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REVIEW

Cellular immune responses against hepatitis C virus: the evidence base 2002

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SUMMARY

Hepatitis C virus (HCV) is an RNA virus which is estimated to persistently infect about 170 million people worldwide. After acute infection, there is an initial period during which long-term outcome is decided. There is strong evidence that the cellular immune responses, involving both $CD4^+$ and $CD8^+$ T lymphocytes, are involved at this stage and it is their effectiveness which determines outcome. What is not understood is what determines their effectiveness. The most important component of this is likely to be some aspect of epitope selection, itself dictated by host MHC. Thus, to understand host immunity to HCV, we need to have a detailed understanding of the peptides involved in T lymphocyte responses. In this review, we discuss the peptide epitopes that have been identified so far, and their potential significance. We relate this to a scheme of host defence which may be useful for understanding natural and vaccine-induced immunity.

Keywords HCV $CD8^+$ T lymphocyte $CD4^+$ T lymphocyte HLA epitope immune escape

INTRODUCTION

The virus HCV is a member of the flavivirus family – positive stranded RNA viruses, which include relatives such as viruses causing dengue and yellow fever. Its origin is obscure but certain strains have probably been circulating in human populations for hundreds of years [1]. Unlike many of its relatives, it is able to set up persistent infections. This, coupled with lifestyle changes and a number of iatrogenic disasters, have led to its recent spread worldwide. In the west it is most common amongst IV drug users (both current and those who may have given up decades ago), as well as recipients of blood products in the prescreening era. In some countries, such as Egypt, it appears to have been spread through needles used for medical programs and high rates in other countries may exist for similar reasons [2].

HCV replicates mainly in the liver. Some negative strand RNA, indicative of intracellular replication, has been found in dendritic cells (DCs) [3]. Even in those who clear virus from the blood (spontaneously or after treatment), there is a possibility that some viral RNA persists in the liver – similar to HBV. Direct detection of HCV is currently only possible by PCR, as culture methods from *ex vivo* samples are not routinely successful, even though *in vitro* 'replicon' (self-replicating RNA constructs) have been established [4].

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Clinical features

After inoculation, unlike Hepatitis A and B, the acute illness caused by HCV is not well documented. This is partly because it is genuinely milder, and possibly it is poorly recognized by physicians, and also those in the current western risk groups may not present to hospital. This is unfortunate, as it now appears that early intervention is of benefit [5]. When it has been documented, or in animal models, the following features are apparent [reviewed in 2]:

- The peak of viraemia may take several weeks to arise [6,7]. The liver inflammation/liver enzyme level in the blood (usually measured as ALT or AST) does not parallel the viral load – consistent with the idea that at this stage much of the liver damage is not caused directly by the virus.
- Resolution of the viraemia (accompanied by cellular immune responses) is associated with liver inflammation and in some but not all cases, clinical jaundice. The level of ALT may be 500–2000 IU/l, compared with HBV where it may be 5 or 10 times higher [8–10].
- After this period, viraemia either persists or the individual, in about 15% of cases, becomes RNA negative in the blood. There may be a state of 'instability' where virus may become undetectable in blood temporarily and then reappears [11].
- If viraemia is established, the level of viraemia does not correlate with progression of disease, unlike HIV. Disease progression is measured by the development of liver inflammation, as assessed by blood ALT and histological indices of lymphocytic infiltration and also by creeping fibrosis. The extent of these vary widely between individuals, and apart from a few factors

such as alcohol and coinfection (for example with HIV), the basis of this variation is not understood.
- In those where disease has progressed, therapy (in the form of interferon-alpha and ribavirin) may lead to long-term clearance of virus from blood, with accompanying improvement in liver histology. The effects of the drugs are not entirely understood, and it is likely that in addition to antiviral activity they influence the immune response, both directly, and indirectly through lowering viral load [12].

Cellular immunology of HCV

HCV, like other viruses, induces multiple immune effector responses, but this review focuses primarily on T lymphocytes. There is good evidence that both $CD4^+$ and $CD8^+$ T lymphocytes play a major role in determining outcome after acute infection and therefore in the long term. This comes from the following observations.

