Hepatitis C virus dynamics and pathology: the role of CTL and antibody responses

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This paper investigates the role of CTL and antibody responses in hepatitis C virus (HCV) dynamics and pathology. Mathematical models suggest that a strong CTL response is required for resolution of HCV infection and that a weak CTL response can result in persistent infection. According to the model, establishment of persistent infection is accompanied mainly by an ongoing antibody response, while CTLs are not maintained at high levels. In the model, this outcome correlates with absence of pathology. Persistent infection in the face of an ongoing antibody response can result in evolution of antigenic escape. According to the model, evolution towards escape from antibodies can shift the balance of immune responses so that the weak CTL levels become increasingly more dominant relative to antibodies. This shift results in onset of liver pathology as the virus evolves towards increased levels of antigenic escape. Therefore, the relative balance of the immune response can be a decisive factor that determines whether patients are asymptomatic or whether pathology is observed. Virus evolution can shift this balance towards pathology over time. Theoretical results are discussed in the context of published data.

INTRODUCTION

Hepatitis C virus (HCV) infection can lead to two qualitatively different outcomes (Hoofnagle, 1999): in a small fraction of patients (15% of cases), infection is controlled and cleared from the blood; in the rest of the patients, chronic infection is established, which eventually results in liver pathology. Disease develops after an asymptomatic phase that can last for decades. These two types of outcome are characterized by different immunological profiles (Cooper et al., 1999; Lechner et al., 2000a, b, c; Thimme et al., 2001). Virus clearance after acute infection is associated with strong and polyclonal CD4 T cell responses, as well as sustained CTL responses. Significant virus diversification is not observed in these cases. Chronic infection is characterized by weak CD4 T cell proliferative responses and the absence of sustained CTL responses. Antibody responses develop but the virus has been shown to evolve antigenic escape mutants relatively quickly (Farci et al., 2000).

The role of CTL and antibody responses in HCV infection is not fully understood yet (Farci et al., 2000; Klenerman et al., 2000). According to one hypothesis, antibody responses have the potential to control infection but evolution of antigenic escape allows the virus to persist in the host. Alternatively, it can be argued that CTL responses are required to resolve the infection and that virus persistence is caused by weak CTL responses. Persistent virus replication in the presence of an ongoing antibody response leads to the generation and selection of escape mutants. Here, a mathematical model is used to study the dynamics between HCV and both CTL and antibody responses. The dynamics during acute infection are first examined. Next, chronic infection and the effect of virus evolution on pathology and disease progression are considered.

METHODS

Interactions between HCV and the immune system were studied with mathematical models. Interactions between replicating virus, liver cells and different types of immune responses (CTL and antibodies) are highly complex and non-linear. Thus, the outcome of infection is frequently counterintuitive and cannot be understood by verbal arguments. Mathematical models take us beyond verbal or graphical reasoning and provide a solid framework upon which to build experiments and generate hypotheses. The models are based on a set of assumptions that are spelled out clearly. The mathematical analysis allows us to follow these assumptions to their precise logical conclusions. Here, ordinary differential equations that describe the change of populations over time are considered. The following populations are examined: uninfected liver cells (x), free virus (v), infected liver cells (y), an antibody response (w) and a CTL response (z). The exact form of the equations will be described in the results section and the assumptions are
Susceptible host cells are produced at a rate \( \lambda \), die at a rate \( dx \) and become infected by virus at a rate \( \beta xv \). Infected cells die at a rate \( ay \) and are killed by the CTL response at a rate \( pyz \). Free virus is produced by infected cells at a rate \( ky \), decays at a rate \( uv \) and is neutralized by antibodies at a rate \( gvw \). Antibodies develop in response to free virus at a rate \( gvw \) and decay at a rate \( hw \). CTLs expand in response to viral antigen derived from infected cells at a rate \( cyz \) and decay in the absence of antigenic stimulation at a rate \( bz \). Infection requires that the basic reproductive ratio of the virus \( (R_0 = \beta kl/dau) \) is greater than one. In the absence of an immune response, the system converges to the following equilibria: 

\[
\begin{align*}
\dot{x}^{(0)} &= au(\beta k), \\
\dot{y}^{(0)} &= (\lambda f b k - dau)/a f k, \\
\dot{v}^{(0)} &= ky^{(0)}/u, \\
\dot{w}^{(0)} &= 0, \\
\dot{z}^{(0)} &= 0.
\end{align*}
\]

Now, one assumes that immune responses can potentially develop. This requires the following conditions: \( cy^{(0)} > b \) and \( g z^{(0)} > h \). In this case, the three outcomes below can be observed.

