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Anne M. Mirza
University of Massachusetts Medical School

Ronald M. Iorio
University of Massachusetts Medical School

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A Mutation in the Stalk of the Newcastle Disease Virus Hemagglutinin-Neuraminidase (HN) Protein Prevents Triggering of the F Protein despite Allowing Efficient HN-F Complex Formation

Anne M. Mirza, Ronald M. Iorio

Department of Microbiology and Physiological Systems, Immunology and Microbiology Program, University of Massachusetts Medical School, Worcester, Massachusetts, USA

Newcastle disease virus (NDV)-induced membrane fusion requires formation of a complex between the hemagglutinin-neuraminidase (HN) and fusion (F) proteins. Substitutions for NDV HN stalk residues A89, L90, and L94 block fusion by modulating formation of the HN-F complex. Here, we demonstrate that a nearby L97A substitution, though previously shown to block fusion, allows efficient HN-F complex formation and likely acts by preventing changes in the HN stalk required for triggering of the bound F protein.

Newcastle disease virus (NDV) is a member of the negative-stranded, RNA-containing Paramyxoviridae (1). Similar to most paramyxoviruses, NDV-induced membrane fusion requires an interaction between the hemagglutinin-neuraminidase (HN) attachment and fusion (F) proteins (1).

The ectodomain of HN consists of a stalk supporting a terminal globular head (1). Whereas the globular domain mediates binding to sialic acid receptors, catalyzes neuraminidase activity (NA), and contains all the antigenic sites (2, 3), the stalk mediates the F interaction (4–7). Mutations in NDV HN stalk residues A89, L90, and L94 specifically modulate fusion with no effect on hemadsorption (HAd), NA, or antigenic structure (8). For these mutations, the extent of fusion correlates directly with the amount of the HN-F complex detectable at the cell surface (9), consistent with these residues contributing to the F interaction. This is supported by the surface exposure of all three residues in the crystal structure of the tetrameric NDV HN ectodomain (10).

However, in this structure these residues appear to be shielded from contacting F by the base of the globular head. This has led to the hypothesis that receptor binding may induce a conformational change that shifts the heads up to expose the F-interactive domain (11). This is consistent with the idea, based on ER retention (12) and attachment-deficient mutants (13), that HN does not interact with F until triggered to do so by receptor binding (9), though evidence to the contrary has recently been published (14).

Whereas the F protein must undergo a dramatic conformational rearrangement to trigger fusion (1), it is difficult to envision this taking place while F is still HN bound, suggesting that the HN-F complex may be transient. We sought to identify HN stalk mutations that would allow formation of the complex, but block fusion triggering, reasoning that such mutations might make it possible to capture an intermediate in the fusion-triggering cascade, which could serve as a reagent to probe the structure of the complex.

HN stalk residues R83 and L97 are also surface exposed (10) and R83N and L97A mutations also specifically inhibit fusion (15, 16). Although the fusion deficiency of R83N-mutated HN correlated with loss of the ability of the protein to interact with F (16), the F-interactive capability of L97A-mutated HN was not previously determined. Thus, we have characterized the effect of A and T substitutions for residues R83 and L97. Also, reasoning that the triggering cascade is transmitted from the receptor binding site in the head to the F-interactive domain in the stalk, we have determined the effect of alanine substitutions for additional residues immediately membrane-distal to L97 for their effect on HN function. A95R and -S substitutions were previously shown to have little or no effect on fusion (8) and an L96A-mutated protein retained about 1/3 of wild-type (wt) activity (15). Therefore, these residues were not investigated.

Using NDV HN in pBluescript SK(+) (Strategene Cloning Systems, La Jolla, CA) and the QuikChange site-directed mutagenesis kit (Strategene, La Jolla, CA), we prepared HN mutants with substitutions of R83A or -T, L97A or -T, N98A, T99A, or E100A. The presence of each mutation was confirmed by sequencing.

Wild-type and mutated HN proteins were expressed in BHK-21 F cells, using the vaccinia virus-T7 RNA polymerase expression system (17). Cells were seeded in six-well plates at 4 × 10⁵ cells/well 1 day prior to transfection and assays were performed at 24 h posttransfection. Cell surface expression (CSE) was determined by the University of Massachusetts Medical School Flow Cytometry Laboratory, using a mixture of conformational HN-specific monoclonal antibodies (MAbs) (18–21). Receptor binding and NA were determined, respectively, by HAd of guinea pig erythrocytes (Bio-Link Laboratories, Liverpool, NY) (8) and cleavage of 2-((4-methylumbelliferyl)-α-D-N-acetyleneuraminic acid (MUN) (22). Fusion was quantitated using a content-mixing assay (8).