- Clearance of acute infection in both man and in chimpanzee models is accompanied by strong $CD4^+$ and $CD8^+$ T cell responses against numerous HCV derived antigens [11,13–16]. The evidence was obtained first for $CD4^+$ T cell responses and initially some specific epitopes were highlighted as potentially 'protective' [13]. Although these do appear to be targeted, this is not exclusive and responses to other gene products are also seen [17]. The strength of the $CD8^+$ T cell response against one epitope when measured using a tetramer, may be up to 8% of the total $CD8^+$ T cells, and can include responses to at least 8 separate epitopes [10]. By ELISpot analysis, the $CD4^+$ T cell responses appear to be of a similar magnitude [10].
- The timing of these responses appears to correlate with resolution of viraemia in those cases where virus is cleared. The level of activation of HCV-specific T cell responses (assessed by CD38 expression) correlates with the degree of liver inflammation analysed by blood ALT levels [9].
- There is an association between possession of specific HLA genes (DRB1*1101 and/or DQ1*0301) and spontaneous clearance of virus [18]. This strongly suggests that selection of particular epitopes is associated with better initial control of viraemia. Those bearing HLA DQ1*0301 (which is in tight linkage disequilibrium with DRB1*1101) were found to be more likely to possess significant HCV-specific $CD4^+$ T cell responses, further evidence that the responses in these individuals are more robust [19].

So much for successful responses – which are in fact the exception. The mechanism for viral persistence, i.e. failure of T cell responses in the majority of patients, is not yet clear. Studies of those who go on to develop persistent infection have highlighted the weak $CD4^+$ T cell responses, although it is not clear yet whether persistence of virus causes attenuation of T cell responses or vice versa [11,19,20]. Re-emergence of $CD4^+$ T cell responses upon clearance of virus with interferon-alpha/ribavirin therapy suggests the latter –, i.e. suppression of T cells by virus may be important [12].

The picture with regards to $CD8^+$ T cell responses is even less clear. $CD8^+$ T cell responses have been observed in the acute phase of infection in those who fail to clear virus at levels of 1–3% of $CD8^+$ lymphocytes against 1–2 separate T cell epitopes [7,9]. Whether these are the main epitopes targeted in these individuals and how, overall, the responses differ in magnitude between clearers and nonclearers is not known. It appears that failure to clear virus is not due to failure to mount any CTL response whatsoever, although, like $CD4^+$ T cell responses, these may be poorly maintained in the face of ongoing viraemia [9]. The overall quality of the response may differ in terms of magnitude or breadth – or, importantly, peptide selection. It is this latter issue that forms the focus of this review.

Peptide epitopes in HCV

The assessment of the exact epitopes used in an individual's HCV-specific response is likely to be crucial in understanding the overall role of $CD8^+$ and $CD4^+$ T cells in control of disease and in approaching vaccine design. There are two broad reasons for this – one biological (i.e. it affects outcome, see below) and one pragmatic.

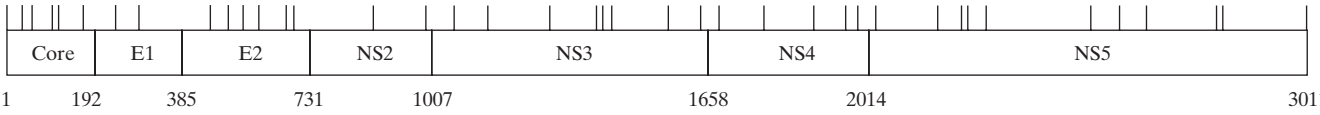
On the pragmatic level, too many studies have focused on too few epitopes. Even those using a larger number of epitopes have tended to limit themselves to those restricted by HLA-A2. There is clear evidence from detailed studies of individual patients in acute and chronic disease that this approach misses many responses [21]. This means that it becomes difficult to draw conclusions as to the overall role of CTL responses from clinicopathological studies – as we have not obtained in each individual an accurate assessment of their magnitude. As an example, a single 'clearer' who made 8 separate CTL responses made only one to a previously mapped epitope (restricted by HLA-A2), and even this was not the dominant HLA-A2 restricted response [10], and similar findings are obtained in studies of those persistently infected [21]. This is similar to the situation in HIV where an epitope in p17 gag (SLYNTVATL) has been found to be frequently targeted in HLA-A2 positive individuals, but during acute disease, it is rarely seen – other epitopes are likely to be much more important at this critical stage [22].