(i) The CTL response develops and the antibody response cannot become established. This is because the CTL response is strong and reduces virus load to levels that are too low to stimulate the antibody response. It is described by the following equilibria: 

\[
\begin{align*}
\dot{x}^{(1)} &= \lambda uc/dg, \\
\dot{y}^{(1)} &= b c, \\
\dot{v}^{(1)} &= ky^{(1)}/u, \\
\dot{w}^{(1)} &= 0, \\
\dot{z}^{(1)} &= (by^{(1)} + ay^{(1)})/py^{(1)}. 
\end{align*}
\]

This outcome is attained if \( gb/h > c < h \) and \( gb/h > c > h \).

(ii) The antibody response develops and a sustained CTL response fails. This is because the antibody response is strong relative to the CTL response and reduces virus load to levels that are too low to stimulate the CTLs. This is described by the following equilibria: 

\[
\begin{align*}
\dot{x}^{(2)} &= \lambda g/dg, \\
\dot{y}^{(2)} &= \beta h z l/([a (d g + h b)]), \\
\dot{v}^{(2)} &= h g, \\
\dot{w}^{(2)} &= ky^{(2)} - uv^{(2)}/qu^{(2)}, \\
\dot{z}^{(2)} &= 0.
\end{align*}
\]

It is attained if \( gb/h < c < h \) and \( gb/h > c < h \).

(iii) Both CTL and antibody responses develop. These equilibria are described by 

\[
\begin{align*}
\dot{x}^{(3)} &= \lambda g/dg, \\
\dot{y}^{(3)} &= b c, \\
\dot{v}^{(3)} &= h g, \\
\dot{w}^{(3)} &= ky^{(3)} - uv^{(3)}/qu^{(3)}, \\
\dot{z}^{(3)} &= (ax^{(3)} + ay^{(3)})/py^{(3)}. 
\end{align*}
\]

It is attained if \( gb/h > c > h \) and \( gb/h > c > h \).

These outcomes are thus governed by competition between CTL and antibody responses for the virus population. This is because the virus population is a resource that both CTL and antibodies require for survival. Competition can result
either in the exclusion of one branch of the immune system or both branches may co-exist. Competition among immune responses has been documented experimentally (Borghans et al., 1999; Freitas & Rocha, 2000) and similar dynamics have been described by Arnaout & Nowak (2000).

A note of clarification: while in the model, a response can go extinct because of competitive exclusion, this does not necessarily correlate with the absence of measurable responses in vivo. In practical terms, it means that one response is significantly more dominant than the other. Many factors that are not included in the model, such as the spatial environment of the body, can result in the persistence of the competitively inferior and subdominant response at low levels.

**Dynamics of acute HCV infection**

This model assumes that, upon infection, the virus population is homogeneous and diversity has not accumulated yet. As the virus population grows, both CTL and antibody responses will start to expand. The outcome of the dynamics in acute infection depends on the relative strengths of the responses. According to the above analysis, three outcomes are possible (Fig. 2): (i) the CTL response is dominant relative to the antibody response and the CTLs will clear the infection; (ii) both the CTL and antibody responses are equally strong. This is also likely to result in resolution or clearance of infection; (iii) the CTL response is weak relative to the antibody response. Thus, the antibody response dominates. If one assumes that HCV is weakly cytopathic (Layden et al., 1999, 2000; Neumann et al., 1998), the antibody response is unlikely to clear the infection. The reason is that while free virus particles are removed, a relatively large pool of infected cells remains because they do not become killed. Hence, the result is persistent infection in the presence of an ongoing antibody response.

If the virus does persist, the model suggests further that the host will be asymptomatic. The reason is that this scenario is associated with the dominance of an antibody response. The degree of pathology is measured by the number of liver cells. A persistent infection with a weakly cytopathic virus in the presence of an antibody response does not cause significant tissue damage because there is little killing of virus-infected cells.

To summarize, if the virus is assumed to be weakly cytopathic, the model suggests that a strong and sustained CTL response is required to clear the infection. CTL-mediated clearance of infection can also be associated with the presence of antibody responses if these are sufficiently strong. On the other hand, if CTL responses are weak, persistent infection in the face of a dominant and ongoing antibody response is observed. The model suggests further that this persistent infection is initially asymptomatic. The notion that sustained T cell responses are required to control
HCV infection is strongly supported by experimental and clinical studies (Cooper et al., 1999; Lechner et al., 2000c; Thimme et al., 2001).

