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Research Article

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Study of Newcastle Disease Virus as an Immunostimulatory Agent for Anticancer Effect

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ABSTRACT

Introduction: Ineffective response of conventional therapies for the treatment of cancer has dictated the search for new treatment strategies.Emerging mode of cancer therapy likeoncolytic virotherapy employs oncolytic viruses (OVs) like Newcastle disease virus which can selectively kill cancer cells through direct lysis and can also trigger a potent anti-tumor immune response.

Aim: To study the immunostimulatory effect of NDV9B on cancer cell lines MCF 7, MDA MB-231, A549, PC3, and normal HEK-293 cell line.

Methodology: UV inactivated NDV9B HAU 10 and 20was used to infect human PBMC. Post infection the stimulation of cytokine IFN-α, IFN-γ and TRAIL was quantified using ELISA. These activated PBMC was co-cultured with the cancer and control normal cell lines to check tumor neutralization activity.

Results: UV inactivated NDV9B triggered cytokines secretion in PBMCs. The concentration of cytokine IFN-α, IFNγ, TRAIL/TNFSF10 was found to be 239.57 pg/mL, 14.68 pg/mL, 61.17 pg/mL respectively infected with 20 HAU. Activated PBMCs when co cultured with cancer cells showed maximum cytotoxicity of 11.75 % in MDA MB231 cancer cell line.

Conclusion: NDV9B was found to stimulate cytokines in PBMCs and these PBMCs successfully brought about low level of cytopathic effect in MDA MB231.

Key Words: Oncolytic virotherapy, PBMCs, Newcastle disease virus, Immunostimulatory, Cytokine, Tumor neutralization

INTRODUCTION

Cancer is a genetic disease caused by underlying mutations(both somatic and germline gene.[1] Global cancer burden assenting to the GLOBOCAN 2020 estimated 19.3 million new cancer cases and almost 10.0 million cancer death occurred in 2020.[2] Metastasis along with proliferative signaling, growth suppressor evasion, replication immortality etc., are the hallmarks of cancer. Current modalities of treatment will continue to be the backbone of cancer therapyin the future. However, none of these existing treatment modalities can effectively control metastatic cancer. The therapy of choice needs to be specific as well as effective in reaching every part of the body. Therefore, immense efforts to cure cancer have been focusing on searching novel drugs, virotherapy, exploring immune pathways to better understand signaling molecules etc.[3,4,5]

NDV causes direct oncolytic effect on tumor cells, it also presents some very robust immunomodulatory properties for the anticancer immune response. NDV strains can be classified, based on their oncolytic mechanism, as lytic and non-lytic for mammalian cells.Both lytic and non-lytic strains are known to trigger immunostimulatory effect in mammalian cells.[6]

NDV shows the presence of two integral membrane proteins, the fusion (F) and hemagglutinin neuraminidase (HN) proteinswhich are the major immunogenic proteins of the virion.[7,8] It has been observed that NDV stimulates the production of cytokines, such as IFN-α, IFN-γ, IL-2 IL-1 etc. leadingto activation of natural killer cells, sensitized T cells, and macrophages. NDV infection also activates dendritic cells which increase the expressions of costimulatory molecules and stimulate T cell response.[9] Various studies have shown TNF-related apoptosis-inducing ligand (TRAIL) expression in NDV activates peripheral blood mononuclear cells (PBMC), dendritic cells, and Natural killer cells which indicates that (Tumor necrosis factor) TNF induced apoptosis could also be an important mechanism in oncolytic NDV induced apoptosis.[10]

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In this study, we have aimed to quantify the main cytokines stimulated by UV inactivated NDV9B in PBMCs and further tested these charged PBMCs for any cytopathic effect in cancer and normal cell line.

MATERIALS AND METHODS

Cell line and Cell culture

The cancer cell lines used in the study are breast cancer-MCF-7 and MDA MB-231, lung cancer- A549, prostate cancer-PC3, and normal human embryonic kidney cell line-HEK-293. These cell lines were procured from NCCS, Pune. DMEM supplemented with heat inactivated 10% FBS and 1% penicillin/streptomycin was used. The cell lines were maintained in complete DMEM media at 37°C in a CO_2 incubator.

Isolation of PBMCs

PBMCs were isolated from heparin anticoagulated blood. Ficoll–Histopaque®-1077 (Sigma-Aldrich, St Louis. MO, USA) gradient separation method was used for isolation. Trypan blue dye exclusion assay was performed to determine viability and cell number to be seeded.