Thus, it is likely that there are, as for HIV, a reasonably large number of epitopes available for targeting by any individual, dependent on their HLA type, and it is not possible to assess the complete response using only a restricted number of peptides. It may not be possible in each case to map the entire array of responses, as this is labour intensive and expensive in terms of peptides, as well as being hampered by the relatively weak responses seen in many patients. Therefore, what is minimally required, is a 'customised' mapping for each patient using peptides based on the full HLA type and viral genotype. This is not perfect, but should improve the yield of responses obtained and provide a better sense of the breadth and relative importance of the T lymphocyte response. The process should improve over time as individual peptides are mapped and confirmed between groups, provided a clear and up-to-date database is available.

HCV-specific epitopes – the evidence base

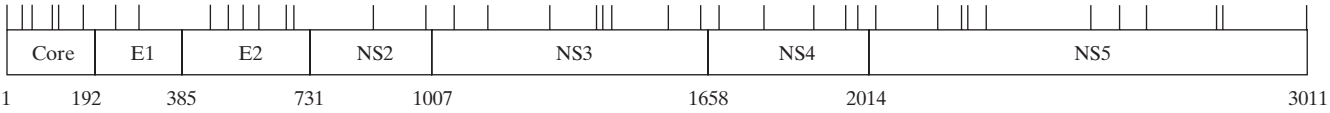
We have compiled a table of HLA Class I-restricted HCV epitopes to enable researchers in the field to establish panels of peptides suitable for their patients – and thus address the issue of the role of HCV-specific T cells comprehensively (Table 1). Table 1a shows the currently published peptides restricted by HLA-A2. The figure above the table illustrates the position of these epitopes, which are spread throughout the genome without any obvious clustering. Many of these were generated by computer predictions and have been subsequently validated in patients. However, many have also not been observed frequently or using *ex vivo* assays. The use of *ex vivo* assays is relevant since responses which only appear after multiple restimulations may also be seen

Table 1. HCV CTL epitopes restricted by HLA-A2 (a) or other alleles (b). Amino acid positions and sequence are based on the HCV-1 isolate (Pubmed accession no. P26664). These peptides have been recognized by human PBMC's using cytolytic assays (technique A), and/or ELISPOT assays/intracellular cytokine staining (e.g. IFN-γ) (technique B) and/or tetramer staining (technique C). Epitopes for which the HLA restriction and the optimal peptide (by testing shorter and longer peptides in dilution rows) have been defined are typed in bold



Protein	Amino acid position	HLA-restriction	Amino acid sequence	Technique	Reference
(a) HCV CTL epitopes restricted by HLA-A2					
Core	35–44		YLLPRRGPRL	A,B	[20,23–34]
			YLLPSRGPKL	A	[32]
Core	90–98		GLGWVGWLL	A	[31]
Core	132–140		DLMGYIPLV	A,B,C	[23,24,29–31,34–39]
			ALMGYIPLV	A	[36]
			DLMGYIPAV	A	[36]
	131–140		ADLMGYIPLV	A,B	[20,25–28,40]
Core	156–165		RVLEDGVNYA	A	[31]
Core	177–185		FLLALLSCL	A	[31]
Core	178–187		LLALLSCLTV	A,B	[24–26,41]
			LLALLSCLTI	A,B	[20]
E1	220–227		ILHTPGCV	A,B	[38,40]
E1	257–266		QLRRHIDLLV	A,B	[27,28,40]
			AIRRHVDLLV	B	[20]
E1	285–293		FLVSQLFTF	B	[39]
E1	363–371		SMVGNWAKV	A,B	[38,40]
E1	398–407		SLASLFTQGA	A	[42]
E2	401–411		SLLAPGAKQNV	A,B	[38,40,41]
E2	614–622		RLWHYPCTV	A,B	[39]
E2	686–694		ALSTGLIHL	A	[23]
E2	723–732		LLFLLLADA	A,B	[29,34]
E2	725–734		FLLLADARV	A,B	[23,29,30,34]
NS3	1073–1081		**CINGVCWTV**	A,B,C	[9,10,21,25–28,32,33,40,41,43–47]
			CVNGVCWTV	A,B	[20]
			CVVGVCWTA	A	[32]
NS3	1131–1139		YLVTRHADV	A,B	[29,34]
NS3	1169–1177		LLCPAGHAV	A	[25,27,28,40]
			LLCPSGHVV	A	[20]
NS3	1287–1296		TGAPVTYSTY	A	[48]
NS3	1406–1415		**KLVALGINAV**	A,B,C	[9,25–28,32,33,40,41,43,47,49]
			KLSGLGINAV	B	[7]
			KLVSLGVNAV	A	[32]
			KLSGLGLNAV	A	[32]
			KLVALGVNAV	A	[32]
NS3	1585–1593		YLVAYQATV	A,B	[23,30,34]
NS4	1661–1669		VLVGGVLAA	A,B	[29,34]
NS4	1666–1675		VLAALAAYCL	A	[30,41]
NS4	1769–1777		HMWNFISGI	A,B	[23,29,30,34]
NS4	1789–1797		SLMAFTAAV	A,B	[25,27,28,40]
			SLMAFTASV	A	[20]
NS4	1807–1816		LLFNILGGWV	A,B,C	[9,20,23–30,32,34,35,40]
			VFFNILGGWV	A	[32]
NS4	1851–1859		ILAGYGAGV	A,B,C	[20,23,24,27–30,34,40]
NS4	1915–23		WMNRLIAFA	B	[29]
NS4	1987–95		VLDSFKTWL	A	[41]
NS5	2141–2149		LLREEVSFRV	A	[41]
NS5	2221–2231		SPDAELIEANL	A	[41,46]
NS5	2252–2260		ILDSFDPLV	A,B	[25,26,40]
NS5	2578–2587		RLIVFPDLGV	B	[34,40]
NS5	2594–2602		ALYDVVTKL	A,B,C	[10,20,41]
NS5	2727–2735		GLQDCTMLV	A,B	[24,27,28,32,33,40]
			GLQDCTMFV	A	[20]

