Mechanisms of immune escape in viral hepatitis

Introduction
WHY IMMUNE ESCAPE IS IMPORTANT
Knowledge of the molecular virology of the hepatitis viruses and the responses they elicit has emphasised the importance of host immunity in resolving infection and mediating liver damage. Many viruses cause cytopathic effects, and research has focused on the mechanisms of viral detection by the immune system. Understanding the molecular and cellular immune responses is crucial in developing effective therapeutic strategies. Although the mechanisms of immune escape by hepatitis viruses is increasing, considerable insight comes from the study of other viruses, some of which can cause persistent infections. In order to cause chronic infection, viruses must use strategies that enable them to evade or modify host immune responses sufficiently to prevent clearance.

The immune response
Immune responses to viral infection may be divided into those that are innate, mediated by complement, phagocytes and natural killer (NK) cells, and those that are adaptive. Adaptive immunity involves the development of antigen-specific T and B lymphocytes that can mount effective immune responses against the virus. The immune response to viral infection may be divided into acute and chronic phases. Acute infection is typically characterised by rapid clearance of the virus, whereas chronic infection is marked by persistent viral replication and may lead to chronic disease.

Mechanisms of immune escape
Mechanisms of immune escape are crucial for the survival of viruses in the host. Viruses may escape immune recognition by sequestration in sites that are inaccessible to the immune system. HBV, which uses RNA as its genetic material, does not encode a reverse transcriptase and so cannot integrate into host DNA. However, the recent discovery of a complementary DNA of LCMV, a murine RNA virus which has no inherent reverse transcriptase capacity, raises this as a possible but unlikely mechanism for HCV.

Other mechanisms by which viruses may evade immune recognition include:
- **Antigenic drift:** Viruses may change their surface antigens to evade immune detection.
- **Molecular mimicry:** Viruses may mimic host proteins to escape immune recognition.
- **Clonal selection:** Viruses may select for mutations that allow them to evade the immune system.
- **Immune suppression:** Viruses may suppress the immune response, either by downregulating immune responses or by promoting the development of regulatory T cells.

Mechanisms of immune escape are discussed in more detail below.

Mechanisms of immune escape
THE “INVISIBLE VIRUS”—PRIVILEGED SITES AND LOSS OF ANTIGENICITY
Viruses may escape immune recognition by sequestration in sites that are inaccessible to the immune system. HBV encodes a reverse transcriptase that enables the virus to integrate its own DNA within the host genome, becoming invisible to the immune system in the process. HCV, which uses RNA as its genetic material, does not encode a reverse transcriptase and so cannot integrate into host DNA. However, the recent discovery of a complementary DNA of LCMV, a murine RNA virus which has no inherent reverse transcriptase capacity, raises this as a possible but unlikely mechanism for HCV.

Classic examples of immunologically privileged sites are tissues through which lymphocytes traffic in small numbers, if at all, such as the anterior chamber of the eye, the brain and testis. Studies in transgenic mice have shown that the access of T lymphocytes to hepatocytes expressing HBV proteins is limited by the density of the liver parenchyma even though some T cells clearly do make contact with HBV-infected cells. It has been established that HBV may be found in many tissues throughout the body but microvascular barriers, which are not present in the liver, may prevent hepatitis B surface antigen (HBsAg) specific cytotoxic T lymphocytes (CTL) from accessing and attacking HBsAg expressing cells in the kidney or pancreas. Although sequestration of virus in these sites does not result in immune-mediated organ damage, it provides a potential reservoir of virus that may repopulate the liver, facilitating viral persistence. Studies in transgenic mice have shown that the access of T lymphocytes to hepatocytes expressing HBV proteins is limited by the density of the liver parenchyma even though some T cells clearly do make limited contact with HBV-infected cells. It has been established that HBV may be found in many tissues throughout the body but microvascular barriers, which are not present in the liver, may prevent hepatitis B surface antigen (HBsAg) specific cytotoxic T lymphocytes (CTL) from accessing and attacking HBsAg expressing cells in the kidney or pancreas.

Another method of evading immune detection is through the loss of antigenicity. Mutations within the pre-core/core genes of HBV can result in loss of expression of the hepatitis e antigen (HBeAg), effectively removing one of the key targets for the immune response and “hiding” the virus.

No similar mechanism has yet been reported for HCV.