**Dynamics of chronic HCV infection**

To examine how this initially asymptomatic persistent infection can change, resulting in the development of liver pathology, the effect of virus evolution towards escape from antibody responses is examined. The following model extends the previous analysis to include virus escape from antibodies.

\[
\begin{align*}
    x &= \lambda - dx - b \sum_{i=1}^{n} v_i \\
    y_i &= b x v_i - a y_i - p y_i z \\
    v_i &= ky_i - u v_i - q v_i w_i \\
    w_i &= g v_i w_i - h w_i \\
    z &= c z \sum_{i=1}^{n} y_i - b z
\end{align*}
\]

The model assumes that \( n \) virus variants can be generated. These variants vary in antibody epitopes. Antibody escape has been studied mostly in the context of the hypervariable region 1 but the model description applies to any mutation that confers resistance to antibodies. For simplicity, one may assume that the variants only differ in their antibody epitopes; otherwise, they are identical (for example, they replicate at the same rate). The results do not, however, depend on this simplification. Viruses of strain \( i \) are denoted by \( v_i \) and cells infected with strain \( i \) are denoted by \( y_i \) (\( i = 1..n \)). Each virus strain can elicit an antibody response that is specific for this strain, \( w_i \). For simplicity, one may assume that the strength of the antibody response against the individual variants is identical. While the antibody response against the different variants is likely to be characterized by different efficacies, the dynamics in question are not changed by this simplification. A CTL response is also included in the model. It is assumed that the CTL response is cross-reactive and can recognize all antibody escape variants. For the present arguments, antigenic escape from CTL responses does not need to be considered in the model.

How virus evolution influences the dynamics between HCV and the immune responses is investigated from acute infection through the chronic phase, assuming that the CTL response is weak. As outlined above, a weak CTL response...
can result in persistent infection and this leads to the dominance of antibody responses (Fig. 3). Because of persistent replication, variants that escape the antibodies evolve. These new variants, in turn, elicit new antibody responses and, in this way, increased antigenic diversity develops over time (Fig. 3). The outcome of infection as a function of the number of virus strains, \( n \), is given by the following equilibria: 
\[
\begin{align*}
\dot{x}^{(4)} &= \lambda g/(d_g + n\beta h), \\
y_i^{(4)} &= \beta lh \\
v_i^{(4)} &= h/g, \\
w_i^{(4)} &= knh - uv_i^{(4)}/q_i, \\
y_i^{(5)} &= l/n, \\
v_i^{(5)} &= (kgb - nuc)/ncqh, \\
\dot{z}^{(5)} &= [\beta l/h/(c - ab) - d\gamma b]/[pb/(d_g + n\beta h)].
\end{align*}
\]
Thus, as the virus population evolves, virus load increases slightly due to the accumulation of antigenic variants and the antibody response broadens as a result of this diversification. The overall number of liver cells is, however, predicted to remain constant, which corresponds to absence of pathology (Fig. 4). These dynamics change if the number of antigenic variants crosses a threshold given by \( n > badg/\beta h\gamma 0.25 \). If this condition is fulfilled, the CTL response can expand and increase in dominance relative to the antibody response (Fig. 3). Thus, while the CTL responsiveness is weak, the accumulation of mutants that escape from the antibodies increases antigenic drive and this promotes increased expansion of the weak CTL response. However, this CTL response contributes little to virus control. Instead, it marks the beginning of liver pathology (Fig. 4): CTLs kill the infected cells and can thus contribute to tissue damage (Chang et al., 1997). Invasion of the CTL response after accumulation of antigenic diversity is described by the following equilibria: 
\[
\begin{align*}
\dot{x}^{(5)} &= \lambda g/(d_g + n\beta h), \\
y_i^{(5)} &= b/n, \\
v_i^{(5)} &= h/g, \\
w_i^{(5)} &= (kgb - nuc)/ncqh, \\
\dot{z}^{(5)} &= [\beta l/h/(c - ab) - d\gamma b]/[pb/(d_g + n\beta h)].
\end{align*}
\]
The degree of pathology that results from CTL invasion is a function of the number of virus strains (Fig. 4). Continued escape from antibody responses shifts the balance between antibodies and CTLs further towards the CTLs. Since antibodies prevent and CTLs induce tissue pathology, the amount of liver damage increases with the accumulation of antibody escape variants. When the number of antigenic variants crosses a threshold, the CTL response attains maximum dominance relative to the antibody response. This threshold is given by \( n > gkb/uch \). At this point, CTL-induced pathology is expected to be at its maximum (Fig. 4). Furthermore, as this threshold is attained, virus evolution is expected to stop, because target cell limitation does not allow invasion of additional virus variants in the face of significant liver destruction. Note in Figs 3 and 4 that there is no significant change in virus load as the dynamics progress from the asymptomatic phase towards liver destruction. This suggests that the development of disease will not correlate significantly with levels of virus load.