Table 1. (*Continued*)



Protein	Amino acid position	HLA-restriction	Amino acid sequence	Technique	Reference
(b) HCV CTL epitopes restricted by other alleles					
Core	2–9	A11	STNPKPQK	A	[26,50]
	1–9		MSTNPKPQK	A	[21]
Core	28–36	B60	GQIVGGVYL	A	[51]
Core	41–49	B7	GPRLGVRAT	A,B	[21,40,44,45,50]
Core	43–51	A3	RLGVRATRK	A,B	[34,52]
Core	51–59	A3	KTSERSQPR	A,B	[34,52]
Core	88–96	B44	NEGCGWMGW	A	[53–56]
Core	111–119	B7	DPRRRSRNL	B	[40]
Core	169–177	B7	LPGCSFSIF	A	[52]
E1	234–242	B35	NASRCWVAM	A	[21,45,57]
E1	289–297	A3	QLFTFSPRR	A,B	[29,34,52]
E2	460–469	B53	RPLTDFDQGW	A	[21,44]
E2	489–496	B51	YPPKPCGI	A,B	[21,50]
E2	497–507	B35	VPASQVCGPVY	A	[58]
E2	530–539	B60	GENDTDVFVL	A	[46]
E2	569–578	B50	CVIGGAGNNT	A	[21,50]
E2	621–628	A11	TINYTIFK	A	[21,44–46]
E2	632–641	A3	RMYVGGVEHR	A,B	[29,34,52]
E2	654–662	B60	LEDRDRSEL	A	[46]
NS2	827–834	A29	MALTLSPY	A	[21]
NS2	831–840	A25	LSPYYKRYIS	A,B	[10]
NS2	838–846	A23	YISWCLWWL	A	[21]
	838–845		YISWCLWW	A	[44]
NS2	957–964	B37	RDWAHNGL	A	[49]
NS3	1031–1039	A24	AYSQQTRGL	A	[59]
NS3	1069–1077	B35	LPGCSFSIF	A	[58]
NS3	1100–1108	A24	MYTNVDQDL	A	[35]
NS3	1261–1270	A3	TLGFGAYMSK	A	[21,46]
	1262–1270		LGFGAYMSK	A,B	[29,34,52]
NS3	1359–1367	B35	HPNIEEVAL	A	[58]
NS3	1391–1399	A3	LIFCHSKKK	A,B	[29,34,52]
NS3	1395–1403	B8	HSKKKCDEL	A,B,C	[9,21,40,44–46]
NS3	1402–1410	B8	ELAAKLVGL	A	[49]
NS3	1531–1539	B35	TPAETTVRL	A	[58]
NS3	1611–1618	B8	LIRLKPTL	A	[46]
NS3	1636–1643	A11	TLTHPVTK	A	[21,46]
NS4	1744–1754	A25	EVIAPAVQTNW	A,B	[10]
NS4	1758–1766	A25	ETFWAKHMW	A,B	[10]
NS4	1858–1867	A3	GVAGALVAFK	A,B	[34,52]
	1859–1867		VAGALVAFK	A,B	[29,34,52]
NS4	1941–48	B38	AARVTAIL	A	[46]
NS4	1966–76	B37	SECTTPCSGSW	A,B	[10]
NS4	2000–2008	B35	LPKLPGVPF	A	[58]
NS5	2152–2160	B60	HEYPVGSQL	A	[46]
NS5	2161–2171	B35	PCEPEPDVAVL	A	[46]
	2163–2171		EPEPDVAVL	A	[58]
NS5	2218–2226	B38	NHDSPDAEL	A	[46]
NS5	2225–2233	A25	ELIEANLLW	A,B	[10]
NS5	2266–2275	B60	REISVPAEIL	A	[21]
NS5	2510–2518	A3	SLTPPHSAK	A	[21,46]
NS5	2588–2596	A3	RVCEKMALY	A	[21,44]
NS5	2629–2637	B57	KSKKTPMGF	A	[21]
NS5	2794–2802	A3	HDGAGKRVY	A	[26]
NS5	2794–2804	B38	HDGAGKRVYYL	A	[21,46]
NS5	2819–2828	A25	TARHTPVNSW	A,B	[10]
NS5	3003–3011	A31	VGIYLLPNR	A	[21]

