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Adaptability costs in immune escape variants of vesicular stomatitis virus

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Abstract

We have used vesicular stomatitis virus (VSV) to determine the cost of antiserum resistance during escape from a polyclonal immune response. Replication of VSV in the presence of polyclonal antiserum resulted in the selection of antibody-escape mutants, as shown by increased fitness in the presence of antiserum and by increased resistance to neutralization. However, resistance came at a cost of overall fitness loss in the BHK-21 host cells. Sequencing of the surface G glycoprotein showed that two to four mutations were fixed in each population, most of which mapped in the A1 and A2 antigenic sites. Selected resistant populations were passaged as large populations in BHK-21 cells under constant conditions, which would normally lead to fitness increases. Nevertheless, many of the populations showed little or no sign of recovery, although the resistant phenotype was maintained. These results suggest that while antiserum resistance can develop, it may come at a cost in fitness and further limitations in the adaptability of the populations.

Keywords: Fitness; Antibodies; Trade-off; Adaptability; RNA virus

1. Introduction

Error-prone RNA virus replication results in complex populations of closely related mutants known as quasispecies (Domingo et al., 1985; Holland et al., 1992). In addition, RNA viruses can replicate very fast and to very high titers, allowing rapid adaptation to changes in the environmental conditions (Domingo et al., 2001). This potential has been exploited to answer many basic questions of evolutionary processes using RNA viruses as models (Domingo et al., 2001; Elena and Lenski, 2003; Novella, 2003). One of the most significant forces that viruses infecting vertebrates have to face during replication is the immune response of the host. Viruses typically respond to this challenge by the accumulation of mutations that abrogate antibody recognition and allow escape. Escape variants become antigenic targets of new specificity and induce more antibodies, which constitute a new selective pressure and promote further mutation accumulation in the virus. Successive waves of host antibody response and virus escape represent an evolutionary race between host and virus. This race conforms to the Red Queen hypothesis (VanValen, 1973) in that "it takes all the running you can do to stay in the same place." Thus, the inability of the host to mount an effective immune response or inability of the virus to develop resistance may result in

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death or extinction. Among human pathogens, human immunodeficiency virus type 1 (HIV-1) and hepatitis C virus (HCV) are two well-known illustrations of this type of interplay between viruses and the immune system of individual hosts (Brander and Walker, 2003; Shimizu et al., 1994). At an inter-host level as in human populations antibody-driven evolution is best exemplified by influenza A virus (Webster, 1999).

Understanding antibody escape is of fundamental importance for the rational design of antiviral strategies. How can viruses escape from a polyclonal immune response, presumably targeted against multiple antigenic sites? What is the cost of antibody escape? We addressed these questions using a well-developed and very successful model of RNA virus evolution, vesicular stomatitis virus (VSV). The prototype of the Rhabdovirus family, VSV is an enveloped virus with a negative-sense single-stranded RNA genome of 11,161 nucleotides with five genes. The nucleocapsid is composed of the RNA and three viral proteins, the nucleoprotein (N) and the polymerase (L and P proteins), and it is bound to an external membrane envelope by the matrix (M) protein. The only external protein is the G glycoprotein, which is the main target for antibodies (Kelley et al., 1972). VSV has been one of the best the models to dissect the processes controlling gene expression in mononegavirales (Barr et al., 2002; De et al., 1997; Rose and Whitt, 2001). It has also proved a unique system to test general principles of population genetics, as well as specific topics of viral evolution (Domingo et al., 2001; Novella, 2003).

The antigenic structure of VSV Indiana serotype has been well characterized (Lefrancois and Lyles, 1982a, 1982b; Nagata et al., 1992; VandePol et al., 1986; Volk et al., 1982) and comprises four or five antigenic sites. Earlier work demonstrated the ability of VSV populations to escape polyclonal antibodies and described the molecular basis of escape (VandePol et al., 1986). However, the effect of escape selection on viral replicative fitness was not determined. To answer this question we allowed extensive replication of VSV populations in the presence of rabbit polyclonal antiserum and characterized the evolved progeny. Our results showed that resistance developed quickly, but at a fitness cost and at an additional cost in adaptability.

2. Materials and methods

2.1. Cells and viruses

BHK-21 cells and wild type VSV Indiana serotype (Mudd–Summers strain) were used in this study. Fitness assays were done using monoclonal antibody resistant mutant (MARM) U as neutral reference virus. Methods for cell culture and virus replication have been previously described in detail (Duarte et al., 1994; Holland et al., 1991).

2.2. Immunization of rabbits and neutralization assays

Two New Zealand rabbits (labeled 160 and 161) were immunized with 100 μg of VSV in complete Freud's adjuvant. Animals were boosted three and 6 weeks after the first inoculation and bled after the second boost. Antisera obtained from bleeding were lyophilized, and resuspended in deionized water prior to use. Neutralization titers were determined by mixing one volume containing 2×10^4 infectious particles with one volume of antiserum at several dilutions and incubating the mixture at 37 °C for 30 min. After incubation virus was titrated by plaque assay on BHK-21 monolayers. Antiserum neutralization titers are expressed as the concentration of serum causing 99% inhibition compared to a control incubated with no serum.

2.3. Virus passages and fitness assays

A wild type VSV stock was diluted to 4×10^5 PFU/ml and mixed with an equal volume of antisera at a concentration causing 99% reduction of virus titer. The appropriate dilutions were 0.45×10^{-4} for antiserum 160 and 0.55×10^{-4} for antiserum 161, and this treatment resulted in a virus population size of 2×10^3 PFU at each passage. After incubation for 30 min at 37 °C, mixtures were used to start six passage series (labeled 160A–160F or 161A–161F) for each antiserum by infection of six BHK-21 monolayers as described (Holland et al., 1991). A lower concentration of antiserum (0.5×10^{-5}) was added to the overlay medium to keep the selective pressure during replication. After complete cytopathic effect (24–48 h), the progeny virus was diluted, mixed with antiviral serum, and used for a second passage, and this process was repeated until 30 passages were reached. As virus resistant mutants appeared, antiserum concentration was gradually increased to keep population size constant. Virus populations were labeled with the number of the antiserum used followed by the letter of the series. For example, 160A corresponds to series A of virus passaged 30 times in the presence of antiserum 160. Six control series were passaged in parallel in the absence of anti-VSV serum; in these infections the virus was further diluted to reach a similar population size per passage (i.e. 2000 PFU) than in the passages in the presence of anti-VSV serum. These were labeled A through F. Four of the populations resulting after 30 passages in the presence of each antiserum were further passsaged. Two populations selected in the presence of each antiserum were randomly picked and passaged ten times in the presence of a constant antiserum concentration (the same as was employed for the 30th passage) and in the absence of antiserum. These were populations 160B and 160C, which were further passaged in the presence of a constant concentration of antiserum 160 or in its absence, and populations 161C and 161E, which were passaged in the presence of a constant concentration of antiserum 161 or in its absence. In some cases, after antiserum treatment, the virus still had to be diluted to keep the population size constant. These populations were labled