Abbreviations used in this paper:
- HBV, hepatitis B virus; HCV, hepatitis C virus; EBV, Epstein-Barr virus; LCMV, murine lymphocytic choriomeningitis virus; NK, natural killer; APC, antigen presenting cells; MHC, major histocompatibility; HBsAg, hepatitis B surface antigen; CTL, cytotoxic T lymphocytes; HBeAg, hepatitis B e antigen; HSV, herpes simplex virus; ER, endoplasmic reticulum; APL, altered peptide ligands; TNF, tumour necrosis factor; IL, interleukin; IFN, interferon; TCR, T cell receptor; TAP, transport associated protein.
ESCAPE FROM ANTIBODIES

Antibodies directed against viral antigens lead to immune clearance in many infections. In acute HBV infection antibodies that bind HBV envelope proteins, including HBsAg, lead to clearance. This antibody response is a T cell dependent process. Antibodies directed against HBsAg are thought to complex with free virus particles, removing them from the circulation and possibly preventing their attachment and uptake into susceptible cells. They also contribute to many of the extrahepatic syndromes of HBV infection. A number of reports have described the emergence of HBV strains with mutations in the envelope gene that lead to a loss of detectable HBsAg expression and viral persistence in the face of anti-HBsAg antibodies.8-10 Antibodies to HBV nucleocapsid antigens HBeAg and HBcAg are found in both acute and chronic infection and do not seem to neutralise viral infectivity. In HCV clustering of mutations has been described in the hypervariable region of the E2 envelope gene which encodes antibody binding domains. It is thought that these mutations encode changes in the HCV envelope protein enabling it to avoid antibody binding that may block infection or aid clearance. Studies of HCV infection in chimpanzees have provided evidence of a correlation between the emergence of envelope mutations and the development of envelope specific antibodies.11

Antibodies recognise epitopes presented in specific conformations. In HIV infection single amino acid substitutions distant from defined epitopes that alter the shape of the envelope protein lead to a loss of binding antibody recognition.12 Similar consequences must be assumed to arise from point mutations distant from the recognised epitopes in the HBV and HCV envelope proteins.

INTERFERING WITH ANTIGEN PROCESSING AND PRESENTATION TO T LYMPHOCYTES

The endogenous and exogenous antigen presenting pathways that generate peptides and present them to T cells have are now well understood (fig 1).13 14 Viruses have evolved strategies to evade each step in these pathways, including mutations in amino acids flanking epitopes that influence peptide processing, downregulation of peptide transporter genes,15 and downregulation of MHC expression. Herpes simplex virus (HSV) causes retention of MHC class I proteins in the endoplasmic reticulum (ER), possibly through interference with the TAP (transport associated protein) transporter. Only some of these mechanisms have been investigated in HBV and HCV infection. A number of viruses encode protein analogues of host cytokines, discussed later, which have wide reaching consequences for the immune response including downregulation of antigen processing and presentation.16

ALTERING IMMUNE RECOGNITION BY T LYMPHOCYTES

Recognition of antigens by T lymphocytes requires both binding of antigenic peptides by MHC molecules and interaction of MHC–peptide complexes with the T cell receptor (TCR). Mutational changes in the amino acids constituting peptide epitopes are likely to interfere with MHC binding if they occur at residues that anchor the peptide in the antigen binding cleft (anchor residues), or T cell recognition if they occur at TCR contact residues. Each amino acid is encoded by a three nucleic acid codon. As a result of errors in copying the viral genome, each round of replication may introduce nucleic acid substitutions into the genetic code. Some of these will generate the code for an amino acid change (coding changes). Of these coding changes some will alter the structure or function of proteins which are essential to the virus and will thus be lethal. However, non-lethal mutations, occurring within immune epitopes, that result in changes in amino acid may alter the affinity of a peptide for a MHC molecule or a TCR for a MHC–peptide complex incorporating that peptide. This loss of affinity for peptide for MHC or TCR is likely to lead to a loss of immune recognition. Genetic mutation as a means of immune escape is highly attractive to viruses such as HBV and HCV that have a reverse transcriptase and an RNA polymerase to copy their genomes. Unlike DNA polymerases these enzymes lack a proofreading capacity and thus permit the introduction of many errors
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Each time the genome is copied. This is especially true for HCV, which not only uses an RNA polymerase to copy its genome but also replicates at very high rates and generates many quasispecies in a single infection.

These mutations are termed "immune escape mutations" and they may lead to immune escape by at least three different mechanisms (fig 2). Mutations at anchor residues may lead to a loss of MHC binding and thus a failure of antigen presentation. Mutations occurring at TCR contact residues, or those influencing the conformation of TCR contact residues may destroy T cell recognition and thus prevent T cell activation. A third type of escape mutation has been described in which the peptide is subtly altered so that it may still bind the same MHC molecule and engage the same antigen specific TCR but with very different conformation. In this scenario, TCR engagement leading to T cell activation, engagement by the altered peptide ligands (APL) renders the T cells unresponsive. This property of APL is called antagonism and has been described for both class I and class II restricted T cells in a range of viral infections.

