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Analysis of 'driver' and 'passenger' CD8$^+$ T-cell responses against variable viruses

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Variable viruses, such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV), persist despite host immune responses directed against them. Numerous lines of evidence have suggested that antiviral CD8$^+$ T-cell responses are key among these immune responses, but these vary widely in their ability to contain virus. We propose that only a proportion of responses may exert significant antiviral pressure ('driver' responses), leading to control over viral replication (protection) and/or, ultimately, selection of escape mutants. Another set of responses may exert only weak pressure on the virus ('passenger' responses): these neither protect nor select. To examine this we have analysed (using established databases of HIV and HCV sequences and cytotoxic T-lymphocyte (CTL) epitopes, and published experimental datasets) two important features —predicted binding of the epitope to major histocompatibility complex molecule and observed variability of the epitope—that might distinguish such responses. We find that a high predicted binding estimate could only explain a limited set of 'driver' responses associated with protection or selection. There is statistical evidence that readily defined (and non-protective) CTL responses target regions associated with lower levels of viral variability. Taken together, this suggests that a large number of well-documented responses may represent 'passengers' and we propose a mechanism that might explain their presence.

Keywords: human immunodeficiency virus; hepatitis C virus; immune escape; CD8$^+$ T cells

1. INTRODUCTION

Despite nearly two decades of investigation, we still lack a human immunodeficiency virus (HIV) vaccine and many basic questions about HIV pathogenesis remain unanswered. Much recent work has focused on the CD8$^+$ T-cell response as it is recognized that this plays a major role overall in antiviral defence (McMichael *et al.* 2000).

The emergence of cytotoxic T-lymphocyte (CTL) escape mutations in many studies is testament to the important selective force that CD8$^+$ T cells exert on the virus (Goulder *et al.* 1997). However, such studies also throw up important questions about the role of CD8$^+$ T cells in protection against disease progression overall: not all are associated with the generation of escape mutants. Are such CTL responses protective in the sense that the virus has been unable to generate suitable escape sequences and may thus remain under surveillance; or are they irrelevant because generation of escape mutants provides no selective advantage to the virus *in vivo*?

Recently we proposed a distinction between, on the one hand, 'driver' responses and, on the other hand, 'passenger' responses (Klenerman *et al.* 2002). The former exert a significant antiviral effect that might lead to protection or selection. The latter exert little antiviral effect on the virus and do not lead to control or selection of escape mutants. In this study we have addressed the following hypotheses.

(i) 'Driver' responses may be distinguished by strong binding of peptide for the major histocompatibility complex (MHC). In this case, highly sensitive T cells may potentially be available to exert significant antiviral pressure.
(ii) 'Driver' responses may be distinguished by targeting of regions that are of low variability. If such regions are under structural constraints, it may be that protection is more easily achieved.

We focused on three areas where 'driver' and 'passenger' responses have been most obviously identified: an analysis of human leucocyte antigen (HLA) association with protection (Carrington & O'Brien 2003); recent data in which evidence of HLA-associated selection was identified (Moore *et al.* 2002); and a recently reported case of superinfection in which certain responses appear to be 'passenger' responses (Altfeld *et al.* 2002).

Because many of the same arguments outlined above regarding the role of driver and passenger CTL apply to hepatitis C virus (HCV), we also performed an analysis of the CD8$^+$ T-cell responses in this disease.

2. METHODS

(a) *Analysis of cytotoxic T-lymphocyte epitopes and sequences*

To analyse a set of epitopes that was comprehensive and robust, we employed a consensus 'best-defined' list (Korber *et al.* 2001) and a set of sequences from clade B viruses (Kuiken *et al.* 2001). Predicted off-rate of peptide from the MHC molecule was analysed using a Web-based package (http://bimas.dcrt.nih.gov/molbio/hla_bind). In some cases, we cross-checked the consistency using an alternative program (http://syfpeithi.bmi-heidelberg.com/Scripts/MHCServer.dll/CheckEp.htm). Analysis of the variability was performed by comparison of conserved and mutated sites within the database described above, compared with clade B consensus sequences. We used for HCV analysis a recent review aimed at synthesizing the CTL data for HCV (sequence data compiled in Ward *et al.* 2002).

3. RESULTS

(a) *Hypothesis 1: analysis of predicted binding of 'driver' epitopes*

(i) *Protection*

We first analysed the association between predicted binding of well-described epitopes and HLA. (For a full listing, see electronic Appendix A, available on The Royal Society's Publications Web site.) Figure 1 reveals a clear outlier among epitopes restricted by HLA B27. The predicted off-rate of peptides binding this protective allele is significantly higher than that of all other peptides restricted by other HLA molecules ($p = 0.001$; Mann–Whitney test).