Newcastle disease Virus:

NDV9B was procured from Indian Veterinary Research Institute Izatnagar (IVRI), Bareilly, UttarPradesh. It was found to have potent oncolytic effect after initial screening and so was further studied for its immunostimulatory potential. Immunostimulatory effect of UV inactivated NDV9B was studied on human PBMC.

UV inactivation of NDV9B

NDV9B was exposed to UV light at wavelength of 254 nm, power 220µw/cm^2 at distance of 10 cm for 10, 20, 30 and 40 minutes in 30 mm petri plates. This UV exposed virus was then injected in 10-day old country embryonated eggs allantoic fluid and incubated at 37°C for 4 days to check for embryo death. The negative control eggs were injected with sterile PBS and positive control eggs were injected with non-UV exposed virus. The inoculation was performed in triplicates.Further the allantoic fluid was also harvested and checked for hemagglutination.

For human PBMC stimulation, 10^5 cells were seeded with 1 ml of supplemented RPMI-1640 medium. The cells were then incubated in 37°C/ 5% CO_2 incubator for 4 hours for acclimatization after which the cells were infected with HAU 10 and 20 of UV inactivated NDV9B, PBS (vehicle control) for 24 hrs. Post stimulation, supernatants from all groups were obtained for cytokine quantification.

ELISA

Measurement of IFN-α, IFN-γand TRAIL was estimated from supernatants by commercial ELISA kits from Elabscience Biotechnology Co.Ltd, USA.Concentrationof cytokines was determined by plotting a standard curve using the software curve expert 1.4.

Tumor neutralization (cytolytic effect of activated human PBMC on cancer cell lines):

The cancer and normal cells were seeded at concentration of 5x10^{4}and incubated for 24 hours at 37°C and 5% CO2. PBMC untreated and treated with UV inactivated NDV9B were cultured for 24 hours. After the incubation period, the PBMCs were co-cultured with cancer cell lines and the normal cellline atthe ratio of effector cell (PBMC) tothetarget (cancer/ normal) cell E/T ratioof 5 to1 for 72 hours. Post incubation media from all wells was removed and washed with sterile PBS. MTT was added and incubation carried out for 4 hours after which DMSO was added into each well and the plate was read by using ELISA microplate reader at wavelength 540 nm.

Statistical analysis

Statistical analysis was carried out using SPSS software, version 21.0 (SPSS, Chicago, IL, USA).Data presented for the MTT assay is mean ± SE. One way ANOVA with Dunnett's post hoc test was applied. Bonferroni's post hoc test was carried out for tumor neutralization assay. Significance was determined at p value < 0.05.

RESULTS

Table 1 shows NDV9B strain exposed to different concentrations of UV power at different time intervals. (+) represents live and (−) represents dead country eggs.

Each UV inactivated virus aliquot was inoculated in country eggs and incubated for 4 days at 37°C. UV inactivation for 30 minutes was found to be effective as no embryo death was observed even up to 4 days and also hemagglutination in chicken RBCs was observed.

Cytokine IFN-α, IFN-γ and TRAIL were quantified after 24 hrs of stimulation with NDV9B. As, shown in table 2 significant secretion of IFN-α,IFN-γ and TRAIL was stimulated by LPS in PBMC. Both dosages HAU 20 as well as 10 were significantly effective in stimulating IFN-α in PBMCs. However, IFN-γ and TRAIL were stimulated at low concentration by both the viral dosage.

As seen in Figure 1 maximum cytolytic effect was seen in MDA MB231 as compared to other cancer cell lines and normal cell line HEK293 tested.

DISCUSSION

Infection of tumor cells by NDV led to increase in tumor cell immunogenicity. A prospective, randomized, controlled clinical study of post-operative immunization with the autologous tumor vaccine ATV-NDV revealed evidence for clinical effectivity and long-term survival for colon cancer patients.[11]

In this study we have explored the possible role of NDV9B in immunostimulatory effect and same was determined by studying the cytokine activation by NDV9B.Co culture of these activated PBMC with cancer and normal cell line further established potencyof NDV9B as immunogenic agent.

UV inactivation of NDV to study its immunostimulatory effect was 254 nm, 2mW, 7cm distance for 5 mins.[12,13] Other studies by Zeng et al. and Ahmad et al.have carried out inactivation at UV lamp (245 nm, 2 mW/cm^2 for 30 mins and 15 mins respectively.[14,15] However, our methodology of UV inactivation at 254 nm, power 220μW/cm^2 at distance of 10 cm for 30 mins served the purpose as well as hemagglutinin activity was intact.