Thus mutations within viruses that result in loss of MHC-peptide binding, TCR-MHC-peptide binding or altered TCR-MHC-peptide binding may all facilitate immune escape. Mutations have been described in T helper cell epitopes in HBV core protein that may be beneficial to the host in that loss of antigen recognition may result in a milder hepatitis. CD8+ T cell antagonism was first described for a HBV core altered peptide ligand. Antagonism by HCV CTL epitopes has been described both in chimpanzees and in human infections, but where this phenomenon has been studied in populations it has been found to occur infrequently. The importance of peptide antagonism as a means of immune escape has still to be established.

CONTRIBUTION OF MHC AND TCR REPertoire

Both in chronic hepatitis B and C epidemiological studies have suggested that host determinants influence disease chronicity. The implication that immune escape is more likely to occur in some individuals than in others has prompted the search for hereditary and acquired immune determinants that might aid immune escape. Several studies have revealed associations between HLA and protection against chronic HBV or HCV infection. These associations may be explained in several ways. Although a virus will encode a number of peptides capable of binding to an individual patient’s HLA molecules some viral proteins may lack epitopes for specific HLA molecules. A patient expressing HLA molecules incapable of presenting early or early-immediate viral proteins (those that do not require translation of the viral genome) may not be able to mount a response to the first wave of infection, facilitating immune escape and chronicity of infection. Examples of this phenomenon include HBV core protein which contains only a single HLA-A2 epitope and HCV core which contains only one HLA-B7 epitope.

The ability of a T cell to respond to an MHC-peptide complex is determined by the specificity of the TCR. The TCR repertoire is shaped by the host MHC background and self peptides to ensure that T cells express TCRs that recognise foreign peptides presented by self MHC but not self peptides or foreign MHC. The selection of TCR repertoire results in the deletion of 95% of T cells during T cell ontogeny, thus it is possible that some viral proteins will not be recognised by certain individuals due to gaps in the T cell repertoire. Due to the role of HLA molecules in repertoire selection this phenomenon may appear to be associated with specific HLA types.

In neonatal HBV infection developing HBV antigen specific T cells encounter HBV proteins as "self" antigens and undergo clonal deletion resulting in immune tolerance of HBV and chronic infection. In this situation, vertically transmitted HBV escapes immune elimination by harnessing the natural process of tolerance. Interestingly this tolerance can be broken by subsequent immunisation with HBsAg, a situation analogous to that in LCMV infection.

T CELL ACTIVATION

Efficient activation of T cells requires the engagement of a variety of ligand/receptor molecules in addition to the interaction between TCRs on the surface of T cells by MHC-peptide complexes on APCs. These molecules contribute to both adhesion between T cells and APCs and co-stimulation of T cells. Transient TCR engagement that is not accompanied by a second signal leads to T cell anergy and immune tolerance. Interference with the expression or interaction of these co-stimulatory molecules offers a potential mechanism for hepatitis viruses to evade host immunity.

Figure 2 MHC-peptide:TCR interactions and consequences. Peptide interactions with MHC and TCR are represented. (A) MHC binds peptide and presents it to TCR leading to recognition by the T cell and activation. (B) Peptide binds to MHC but a changed TCR contact residue results in loss of recognition by the T cell. (C) Changes in "anchor residues" prevent the peptide from occupying the MHC antigen binding cleft and so the peptide is neither presented nor recognised. (D) Antagonism. The peptide binds to the MHC molecule and engages the TCR but, because of a subtle change in a TCR contact residue, the T cell is not activated but antagonised.

DISRUPTING THE FUNCTION OF ACTIVATED T CELLS

Once T cells have been stimulated by antigen recognition they become functionally active. Most CD4+ T cells secrete cytokines that stimulate humoral (Th2) or cellular immune responses (Th1). CD8+ T cells exhibit MHC restricted antigen specific cytotoxicity or secrete cytokines, or both. Not all T cell epitopes are equal. Some epitopes elicit vigorous polyclonal T cell responses in the majority of infected individuals whereas others are poorly immunogenic and some may only be revealed by peptide stimulation in vitro. The former are termed immunodominant epitopes, the second are subdominant and the latter are cryptic epitopes. Loss of an immunodominant epitope may be sufficient to establish persistent infection.

