Causes of HIV diversity

The human immunodeficiency virus (HIV) is characterized by enormous genetic flexibility, which gives rise to drug resistance, escape from immune responses and failure of vaccination attempts. There is much discussion about the factors con- tributing to viral diversity in individual infections. It is clear that the high error rate of the reverse transcriptase1 and the high turnover rate of virus2 generate vast numbers of different virus mutants. The diversity of viral quasispecies, however, is shaped by a combination of mutation and selection forces. The main selective forces that have been proposed to drive HIV diversity are the immune response, cell tropism and random activation of infected cells.

To quantify selection pressures on the HIV quasispecies, we have analysed the synonymous (amino-acid preserving) and non-synonymous (amino-acid changing) nucleotide substitution pattern in the HIV-1 envelope gene of an infected haemophilic patient followed since seroconversion for 7 years3. We compared 67 non-identical plasmid-derived RNA sequences over a stretch of 251 bases, including the V3 loop and flanking regions. We derived the mean number of nucleotide substitutions per synonymous site, \( d_s \), and per non-synonymous site, \( d_a \), for all pairwise comparisons of sequences in the samples taken at years 3, 4, 5, 6 and 7 after infection. At year 7 these sequences were completely homogenized.

The figure shows \( d_s, d_a, d_s/d_a \) and the CD4 cell count for years 3, 4, 5, 6 and 7 after seroconversion. As expected, \( d_s \) increases with time, due to accumulation of synonymous substitutions. The low value of \( d_s \) at year 3 may indicate that a selective sweep occurred in the viral population shortly before this time. It is interesting that \( d_s \) initially exceeds \( d_a \) but thereafter increases much more slowly, if at all. At year 3 the \( d_s/d_a \) ratio is about 0.1, indicating strong positive selection for amino-acid change. As the infection progresses this selection pressure declines markedly. By year 7 the ratio is about 1.25, indicating weak negative selection against amino-acid change. The pattern of increase of \( d_s/d_a \) agrees well with the pattern of decrease in the CD4 cell count. Whenever the CD4 cell count decreases, the \( d_s/d_a \) ratio increases.

A varying selection pressure is compatible with the notion that the immune responses select for viral diversity. Early in the infection the immune system will respond strongly against common viral variants and hence favour rare mutants, thereby providing a strong positive selection pressure for diversification. As the immune system declines, this selection pressure should become weaker.

The pattern of decreasing posi- tive selection, however, is also consistent with the notion that most viral diversity in the V3 region is caused by adaptation for various tropisms4. Serocon- version, the patient carries a strongly homogenous virus pop- ulation (in the V3 region), that diversification occurs as HIV infects many different cell types and tissues in the human body. Initially this would provide strong-positive selection pressure which would decline when the virus has gener- ated many variants with specific cell tropisms.

The observed pattern rejects the hypotheses that HIV diversity in V3 are purely neutral or simply generated by random activ- ity of infected cell clones5. Such mechanisms would act equally on synonymous and non- synonymous substitutions and therefore predict a constant ratio of \( d_s/d_a \) during infection.

Phylogenetic analysis of the viral sequences reveals a division into two major lineages after year 3 (see ref. 4), which correspond to preferentially macrophage-tropic or T-cell-line-adapted phenotypes6. Comparison of the sequences in these two lineages (regard- less of the sampling time point) reveals that the \( d_s/d_a \) ratio is significantly lower in the T-cell-line-adapted sequences than in the macrophage-tropic sequences (0.59 ± 0.07 and 1.39 ± 0.87, respectively). This result was independently confirmed by analysing 177 additional V3 sequences from 133 different donors7. For this dataset we found \( d_s/d_a \) ratios of 0.52 ± 0.16 for the T-cell-line-adapted sequences and 1.34 ± 0.45 for the macrophage-tropic sequences.

This result can be explained if we assume that the immune response acts more strongly against the T-cell-line- adapted variant8. In the context of the cell-tropism hypothesis, we would have to assume that T-cell-line-adapted variants evolve a larger variety of cell tropisms, which seems unlikely. Therefore, it is plausible that immune selection has a greater effect on V3 diversity than cell tropism (but the two factors may be inter- linked).

This is only the patient studied so far in sufficient detail to provide estimates of \( d_s/d_a \) ratios (with reasonably small stan- dard deviations). More data of this kind are urgently need- ed if we are to understand the evolution of HIV in individual infections and its conse- sequences for pathogenesis.

Sebastian Schaller
Edward C. Holmes
Artur M. Nowak
Department of Zoology
University of Oxford,
South Parks Road
Oxford OX1 3PS, UK

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