Envelope glycoproteins in NDV play an important part in stimulation of the PBMCs to trigger induction of cytokines involved in anticancer activity.[16,17]

We observed highest induction of IFN-α as compared to IFN-ν and TRAIL. Stimulation of IFN-αin 24 hours by a recombinant strain rNDV-P05 was observed in the range of 200 pg/mL- 300 pg/mL when PBMCs were infected at 10 and 20 HU of virus.Our study shows concurrence in the levels of IFN-α mediated innate immune response in PBMCs which can contribute in tumor clearance.[18] NDV 73-T strain infection in human PBMCs also projected elevated level of IFN-α.IFN-γ, cytokine of adaptive immunity was found to be stimulated at low but detectable levels in 24 hours post infection with UV inactivated NDV9B at both 10 HAU and 20 HAU. A study by Lam et al. showed highest level of IFN-γ 44.221±0.903pg/ml at titer 10HAU and stimulation was observedafter 48 hours of NDV treatment. Besides this, only recombinant NDV isolates like rNDV-P05, NDV-rec IL2, MEDI5395, engineered to express granulocyte–macrophage colony-stimulating factor (GM-CSF), exhibited enhanced IFN-γ levels.[19] We believe a prolonged incubation with the virus would have boosted the IFN-γ levels. T cells, monocytes and dendritic cells express TRAIL. This is improved after IFN-α/β or -γ stimulation in PBMCs.[14] Research by Washburn et al. have concluded that human monocyte infection with NDV ulster strain resulted in increased TRAIL induction.[20] Another study confirmed that IFN-α promoted the production of soluble as well as membrane bound TRAIL.[21] Our study showed heightened IFN-α but low IFN- γ stimulation. Also, we have checked only the levels of soluble TRAIL in this study and found detectable but not significant stimulation of TRAIL as compared to LPS.

PBMC effector to MCF-7 target (E/T) ratio 5:1 has proved to be effective in reducing cell viability to 69.66% after treatment with activated PBMC (infected with 8 HAU of velogenic NDVAF2240 strain). We similarly explored the E/T ratio 5:1 with four cancer and one normal cell line to activated PBMCs (infected with 20 HAU OF UV inactivated NDV9B). Contradictory to the previous study by Lam et al. no major cytotoxicity was observed in MCF-7 cell line.[10] However, MDA MB231 showed decrease in cell viability by 11.75%. In our previous study we have established the maximum cytopathic effect of velogenic NDV9B in MDA-MB231. A similar trend was observed even in immunostimulatory effect on MDA MB 231.

CONCLUSION

This study has effectively explored the potential of wild type UV inactivated velogenic NDV9B strain as immunostimulatory agent. Although, further studies are required to optimize the stimulation of PBMCs as well as screen other effective cytokines stimulated by NDV. This will lead to better understanding the exact signaling molecules involved in immunomodulatory effect of NDV9B.

Table 1: UV inactivation of NDV9B:

Time of exposure NDV9B (minutes)	Day 1	Day 2	Day 3	Day 4
10	+	+	-	-
20	+	+	-	-
30	+	+	+	+
40	+	+	+	+

Table 2: Immunostimulatory effect of NDV9B

Isolate	Cytokine concentration (pg/mL)		
NDV 9B	IFN-α	IFN- γ	TRAIL
HAU 20	239.57 ±7.36 *	14.68±2.62	61.17±21.43
HAU 10	154.83 ±9.69 *	7.63 ±0.807	7.15± 1.87
Positive control (LPS)	266.36 ± 21.70 *	27.46 ± 4.65*	276.86 ± 41.58*
Negative control	0	6.62± 1.052	0

Cytokine concentration estimated by ELISA. * p<0.001 when compared with the respective negative control.

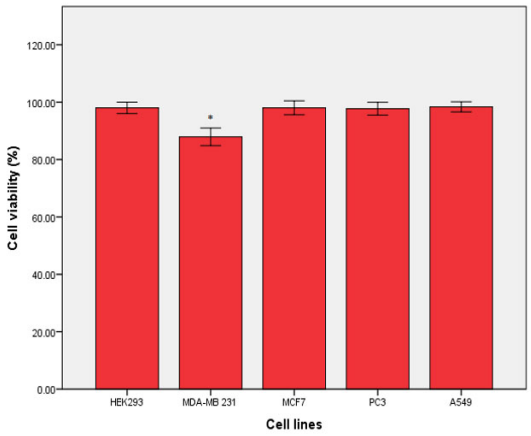

Figure 1: Tumor Neutralization assay- the cell viability after co culture with PBMC at E/T ratio 5 to1. p<0.05 when MDA MB231 compared to other cancer and normal cell line.

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Conflict of Interest: None

Authors' Contribution: Pathak U- Execution, data analysis and article writing; Malik N- Article review and overall supervision; Pal RB – Study Planning, article review, overall supervision.

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