Maximal stimulation by immunodominant epitopes may result in T cell exhaustion whereby T cells become unresponsive following prolonged stimulation or even after weak proliferation. This outcome is favoured by a high viral load. Alternatively prolonged maximal stimulation of immunodominant T cells may lead to T cell death by apoptosis leaving only T cells that recognise subdominant epitopes. These T cells may be capable of controlling viral replication but incapable of eliminating infection, a situation common to T cell responses in both chronic hepatitis B and C.


Recently the phenomenon of original antigenic sin, initially described in antibodies, has been reported as a feature of T cell responses to viruses. This term describes what happens when the immune system is confronted by a variant of a previously encountered immunogen. Instead of generating a fresh response to the new variant, the host responds with the antibodies and T cells previously elicited by the original wild type immunogen. This results in an ineffectual response to the new variant. It is unclear whether this is the result of memory CTL destroying APCs that are trying to present the new variant, APCs being deactivated by interferon (IFN) γ or IFNα and IFNβ producing memory T cells, or antagonism of variant CTL by wild type peptides. The phenomenon is a highly attractive mechanism for viruses that generate variant quasispecies such as HCV and it may prove to be an important mechanism of immune escape in chronic hepatitis C. Original antigenic sin may also be important in vaccine design as it may render ineffective vaccines that stimulate responses to single peptide epitopes.

Antigen specific activated T cells may impair the host immune response by eliminating APCs or other antigen specific T cells. Hepatitis virus specific HLA class II restricted CTL capable of killing cells that have taken up HBV or HCV may eliminate APCs in a manner analogous to that seen in HIV. HBCAg specific CD8+ T cells that can suppress the CD4+ proliferative T cell response have been detected in the livers of patients with chronic hepatitis B. The exogenous and endogenous antigen processing pathways are not entirely exclusive. Exogenous HBSAg can enter the class I MHC processing pathway of a variety of cell types which may then be killed by HBSAg specific CTL.

**Viral Proteins as Molecular Mediators of Immune Escape**

Once viral infection of host cells is established the translation and expression of viral proteins may release molecular compounds that can interfere with host immunity, enabling the virus to evade immune elimination or control. A number of viruses use these mechanisms of immune escape.

Most viruses induce the expression of host antiviral cytokines including interleukin (IL) 1, tumour necrosis factor (TNF), IL-8, and IFNs α, β, and γ. The actions of these cytokines on host cells are mediated by cytokine receptors. Many viruses have evolved analogues or antagonists of human cytokines and their receptors that modulate host immune responses. Knowledge of these mechanisms may provide insight into the strategies available to hepatitis viruses. EBV encodes an IL-10 analogue that both inhibits IFN-γ synthesis and enhances HLA class II expression. Vaccinia virus produces two proteins that bind to IFNs and a molecule that has homology to an IFN type I receptor as well as analogues of IL-1 and IL-6 receptors. Myxoma virus produces a soluble receptor for IFN-γ which blocks its autocrine functions, and macrophage activated MHC class II expression and regulation of B cell differentiation. Other viruses produce specific inhibitors of cytokines. Adenoviruses encode three proteins that block TNF mediated lysis of cells. Some of these activities have been investigated in HBV. HBCAg has been shown to inhibit IFN-β transcription. The polymerase protein of HBV is immunogenic but the terminal protein inhibits cellular responses to IFN-α and IFN-γ.

Investigation of the immunomodulatory actions of HCV proteins is beginning to reveal interesting observations. Adenovirus vector mediated gene transfer of HCV core and envelope genes into murine dendritic cells has revealed defects in their ability to stimulate other immunocytes and in their production of IL-12. Further research is very likely to reveal similar properties of HBV and HCV proteins. HCV core protein has been shown to be capable of binding to the lymphotxin-β receptor, a member of the TNF receptor family, which is involved in apoptotic signalling and may exert immunomodulatory effects.

**Mechanisms of immune escape in HBV and HCV infection**

Examples of the different mechanisms of immune escape used by viruses have been described earlier. Where possible specific references have been made to the hepatitis viruses. However, this cataloguing of mechanisms fails to provide a clear picture of their role during the evolving course of natural infection or explain how they may account for some of the patterns of immune responsiveness observed in HBV and HCV infection.

Studies of the immune responses in acute resolved and chronic hepatitis virus infections have revealed striking similarities between HBV and HCV infection. In acute HBV infection the production of antibodies directed against envelope antigens is associated with the clearance of circulating virus particles. Vigorous polyclonal, multispecific CD4+ T responses to nucleocapsid antigens and CD8+ T cell responses to envelope, nucleocapsid and polymerase antigens are found in patients who clear acute infection. In chronic infection only weak T cell responses can be elicited, suggesting that immune escape has occurred.