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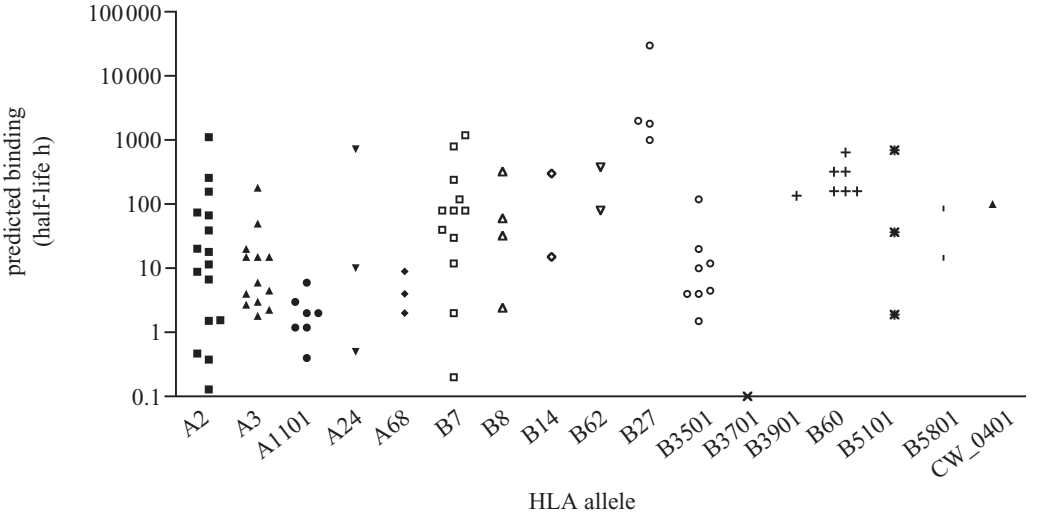

Figure 1. Predicted binding (described as 'bimas score') in relation to HLA alleles. The scale is log and based on the estimated half-life of dissociation generated by the Web-based program (see § 2): the marked difference between HLA B27 and other alleles is signficant by Mann–Whitney analysis ($p = 0.0007$).

(ii) *Selection*

To dissect this issue we used a recently published dataset in which selection had been analysed (Moore *et al.* 2002). In table 1 (in electronic Appendix A), we analysed the predicted binding of epitopes studied in this paper, comparing those that were associated with selection with those that were not. We found that epitopes that had predicted binding levels that were orders of magnitude lower than those of HLA B27-restricted epitopes were nevertheless associated with selection. Within HLA alleles, the differences between epitopes associated with selection and those that were not was not consistent.

A further analysis to address the question of whether responses can be distinguished was performed using a set of responses that are apparently 'passengers' *in vivo*. Altfeld *et al.* (2002) recently described a case of HIV superinfection in which there was loss of control over viral replication despite vigorous CTL responses. In a set of such epitopes, there was no sequence difference between the original and the superinfecting virus, and there was no selection of escape mutants *in vivo* following superinfection. Thus the CTL responses were present at high level but produced neither protection nor selection, thereby defining themselves as 'passengers'. Table 2 (in electronic Appendix A) shows the data for the peptides concerned: these show a wide range that is very similar to that shown in table 1 (in electronic Appendix A).

It seems apparent that 'driver' versus 'passenger' epitopes cannot be typically distinguished on the basis of predicted binding alone. We can therefore reject hypothesis 1 for the general case.

(b) *Hypothesis 2: 'driver' and 'passenger' responses may differ in the variability of the epitopes targeted*

Simple analysis of variability among the database epitopes revealed a wide range as for binding affinities, and which was not clearly associated with HLA (table 5 in electronic Appendix A). Only a limited set of peptides showed significant conservation (more than 90% identity between strains). Using the group of 'passenger' epitopes above (Altfeld *et al.* 2002), we specifically analysed the variability in this group (table 2 in electronic Appendix A). Interestingly, four out of seven of these showed greater than 90% conservation compared with 15 out of 88 of the remaining epitopes ($p = 0.016$, Fisher's test).

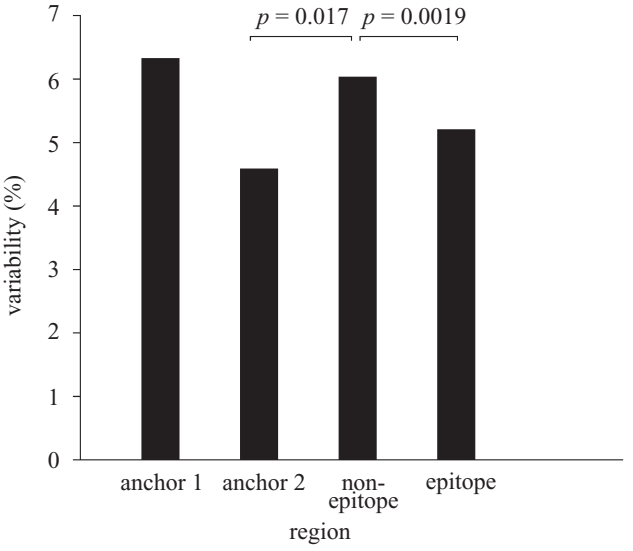

Figure 2. Variability of epitope and non-epitope regions. Analysis of variability was performed at the level of single sites within epitopes or outside epitopes (percentage of mutated residues among all residues in all strains analysed). Envelope gene products were excluded from this analysis to avoid confounding effects due to antibody-mediated selection pressure.