**DISCUSSION**

This paper has used mathematical models in order to examine the role of CTL and antibody responses in HCV dynamics and disease progression. Assumptions of the models are based on data from natural infection and the models explore the consequences of these assumptions for the acute phase and for disease progression in the context of virus evolution. The model accounts for many observations about the dynamics between HCV and the immune system and gives rise to some new insights regarding the relationship between virus evolution, the balance of immune responses and pathogenesis.

The model suggests the following patterns. CTL responses are required to clear the infection. If they fail to clear the infection, however, CTLs are responsible for causing liver pathology. Antibody responses are unlikely to clear the
infection. This is because the virus is thought to be weakly cytopathic and infected cells are not killed at a fast enough rate. Antibody responses are, however, responsible for preventing liver pathology and for keeping the persistent infection asymptomatic. Dominance of antibodies over CTLs ensures that CTL-induced liver destruction is avoided. In general, a weak CTL response can cause tissue destruction if virus replication is not slowed down (Wodarz et al., 2002). Non-lytic immunity slows down the replication kinetics of the virus and thus prevents the occurrence of tissue pathology (Wodarz et al., 2002).

Therefore, the model suggests that following the acute phase, a persistent infection develops if CTL responses are weak. Furthermore, antibodies will initially dominate and this results in absence of symptoms. This dominance in the model is due to competition of the immune responses for the virus population. Virus evolution towards escape from antibody responses, however, allows the weak CTL response to increase relative to the antibody response. This is because accumulation of virus diversity increases the antigenic drive. Note that while this can cause pathology, the model suggests that the degree of increase in the number of CTLs is low because the response is weak. It might, therefore, be difficult to find evidence for this in clinical data. Of further note is that non-specific T cells can also infiltrate the liver and contribute to pathology and this has not been included in the model. The overall message of the model is that evolution of escape from antibodies paves the way for disease progression and liver pathology by shifting the balance of immunity in favour of lytic responses. This is supported by data from immunosuppressed patients who show faster disease progression (McCaughan & Zekry, 2000; Einaiv & Koziel, 2002). Such patients are characterized by a deficiency of antibodies. The disease might be caused by weak CTL responses, which may be maintained after acute infection and cause progressive tissue damage. A possible scenario not captured in the model is that the inoculum upon infection is not a homogeneous virus population, but characterized by some degree of antigenic diversity. This might also speed up disease progression.

The modelling framework helps us understand and interpret experimental data on immune responses against HCV. The role of CTLs and antibodies for the resolution of HCV infection is debated in the literature (Klenerman et al., 2000). Studies of the acute phase of the infection showed that both humans and chimpanzees who cleared the virus from blood developed strong and sustained CTL responses (Chang et al., 2001; Cooper et al., 1999; Lechner et al., 2000a, b, c; Thimme et al., 2001). In chimpanzee studies, CTL-mediated clearance is associated with the absence of strong antibody responses (Cooper et al., 1999) and this supports the notion of competition between the two branches of the immune system. Humans and chimpanzees who developed persistent infection were characterized by an initial CTL response that was not sustained at high levels beyond the acute phase of infection (Cooper et al., 1999; Lechner et al., 2000a, b, c; Thimme et al., 2001). Persistent infection has, however, been observed to be associated with vigorous antibody responses (Farci et al., 2000; Major et al., 1999), again pointing to the occurrence of competition. Thus, it was argued that a strong CTL response is crucial for the resolution of infection. The theoretical results presented here agree with this conclusion. On the other hand, it has been argued that antibody responses are crucial for deciding the outcome of infection (Farci et al., 2000). Farci et al. (2000) showed that patients who resolved the infection showed evolutionary stasis in the virus population. Patients who developed chronic infection showed accumulation of genetic variation in antibody epitopes. It was concluded that escape from antibody responses allows the virus to persist. The importance of virus evolution for persistence has also been stressed by others (Forns et al., 1999; Weiner et al., 1992). The theory presented here argues that antigenic escape might not be the reason for virus persistence, but the consequence of virus persistence. Continued productive infection in the presence of an immune response is the most efficient scenario for the evolution of antigenic escape (Wodarz & Nowak, 2000). This argument does not, however, diminish the importance of antibodies in HCV infection. If a persistent infection is established, the model suggests that antibodies are crucial for keeping the patient asymptomatic and that escape from antibodies allows the balance of immune responses to be shifted in favour of a weak CTL response, which causes liver pathology. Thus, the importance of different types of immune responses should be considered in a broader setting than just in the context of clearing virus during acute infection. The relative balance of antibody and CTL responses can be crucial for deciding whether the persistent infection remains asymptomatic or whether pathology is observed.

