The matrix (M) protein of Newcastle disease virus binds to human Bax through its BH3 domain.

ABSTRACT

The underlying mechanisms by which Newcastle disease virus (NDV) kills cancer cells are still unclear. Recent discoveries have shown that many viruses contain Bcl-2 homology-like domains which enabled their interaction with Bcl-2 family members, and thereby accounting for their virulence and pathogenicity. Alignment of the protein sequences of Malaysian strain of NDV, known as AF2240, with those from members of the human Bcl-2 family showed many similar regions; most notably we found that its matrix (AF2240-M) protein, large (AF2240-L) protein and fusion (AF2240-F) protein all contain BH3-like regions. In addition, there are BH1-like domains in these proteins, where AF2240-F and Mcl-1 share 55% identity within this region. To further investigate our hypothesis that the presence of the BH3-like domains in these proteins may convey cytotoxicity, AF2240-M and AF2240-F genes were cloned into pFLAG and pEGFP.N2 vectors and transfected into HeLa cells. The expression of these constructs promoted cell death. As shown by flow cytometry, AF2240-M protein with deleted BH3-like region showed five-fold decrease in apoptosis. Moreover, the construct containing the N-terminal of AF2240-M showed nearly the same cell death rate as to that of the full-length protein, strongly suggesting that the BH3-like domain within this protein participates in promoting cell death. Moreover, AF2240-M transfection promoted Bax redistribution to mitochondria. Therefore, to determine whether there is any direct interaction between NDV viral proteins with some members of the Bcl-2 family, various constructs were co-transfected into HeLa cells. Co-immunoprecipitation trials showed that the AF2240-M indeed directly interacted with Bax protein via its BH3-domain, as the mutant proteins failed to interact with Bax. AF2240-F failed to interact with any of the tested proteins, although Bcl-XL slowed down the rate of cell death caused by this construct by nearly five-fold. In a parallel experiment, the level of expression of endogenous Bax and Bcl-2 after infection of HeLa cells with NDV was assessed by qRT-PCR, but no statistically significant change was observed. Consequently, the Bax/Bcl-2 ratio at the mRNA level did not alter. Overall, our study has shed additional light into the mechanisms by which NDV induces apoptosis.

Keyword: AF2240; Apoptosis; Bcl-2 homology domain; Fusion protein; HeLa; Matrix protein; NDV; Protein-protein interaction.