To extend this comparison, we analysed whether variation with epitopes differed overall from non-epitope regions of the virus. Figure 2 shows that, interestingly, the degree of variability among sequences falling within well-defined CTL epitopes is lower than that in the rest of the virus (Gp120 excluded; $p = 0.0019$; odds ratio 1.059–1.292). This suggests that overall the epitopes are associated with conservation of viral sequences rather than selection for escape. There was also evidence for specific

conservation of anchor residues: the C-terminal anchor of well-defined epitopes was found to be less variable than average sequences within the database ($p = 0.017$).

(**c**) ***Analysis of hepatitis C virus epitopes***

HCV is a variable virus where T-cell responses play potentially important roles in determining outcome. We used identical methodology for analysis of variation. We observed that there was a significantly lower level of variation in epitopes than in non-epitope regions (E1/E2 excluded; 0.25% versus 1.5%; $p = 0.006$).

4. DISCUSSION

The concept of 'driver' versus 'passenger' epitopes was laid out briefly in a previous manuscript (Klenerman *et al.* 2002) and is an attempt to distinguish CTL responses that have a significant antiviral effect (protection or selection) from those that do not. The aim of the current study is to find simple analytical tools that might distinguish these two. Several caveats must be mentioned: the peptide prediction programmes used may be imperfect, the mapping strategies used tend to rely on consensus sequences, and mutations in regions flanking the epitopes or compensatory mutations may also influence the recognition and selection. Dividing CTL epitopes into two camps is clearly an over-simplification. However, this is an attempt to look globally at the issue.

The data presented suggest that we cannot predict whether a response is likely to be a driver or passenger on the basis of expected peptide affinity/off-rate. There may be a special case for HLA B27, a clearly protective allele. HLA B27 has also generated the best examples of escape and it is likely that these specific responses are the best examples of 'driver' responses available (Goulder *et al.* 1997).

In terms of variability, the results are provocative in that there is an overall association between the presence of an epitope and relative conservation of sequence, especially at the C-terminal anchor. This finding, which is similar to a recently published observation, suggests that easily identified CTL are targeting regions of the virus that differ least from consensus sequences (Yusim *et al.* 2002). The fact that a group of non-protective 'passenger' epitopes showed extreme conservation is particularly striking. This finding adds weight to the argument that measurement of strong responses against conserved epitopes is not a marker of a necessarily effective CTL response *in vivo*.

It could be argued that the reason why such responses are non-protective or non-selective is due to lack of function. However, the responses in the superinfection study (Altfeld *et al.* 2002) were both functional and large. There may potentially be important differences between 'driver' and 'passenger' responses in terms of function, a feature which could be directly addressed experimentally, once they have been well defined within one individual.

The proportion of 'passenger' responses in any one individual's repertoire is still not clear, but the fact that they are there at all demands some explanation. One potential mechanism relies on the observation that the priming or reactivation of T cells may occur on uninfected dendritic cells (DCs). DCs that take up circulating antigen or dead infected cells can 'cross-prime' *in vivo* in several murine models (Carbone & Bevan 1990).

In this case there is an important discrepancy between the surface on which T cells are primed and that on which they act to protect or select. The DC surface is enriched for class I and co-stimulatory molecules and HIV-nef is also well known to downregulate class I on infected cells (Collins *et al.* 1998). Clearly, there is still sufficient recognition on the surface of an infected CD4$^+$ T cell to provide some T-cell triggering *in vivo*: however, this may not be equally efficient for all T cells.

The model therefore suggests that it may be relatively easier to 'cross-prime' or 'cross-present' epitopes on DCs than to recognize the same epitopes on an infected cell. Thus only a subset of primed cells may be efficient antiviral effector cells, which can then act as 'drivers'. For the others—'passengers'—they are markers of viral replication and their levels will be positively correlated with the amount of viral antigen to which they are exposed. This latter point may explain why it has been difficult to reach a consensus on the relationship between antiviral CTL and viral load, because different T-cell populations have a different relationship.

The model may also be relevant to other persistent virus infections where the sites of priming and the sites of effector activity are different. In particular, in the case of HCV or hepatitis B virus, the level of class I on a normal hepatocyte is very low and this might exaggerate any differences seen between priming efficiency and antiviral efficiency (Willberg *et al.* 2003).

To test the model, analysis of recognition of DCs, cross-presenting viral peptides could be performed side-by-side with analysis of recognition of CD4$^+$ T cells using a range of defined 'driver' or 'passenger' responses. If differences are found then it could provide a useful method to distinguish driver from passenger responses. Defining this further will certainly improve our understanding of immune escape and the pathogenesis of HIV, but may also help to design vaccines that generate 'protective' CTL.

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