Each of the mutated proteins is expressed at the cell surface at levels ranging from 71 to 109% of wt (Fig. 1A). HAd activity at 4°C was only minimally affected, with levels ranging from 79 to 109% of wt (Fig. 1B). Although NA was diminished for E100A-mutated...
HN (33% of wt), each of the other mutants exhibited >60% of wt activity (Fig. 1B).

Despite the lack of a significant modulation of CSE, HAd, or NA, several of the mutated proteins were markedly diminished in the ability to trigger fusion (Fig. 1C and Fig. 2). While R83A-mutated HN promotes approximately 20% of wt fusion, the R83T, L97A, and L97T proteins all promote fusion at <6% of the level of wt HN. The phenotype of the L97A protein is similar to that reported previously (15), though at that time fusion was reported as 18% of wt, as determined by counting nuclei in syncytia in Cos-7 cells. The A mutations of residues N98, T99, and E100 all promoted significant levels of fusion (Fig. 1C and Fig. 2) and were not considered further.

Since the fusion deficiency resulting from dimer interface mutations in the globular domain correlates with an apparent disruption of a second sialic acid binding site, detectable by unstable HAd at 37°C (23), we also measured HAd at this temperature. For all the fusion-deficient mutants, HAd relative to that of the wt protein was actually more efficient at the higher temperature than it was in the cold (data not shown). Thus, we conclude that the diminished fusion promoted by these mutants is not due to an effect on either sialic acid binding site.

Having confirmed that three additional amino acid substitutions, R83T, L97A, and L97T, result in a specific decrease in fusion, we next determined the ability of the mutated proteins to take part in the complex with F. This was accomplished using a cell surface coimmunoprecipitation (co-IP)-Western blot assay (23). Briefly, wt or mutated HN was coexpressed on the surfaces of BHK-21F cells with a cleavage site mutant form of F (csmF), surface proteins were biotinylated, the cells were lysed, and HN was immunoprecipitated through its interaction with csmF using an F-specific MAb and detected by Western blotting using an MAb to a linear epitope in HN (21).

Figure 3 shows that the R83T substitution also markedly diminishes the ability of HN to interact with F (Fig. 3). The amount of co-IP is comparable to that of the negative control, A89Q-mutated HN (8). L97T-mutated HN coimmunoprecipitates more efficiently than either of these mutated proteins but still at a lower level than wt HN. However, L97A-mutated HN interacts with the F protein to an extent comparable to that of wt HN (Fig. 3). Efficient expression of HN and F was verified in each sample (Fig. 3).

Taken together, these findings indicate that L97A-mutated HN associates efficiently with F but does not trigger it to promote
forms intracellularly prior to receptor binding, the NDV HN-F complex. This is significant, as it is thought that while the MV H-F complex plays an active role in fusion triggering, it may make it possible to capture an intermediate in the fusion-triggering cascade that could be useful in understanding the structure of the NDV-HN F complex formation but prevents the complex dissociation subsequent to HN-F complex formation and may act by preventing the rearrangements in HN necessary for fusion triggering. This mutation is analogous to the I98A mutation in the stalk of the measles virus (MV) hemagglutinin (H), which also blocks fusion triggering and increases the avidity of the H-F complex (24, 25). This is significant, as it is thought that while the MV H-F complex forms intracellularly prior to receptor binding, the NDV HN-F complex appears to initially form at the cell surface upon receptor binding (reviewed in reference 9). The identification of a trigger that acts similarly in both viral attachment proteins indicates a commonality in paramyxovirus fusion triggering models in the two viruses and is consistent with the stalk domains of both MV H and NDV HN playing an active role in fusion triggering.

Finally, whereas I97A-mutated NDV HN appears to allow HN-F complex formation but prevents the complex dissociation that would presumably be integral to fusion triggering, it may make it possible to capture an intermediate in the fusion-triggering cascade that could be useful in understanding the structure of the HN-F complex.

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**REFERENCES**


22. Tappert M, Smith DF, Air GM. 2011. Fixation of oligosaccharides to a surface may increase the susceptibility to human parainfluenza virus 1, 2 or 3 hemagglutinin-neuraminidase. J. Virol. 85:12146–12159.