in HCV negative individuals and their *in vivo* relevance is less concrete than those seen using direct *ex vivo* tetramer or ELISpot assays [25,45]. Thus, from this large panel of peptides only a handful are likely to be significant in terms of providing an 'anchor' for a successful T cell response. In our hands, three peptides, NS31073–81, NS3 1406–14, and NS5b 2594–2604, appear to be the most commonly recognized and have been identified using *ex vivo* assessments. The first of these may be cross-reactive with an epitope from influenza, although initiation of strong activated responses in several patients with acute disease suggest that the cells are HCV-specific *in vivo* [60].

Table 1b shows peptides derived from studies of other MHC restrictions. This list is proportionately much smaller considering the relative number of alleles involved and this reflects the effort spent in defining new epitopes in these patients, rather than the importance of such responses. It is not clear whether, unlike HLA-A2, one peptide may be immunodominant if a particular restriction element is present as for HLA-B27 in HIV [61]. Much more work needs to be done in this area before any strong conclusions can be drawn. Work using tetramers derived from HLA-B8 and -B7 restricted peptides has given similar results to A2-based tetramers, i.e. most persistently infected patients show weak or absent responses [9,62].

Table 2 shows the variability of some of these epitopes, dependent on viral genotype. Clearly, viral genotype and even subtype needs to be taken into account when identifying the appropriate peptide to use for study. This may be difficult in cases where virus has been cleared from the blood and also in situations, which may not be uncommon, where several viral strains may have been previously encountered. It should be stressed that these variations only apply to bulk sequences from different genotypes and within one individual, viral sequences may vary – potentially under CTL selection pressure.

All the epitopes illustrated do show significant capacity for variation, although the extent varies between epitopes. Thus, targeting of a completely conserved region which cannot mutate at all for reasons for structure or function, may not be an option in vaccine design. However, whether an epitope mutates or not depends on the intensity of CTL selection, its breadth, and crucially, the viral load. Therefore, the issue of epitope selection as a determinant of outcome may be very complex (see below).

These tables should be regarded as 'work in progress'. Such a table could never be used to fully predict the response of an individual patient, but it could potentially cover the most important responses, once comprehensive mapping has been performed in many more individuals. Further tables for $CD4^+$ T cell responses are also required, although beyond the scope of this review.

