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The ectodomain of rabies virus glycoprotein determines dendritic cell activation

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ABSTRACT

The immune evasion of wild-type (wt) rabies virus (RABV) has been attributed to its glycoprotein (G), particularly to their inefficiency to bind/enter into dendritic cells (DCs). However, the domain responsible for G-mediated DC activation is not clear. In the present study, attempts were made to map the domain(s) on the G involved in differential DC activation using laboratory-adapted and wt viruses. Recombinant RABVs with exchange in each of the structural domains such as signal peptide (sp), ectodomain (et), transmembrane domain (tm), cytoplasmic tail (ct) of the G between wt and laboratory-adapted strains were constructed. Characterizations of these recombinant RABVs show that the viruses containing the sp, tm and ct from the wt G are capable of growing in high titer by efficient cell-to-cell spread, similar to laboratory-adapted virus. On the other hand, recombinant virus containing the et domain from wt G was inefficient in cell-to-cell spread and grew in lower levels, similar to the wt RABV. Analysis of DC activation shows that viruses containing sp and tm from wt G are efficient in binding to and activating DCs. However, viruses containing the et domain from wt G are incompetent in binding to and activating DCs. Analysis of the G expression in the infected cells suggests that the level of G expression is regulated solely by the ct domain, indicating the level of G expression and DC activation are governed by different domains. Together, our results demonstrate that G-mediated DC activation is regulated by the et domain while the level of G expression by the ct domain.

Rabies virus (RABV), a member of the genus Lyssavirus in the family of Rhabdoviridae, is a neurotropic virus that causes fatal encephalitis in warm-blooded animals (Jackson, 2003). RABV has a non-segmented and negative-sense RNA genome which is approximately 12 kb in length, comprising five genes that encode the nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and RNA-dependent RNA polymerase (RdRp, also termed large protein, L) (Wunner et al., 1988). RABV G is the only surface protein of the virion. RABV can enter from a peripheral site into the nervous system through the G by interacting with host cell receptors (Lentz et al., 1982; Thoulouze et al., 1998). G is also the only antigen that induces virus-neutralizing antibodies (VNA) (Dietzschold et al., 1978; Johnson et al., 2010). The G protein encodes 524 amino acids (aa) with the first 19 aa at the 5' end as the signal peptide (sp) which auto-cleaves from the G protein precursor. The mature G is comprised of the ectodomain (et, 1–439 aa), the transmembrane domain (tm, 440–461 aa) and the cytoplasmic tail (ct, 462–505 aa) (Kuzmina et al., 2013; Wunner et al., 1988). Wild type (wt) and laboratory-adapted RABVs differ greatly in the level of G expression and cell tropism, wt RABV expresses less G protein than laboratory-adapted viruses (Morimoto et al., 1999).

It has long been known that most rabid patients do not develop VNA at the time of death (Hemachudha, 1994) and this has been confirmed in animals such as skunks (Tolson et al., 1988), dogs (Gnanadurai et al., 2013) and mice (Wang et al., 2011) after experimental infection with wt RABV. However, infection with laboratory-adapted RABV can induce high level of VNA (Li et al., 2012; Rupprecht et al., 2005; Wen et al., 2011; Zhao et al., 2010; Zhou et al., 2013). Thus, wt RABV evades the immune responses by limiting the production of VNA. Further studies have indicated that it is the G that determines the level of G expression (Zhang

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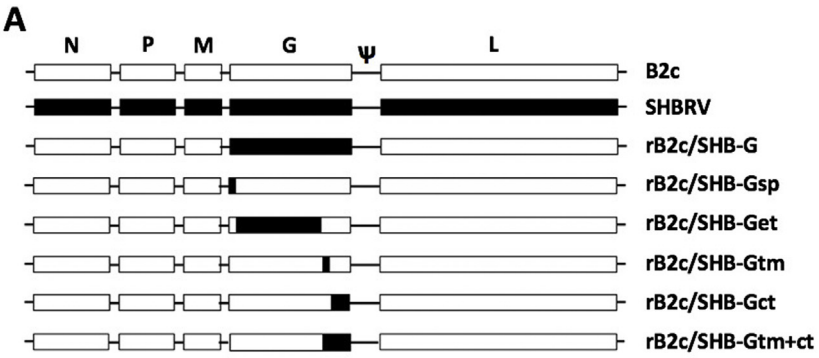

B

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B2c     MVPQVLLFVL LLGFSLCFGK FPIYTIPDKL GPWSPIDIHH LRCPNNLVVE DEGCINLSGF SYMELKVGYI SAIKVNGFTC
SHBRV   .I..A....P ..IP...L.. .......... .......... .S........ ....TS.... .......... ..M.......

B2c     TGVVTEAETY TNFVGYVTTT FKRKHFRPTP DACRAAYNWK MAGDPRYEES LQNPYPDYHW LRTVRTTKES LIIISPSVTD
SHBRV   .......... .......... ........M. ......HD.. I.......D. .......... ....K..... .V......A.

B2c     LDPYDKSLHS RVFPGGKCSG ITVSSTYCST NHDYTIWMPE NPRPGTPCDI FTNSRGKRAS NGNKTCGFVD ERGLYKSLKG
SHBRV   .......... ....S...L. ........P. .........V EA.L..S... .......K.. K.GR..... ..........

B2c     ACRLKLCGVL GLRLMDGTWV AMQTSDETKW CSPDQLVNLH DFRSDEIEHL VVEELVKKRE ECLDTLESIM TTKSVSFRRL
SHBRV   ..K......P .....N.... SI....DI.. .P........ ..H....... .....I.... G...A..... ..........

B2c     SHLRKLVPGF GKAYTIFNKT LMEADAHYKS VRTWNEIIPS KGCLKVGGRC HPHVNGVFFN GIILGPDDRV LIPEMQSSLL
SHBRV   .......... ........N. .......... ......V... .......... ..P....... .......GN. ..........

B2c     RQHMELLESS VIPLMHPLAD PSTVFKEGDE AEDFVEVHLP DVYKQISGVD LGLPNWGKYV LMTAGAMIGL VLIFSLMTWC
SHBRV   Q......... .......... ......D... .......... ..H..V.D.. ....S....L ..S...LAT. I.AIF.I.C.

B2c     RRANRPESKQ RSFGGTGGNV SVTSQSGKVI PSWESYKSGG EIRL
SHBRV   .....TK.T. .GHRES..K. ..AP.N..I. S...L...ES .TGM
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