RNA Virus Quasispecies Populations Can Suppress Vastly Superior Mutant Progeny

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A variant clone of vesicular stomatitis virus recovered from a high-passage, evolving virus population replicated rapidly and produced remarkably high yields of virus, but these variants never dominated during further passages. We show that this clone is highly competitive, but it can overwhelm its progenitor population only when seeded above threshold level during dilute passages.

RNA viruses in general exhibit high mutation frequencies, but some lower frequencies have also been reported (2, 4-12, 16, 18, 28-30, 32, 34, 38, 41; J. Coffin, in E. Kurstak, R. G. Marusyk, F. A. Murphy, and M. H. V. Van Regenmortel, ed., Applied Virology Research, in press). These high mutation frequencies produce heterogeneous quasispecies populations even in virus clones (4, 6, 7, 10, 16, 18, 20, 32-34, 36, 39; Coffin, in press). This facilitates but does not neces-

FIG. 1. Comparison of replication kinetics and relative competitive abilities of clonal virus populations 1 and 4 and their corresponding clone 1 MAR and clone 4 MAR populations in BHK21 cells in culture at 33°C. (A) One-step growth curves of clone 1 and clone 1 MAR. Symbols: ●, clone 1; ○, clone 1 MAR; △, tsG31. (B) One-step growth curves of clone 4 and clone 4 MAR. Symbols: ●, clone 4; ○, clone 4 MAR; △, tsG31. (C and D) Competition experiments between clone 1 and clone 1 MAR (panel C), and clone 4 and clone 4 MAR (panel D). Data for competition between clone 1 MAR and tsG31 and between clone 4 MAR and tsG31 are included for comparison. The different competitors are indicated by letters as follows: clone 1 and clone 1 MAR at low (a) and high (b) multiplicity, and clone 1 MAR and tsG31 at low (c) and high (d) multiplicity (panel C); clone 4 and clone 4 MAR at low (a) and high (b) multiplicity, and clone 4 MAR and tsG31 at low (c) and high (d) multiplicity (panel D). Selection of clone 1 MAR and clone 4 MAR populations with monoclonal antibody 11 was done as previously described for other MAR variants (40). During the competition experiments, the fraction at each passage of MAR mutant resistant to 11 was determined by using agarose overlays containing 11 to avoid underestimates due to phenotypic masking and/or mixing (18). Low-multiplicity passages for competition studies were done after 10⁴-fold dilutions of virus from the previous passage; high-multiplicity passages used undiluted inoculum from the previous passage of virus in BHK21 cells.

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prolonged genetic stability (35, 37) when passaged under conditions (such as dilute passages) which promote relative population equilibrium (i.e., prolonged dominance of high-fitness “master sequences”). Very little is known of the virus population dynamics by which complex quasispecies (11) populations evolve or maintain equilibrium.

During serial undiluted (high-multiplicity) passages at 33°C, vesicular stomatitis virus undergoes rapid and continuous evolution (35). We recently sampled the quasispecies population of genomes present at passage 254 by isolating a number of clones (37). Two of these, clone 1 and clone 4, exhibited superior fitness relative to their original progenitor virus, tsG31, and both were present as a rather low proportion of the population at passage 254 (37). However, clone 1 exhibited remarkably high yields and very rapid one-step growth curves compared with tsG31, wild-type vesicular stomatitis virus, or clone 4 (37). To study the population dynamics of these two clones, we marked them with selectable genetic traits by picking monoclonal antibody-resistant (MAR) clones of each by using the 11 monoclonal antibody (24, 40). The introduction of this genetic marker did not greatly alter the yields of clone 1 MAR (ca. \(10^{11}\) PFU/ml and 20 particles per PFU) or of clone 4 MAR (ca. \(2 \times 10^9\) PFU/ml and 80 particles per PFU). Figure 1A and B compare the replication kinetics (in one-step growth experiments) of clone 1, clone 1 MAR, clone 4, clone 4 MAR, and their progenitor, tsG31. Clearly, the rate and extent of replication of clone 1 and clone 1 MAR exceed those of clone 4 and clone 4 MAR (which only slightly exceeds tsG31 in replication efficiency). The monoclonal antibody resistance marker on clone 1 MAR did not alter its competitive ability (Fig. 1C). When mixed with clone 1 at a ratio of approximately 0.5, clone 1 MAR maintained this starting fraction throughout nine dilute (a) or undilute (b) passages in BHK21 cells. In competition studies with tsG31, both clone 1 and clone 1 MAR quickly dominated the population by the third passage (c and d). Figure 1D shows similar results for clone 4 and clone 4 MAR.

Having demonstrated that clone 1 MAR and clone 4 MAR...
replicate and compete similarly to their unmarked progenitor clones, we next analyzed their ability to compete with each other. Clone 1 rapidly outcompeted clone 4 (Fig. 2). This occurred whether the MAR marker was on clone 4 (a and b) or clone 1 (c and d) and occurred during both dilute and undiluted passage series. This was the expected result because clone 1 produces very large yields and replicates more rapidly than clone 4 (Fig. 1). Finally, we examined the competitive characteristics of clone 1 MAR and clone 4 MAR with which they were seeded back into the complex quasispecies population from which they had been isolated after 254 serial undiluted passages in BHK21 cells (35, 37). Figure 3 shows the results obtained during serial passages in BHK21 cells after clone 1 MAR (A) or clone 4 MAR (B) was seeded into the total passage 254 virus population at starting fractions of 10⁻¹ or 10⁻³ of total virus PFU. The fraction of MAR mutant was measured under II monoclonal antibody in agarose overlays to circumvent phenotypic mixing (18) after both dilute and undiluted passages of virus. Clone 4 MAR was strongly outcompeted under all conditions (Fig. 3B) by the total passage 254 virus population. In contrast, the competition fate of clone 1 MAR depended upon the level at which it was initially seeded into the mixed virus population prior to the first passage in a dilute-passage series (Fig. 3A). With a starting fraction of 10⁻¹ (a) it rose to dominance by passage 5 and thereafter, but with a starting fraction of 10⁻³ (c) it was quickly outcompeted by its complex progenitor passage 254 virus population. Finally, even at a starting fraction of 10⁻¹, clone 1 MAR could not compete with its complex progenitor passage 254 virus (and defective interfering particle) population during undiluted passage series in BHK21 cells [Fig. 3A (b)].

The above results demonstrate that even virus variants of remarkable relative fitness may be hidden and suppressed within a complex quasispecies population of lower average relative fitness. The emergence of potentially dangerous new virus pathogens in nature may often be prevented by such population dynamics. Occasional emergence of novel pathogens may depend upon low-probability bottleneck transmissions in which the suppressed new variant is transmitted as a single particle or as one of several particles, thus exceeding a threshold limitation. Finally, such population dynamics may often confound efforts to correlate observed phenotypic effects (such as disease syndrome, tissue pathology, etc.) with single genome variants of defined sequence. The complex mixture of virus (and defective interfering particle) variants present in the passage 254 population (35, 37) cannot be analyzed completely. This requirement of thresholds for dominance (or even detection) dictates an element of uncertainty in virus sampling during outbreaks in nature.

This work was supported by Public Health Service grant AI 14627 from the National Institutes of Health. J.C.T. is a Fullbright Post-doctoral Fellow.

LITERATURE CITED

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