Success versus failure of T cell responses: the potential role of epitope selection

A simple model for the role of epitope selection in determining outcome in HCV is as follows (Fig. 1). After infection, viral replication takes place within the liver and at this stage is influenced by host innate immune responses, including interferon alpha and intrahepatic lymphocyte populations, such as NK and NKT cells [63,64]. Viral antigen is presented within the liver and in lymphoid organs. Induced responses include $CD4^+$ and $CD8^+$ T cells, the exact responses dictated by host MHC and incoming viral genotype. The magnitude of the responses depends not only on the exact epitopes targeted, but also on initial viral kinetics [65]. Induced T cell populations migrate to the liver, where – in concert with innate responses – they serve to reduce viral load (Positive loop in Fig. 1]. At the same time, viral replication – either through T cell 'exhaustion' [66], a tolerogenic effect of the liver environment [67] or through action of a specific viral gene product such as core, will tend to attenuate T cell responses. The phenomenon of temporarily reduced interferon gamma secretion in HCV-specific populations during the period of most intense activation ('stunning'), has been observed by two groups [7,9]. This may be related to the phenomenon of exhaustion as it is commonly seen in murine models in the 'pre-exhausted' phase, before responses appear altogether [68,69]. Although the exact mechanism is not clear, this can be observed even if $CD4^+$ T cell responses are intact, and it is likely that this is indicative of the 'negative' loop of Fig. 1. The outcome of this race – i.e which loop becomes dominant – depends on peptide epitopes in three ways (illustrated in Fig. 1b-d).

Epitope selection dictates the 'efficiency' of the responses within the liver, which in turn depends on the ability of T cells to recognize low levels of antigen in cells – ideally before they generate new virions [70]. Certain epitope(s) listed in Table 1, or yet to be discovered, may favour this process, whilst others, although providing adequate MHC binding and presentation to T cells, may be less efficient. Unlike HIV, there are no early regulatory genes, such as tat, which provide obvious targets in this respect, but there may be differences between gene products.

Epitope selection may determine how easily escape occurs at the level of an individual peptide. If escape occurs in variable viruses, it is highly epitope dependent, as is clear from detailed studies of HLA-B27-restricted epitopes in HIV [71–73]. Some of these epitopes may fall in regions that are 'constrained' or stable within the virus, due to conserved structure or function and multiple 'compensatory' mutations may need to accumulate, which does not occur readily. Thus, a clear understanding of the epitopes targeted and their escape 'potential' is needed – certain responses may be inherently more or less 'escapable'.

Epitope selection will dictate the breadth of the response, which crucially affects the ability of replicating virus to escape T cell responses. A response which is highly vigorous and efficient may rapidly lose its efficacy *in vivo* if viral load is not quickly contained, as this will generate escape mutation, which has been very clearly illustrated in the LCMV and SIV challenge systems [74,75]. How many effective epitopes need to be targeted is an important question, and one that is critical to vaccine design. In the LCMV model the answer appears to be three, although kinetics of this system are much faster than HCV [76].

Once escape has occurred, it is difficult for cellular responses to regain control due to the phenomena of T cell exhaustion (clonal deletion in the face of continuing viral loads) [66,77] and original sin (inability to generate new responses against emerging variants) [78]. Thus, once this situation is established, chronicity becomes inevitable [79].

CONCLUSIONS

We present here a discussion of the effectiveness of T cell responses in HCV. It is now clear that cellular immune responses play an important role in determining outcome, and what forms the important topic of interest now is why some responses are more effective than others. The main conclusion is that this issue cannot be satisfactorily addressed until more data is available on the exact peptides used by individuals making cellular immune

Table 2. HCV CTL epitope sequences. Dashes indicate identity with the model peptide sequence (HCV-1 isolate–PubMed P26664)

