552.05**	<400.4	<143.53	<61.42	<80.59	**>980.47**	<33.14	14.24	<179.54	<58.32	187.8533	<6000.85	<150.58
HF19 (−)	<161.07	<2247.56	742.9567	497.63	<400.4	143.8967	<61.42	>2223.34	537.97	<33.14	11.11	<179.54	68.57667	<180.45	<6000.85	<150.58
HF19 (0.001)	176.8833	2362.323	804.9	**>11552.05**	<400.4	201.1833	100.8633	**>2223.34**	**>980.47**	<33.14	22.94667	192.4867	102.7733	<180.45	<6000.85	<150.58
Monocyte (−)	<161.07	<2247.56	<460.92	587.48667	<400.4	<143.53	<61.42	167.7667	>980.47	<33.14	12.16667	<179.54	60.66	<180.45	<6000.85	406.96
Monocyte (0.001)	<161.07	<2247.56	<460.92	543.61333	424.1833	<143.53	<61.42	131.1567	>980.47	<33.14	<10.57	<179.54	<58.32	<180.45	<6000.85	180.5667

Notes: Values are the average from triplicate well. (**A**) Huh7, MCF7, MDA-MB-453, NB1RGB, T98G; (**B**) A549, U87MG; (**C**) HEK293, HF19, monocyte. Difference between infected (0.001) and noninfected (−) cells is statistically significant with P-value <0.0001 (bold values).
Abbreviations: NDV, Newcastle disease virus; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; ULOQ, upper limit of quantification; LLOQ, lower limit of quantification; MOI, multiplicity of infection.