Patients with acute resolved HCV infection exhibit vigorous polyclonal, multispecific CD4+ T cell responses. These T cells exhibit a Th1 phenotype. Immunodominance of an NS3 epitope has been reported. By contrast, in chronic HCV infection CD4+ T cell responses are less vigorous and are directed at fewer epitopes whereas those cells that do respond exhibit a Th2 phenotype. CTL responses are heterogeneous both in their vigour and in the multiplicity of epitopes recognised. Epitopes are scattered throughout the genome and no immunodominant CTL epitopes have been identified. In the vast majority of patients CTL responses in chronic hepatitis C seem to be submaximal.

This pattern of T cell unresponsiveness in chronic infection might occur as the result of impaired antigen presentation or impaired T cell responsiveness. There is little evidence as yet to suggest that antigen presentation is impaired in HBV or HCV infection. Certainly presenting HBV or HCV antigens on healthy APC in vitro does not reverse the observed T cell defects. Thus it seems likely that the antigen specific T cell unresponsiveness seen in chronic hepatitis B and C is a property of the T cells themselves.

How might the various mechanisms of immune escape have contributed to these patterns of immune responsiveness? In early infection antigen excess may well contribute to immune suppression by clonal exhaustion or by apoptosis of immunodominant T cells. In HBV infection, responses to HBV nucleocapsid antigens, usually associated with immune clearance, may lead to immune escape via these mechanisms. Because of the lack of symptoms in acute infection it is difficult to study T cell responses in acute HCV. Comparison of the responses found in patients with acute resolved infection and chronic hepatitis C suggests that vigorous responses to immunodominant epitopes are lost in chronic hepatitis C. Emerging evidence of the rapidity of HCV replication suggests that circumstances are right for clonal exhaustion or apoptosis in acute HCV infection.

Under these circumstances the consequences of vigorous responses in early infection would be either viral clearance accompanied by strong memory T cell responses, or viral persistence in the face of weak T cell responses. The
role of virus mutation generating escape variants and antagonist peptides is unclear. It is more likely to be important in HCV infection than in HBV infection due to the greater diversity of HCV quasispecies. Similarly, antigenic drift in quasispecies may allow original antigenic sin to contribute to the failure of the immune response to clear the majority of HCV infections.

Evasion of antibodies through genetic mutation has already been described for HBV and is implied for HCV. The importance and may be possible for HCV even though the virus lacks an antibody-mediated immune escape. Several approaches used by other viruses to evade host immunity have been described and is likely to contribute to immune escape by the hepatitis viruses.

Evasion of the immune system through cellular tropism is likely to play a part in persistence of HBV and HCV infection. Integration into the host genome is used by HBV and may be possible for HCV even though the virus lacks a reverse transcriptase. Thus mechanisms of immune escape seem to play a major role in the success of hepatitis viruses in establishing chronic infections.

Consequences for treatment

The main therapeutic approaches in viral hepatitis are the use of pharmacological agents, such as IFN-α, which are thought to act in part by augmenting host immune responses, and protective and therapeutic vaccination strategies. From the preceding account, augmenting host immunity might be expected to meet both with success and failure. Enhancing the immune response offers a second chance for the host to eliminate the virus. Antigens in hiding may be forced into the open. Weakly reactive T cells may be stimulated to mount effective responses to subdominant epitopes. However enhancing host immunity also provides further opportunities for clonal exhaustion and escape mutation or host cell damage.

Passive transfer of hyperimmune anti-HBV immunoglobulin has met with some success but has been shown to induce the selection of escape mutants. Protective vaccination has proved to be highly effective against HBV infection. The emergence of pathogenic mutant strains is possible but unlikely. Variation in response rates has been observed and seems to exhibit HLA association, suggesting that the antigens in existing vaccines may themselves exhibit immune escape by failing to bind certain HLA alleles. Therapeutic vaccination is likely to confront many of the difficulties encountered by pharmacological approaches. Clearly the use of small numbers of peptide epitopes may invite escape and adequate immune activation may benefit from novel stimulation strategies.

Conclusions

The hepatitis viruses use many different strategies to escape host immunity. Several approaches used by other similar viruses are still to be investigated. Establishing the relative contribution of these mechanisms of immune escape to viral persistence and the immunopathology of HBV and HCV will prove interesting and should provide important insights into more effective treatments for chronic viral hepatitis.

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