HLA Restriction (Location) Sequence	B7 (core 41–49) GPRLGVRAT	A11 (E2621–628) TINYTIFK	A2 (NS31073–1081) CINGVCWTV	B8 (NS31395–1403) HSKKKCDEL	A2 (NS31406–1415) KLVALGINAV	A2 (NS41807–1816) LLFNILGGWV	A2 (NS5B 2594–2602) ALYDVVTKL
P26664 (1a)	---------	--------	---------	---------	----------	----------	---------
AAB67038 (1a)	---------	--------	---------	---------	----------	----------	------S--
P27958 (1a)	---------	--------	---------	---------	----------	----------	------S--
P26663 (1b)	--------P	-V-F----	-V-------	---------	---SG-----	----------	------ST-
P26662 (1b)	---------	-V-F-V--	-V-------	---------	---TG-L---	----------	------ST-
Q00269 (1b)	--T------	-V-F----	-V-------	---------	---SG-----	----------	------ST-
P29846 (1b)	---------	-V-F----	---------	---------	---S---H--	----------	------ST-
BAA14035 (1b)	---------	---F----	-V-------	---------	--TG--L---	----------	------ST-
BAA09075 (1b)	---------	-V-F---T	-V-------	---------	--S---V---	----------	------ST-
CAA03854 (1b)	--------L	-V-F----	-V-------	---------	---SG-L---	----------	------ST-
CAB41951 (1b)	---------	-V-F----	-V-------	---------	Q-SS--V--	----------	------ST-
AAD50312 (1b)	---------	AV-F----	-V-------	---------	---SG-L--	----------	------ST-
BAB18814 (1b)	---------	-V-F----	-V-------	---------	--SS--L--	----------	------ST-
AAL00900 (1b)	---------	-V-F----	-V-------	---------	--SG--L--	----------	------ST-
CAB53095 (1b)	---------	-V-F----	-V-------	---------	--SG--L--	----------	------ST-
P26660 (2)	---------	-V------	T-S--L---	---------	A-RGM-L--	I-L------L	----ITQ--
BAA88057 (2)	---------	---F--H-	T-S-IL---	---------	A-RGM-L--	I-L--M---L	----ITQ--
JQ1303 (2a)	---------	-V------	T-S--L---	---------	A-RGM-L--	I-L------L	----ITQ--
BAB32876 (2a)	---------	-V-F----	S-S--L---	---------	A-RGM-L--	I-L------L	-----TQ--
AAF25613 (2a)	---------	--------	S-S--L---	---------	A-RGM-L--	I-L------L	-----TQ--
P26661 (2b)	---------	-V-F----	S-S--L---	---------	A-RGM-L--	I-L--M---L	----IAQ--
AAF59945 (2b)	---------	NV-F----	S-S--L---	--R------	A-RGM-L--	I-L--M---L	----IAQ--
BAB08107 (2b)	---------	-V-F----	S-S--L---	---------	A-RGM-L--	I-L--M---L	----IAQ--
BAA08911 (2c)	--------A	-V----F-	S-S--L---	---------	A-RGM-L--	I-L--M---L	----ITQ--
BAA04609 (3a)	---------	-VDFRL--	TVG--I----	--------I	--RGM-L--	TM--------	-----IQ--
BAA06044 (3a)	---------	-V-F-L--	TVG--M---	-------KM	--RGM-L--	TM--------	-----IQR-
AAC03058 (3a)	------C--	-V-F-L--	TVG--T----	--------I	--RGM-L--	TM--------	-----IQ--
BAA08372 (3b)	--Q----EV	-V-FS---	TVG--M---	---E-----	--RGM-V--	TM--------	-----IQ--
CAA72338 (4a)	---------	-A-FSV-N	AV---M---	---------	Q-TS--L---	----------	--H--IK-T
CAA73640 (5a)	--K------	-V------	-----M--L	---------	Q-TS--V---	----------	-----AQ--
AAC61696 (5a)	---------	-L------	-----M---	---------	Q-TS--V---	----------	----IAQ--
CAA72801 (6a)	---------	-V-F-LH-	S----M----	---------	--KS--L---	I-L------I	-----TQ--
BAA09890 (10a)	--K-----V	-V------	SVG--M---	---------	Q-TS--V---	-----M----	-----IQ--

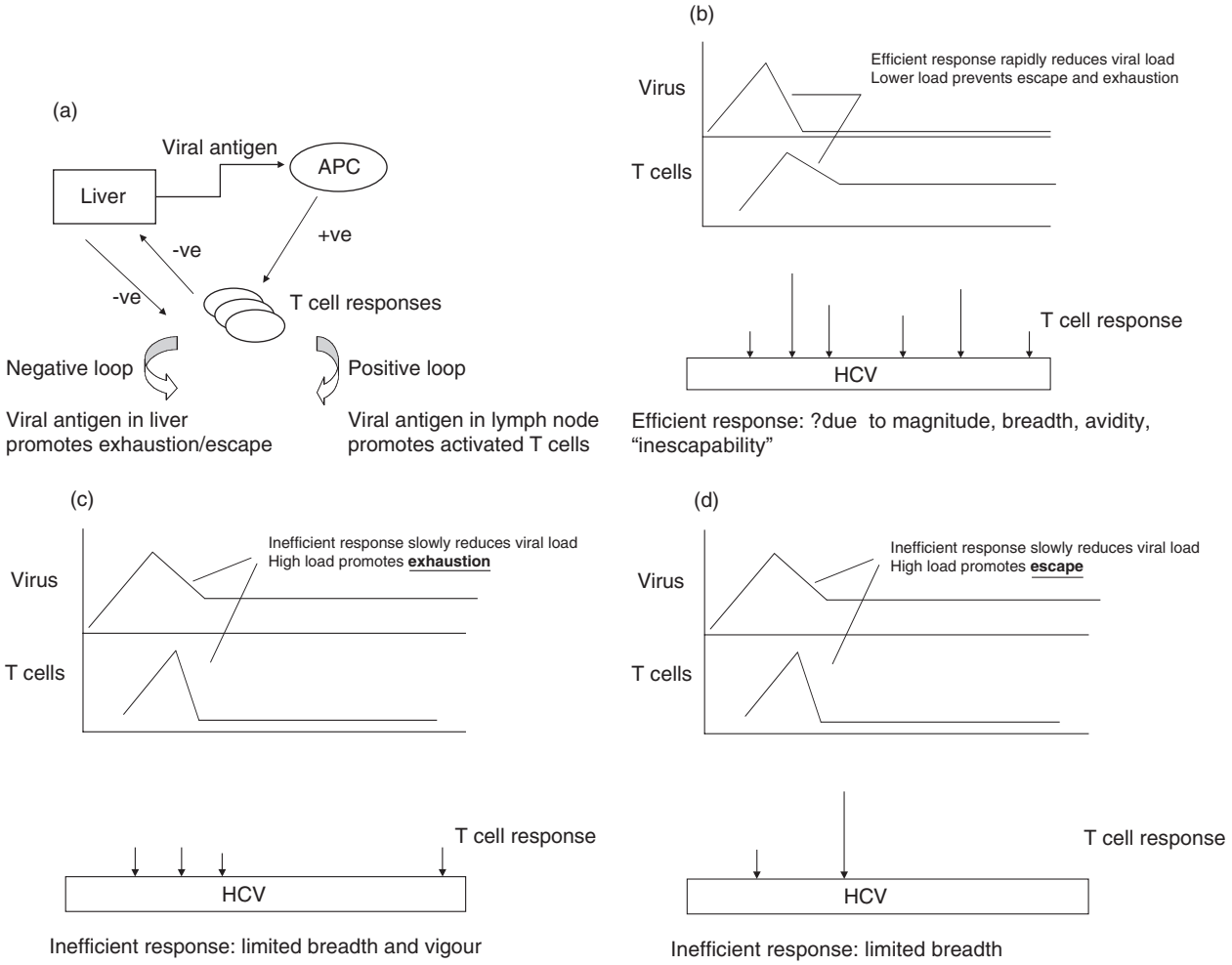


Fig. 1. A simple model for the role of T cell epitope selection in HCV outcome. (a) T cell responses are induced optimally in lymphoid tissue and migrate to the liver to suppress viral replication (positive loop). The liver environment, acccompanied by a high viral load serves to attenuate T cell responses (negative loop). High viral loads will also tend to promote escape. (b) An optimal 'efficient' response rapidly reduces viral load and thus accentuates the positive over the negative loop. The mechanism behind 'efficiency' is not well defined, but includes breadth, vigour, avidity (i.e. the ability to recognize infected cells sensitively), good effector function and targeting of 'constrained' epitopes. (c) An inefficient response fails to lower viral load rapidly and enters the negative loop. In this example the responses lack vigour or avidity, and are then attenuated further in the face of maintained viral loads. (d) An inefficient response fails to lower viral load early and allows for the emergence of escape variants. This will be promoted if the response is targeted at epitopes which can escape rapidly, or if the response is very highly focused.

responses against their own 'endogenous' virus. However, a database of peptides is now emerging that should allow better insights into this complex process. Viral persistence does not depend entirely on T cell epitope selection, as numerous other host and viral factors are involved which are of major importance in controlling viral load. It may not depend entirely on mutational escape, as other issues affecting the efficiency of T cell responses are also likely to be important. However, the simple model presented here suggests this is a major component, and one clearly relevant to infections with other variable pathogens, such as HIV.

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