A central concept in this analysis is that virus evolution towards escape from antibodies might drive disease progression. This concept is very difficult to test with data. Studies have quantified sequence diversity and the rate of virus evolution in patients who differ in the severity of liver disease (Cabot et al., 2000; Curran et al., 2002; Farci, 2001; Lyra et al., 2002; Major et al., 1999; Thomson et al., 2001). Even if virus evolution does drive disease progression – as suggested here – this does not mean that one would expect higher virus diversity or faster evolution in patients with severe compared to mild disease. The amount of virus diversity and the rate of evolution is a complex function of the degree of immune-mediated virus suppression, virus load and the number of susceptible host cells (Wodarz & Nowak, 2000). If virus load is suppressed efficiently, reduced replication does not allow a fast accumulation of mutations. On the other hand, immune-mediated suppression of the virus allows for the co-existence of different antigenic variants (Regoes et al., 1998). If immune responses are diminished, however, competition between virus strains becomes an important factor that can lead to a reduction in virus diversity (Nowak et al., 1991). Moreover, as the number of susceptible cells becomes limiting – for example,
because of liver destruction – virus evolution may slow down or stop because new antigenic variants cannot invade (Regoes et al., 1998). Therefore, if pathology develops as a result of virus evolution towards antigenic escape, it is possible that patients with severe disease show reduced levels of virus diversification and evolution compared to patients with milder disease. Data on virus diversity have given rise to different and contradictory results (Cabot et al., 2000; Curran et al., 2002; Farci, 2001; Lyra et al., 2002; Major et al., 1999; Thomson et al., 2001). Studies with HCV-infected patients report the emergence of antibody escape mutants early after infection (Farci et al., 2000), while data from the chimpanzee model argue against the frequent occurrence of antibody escape mutants during the early stages of the infection (Major et al., 1999). While the chimpanzee data argue against a role of escape for virus persistence, this study does not address the role of escape for disease progression, which occurs over a much longer period of time. Curran et al. (2002) reported a consistent accumulation of amino acid-changing substitutions in patients with mild liver disease. In patients with severe liver disease, however, they reported significantly lower rates of virus evolution. The observation that the virus continuously evolves in patients with mild disease supports the notion that ongoing virus evolution can eventually shift the dynamics towards pathology. A reduced rate of evolution in patients with severe disease is also consistent with the theoretical arguments presented here. As severe pathology develops, the number of susceptible host cells is reduced and this can prevent invasion of further antigenic variants. The hypothesis that virus evolution can drive disease progression is supported further by the observation that the population diversity, as well as the ratio of amino acid changing to silent substitutions was higher in individuals with severe liver disease (Cabot et al., 2000; Curran et al., 2002). Similar patterns have been observed in studies of virus evolution following liver transplants in patients with mild and severe disease (Lyra et al., 2002). Investigation of the expected patterns of diversity and rates of evolution in patients with different severity of disease under various assumptions will benefit from further mathematical modelling.

Another factor that can influence patterns of virus evolution is the ability of the specific immune responses to adapt to the changing virus population. The model assumes that new escape variants can always trigger new antibody specificities. Therefore, the virus continuously evolves away from the immune response. Clinical data from HCV-infected patients suggest that specific CD4 T helper cell responses are impaired (Lechner et al., 2000c). Helper cell impairment can, in turn, compromise the ability of the neutralizing antibody response to adapt to new virus variants (Klennerman et al., 2000). If this happens, virus evolution towards increased antigenic diversity in antibody epitopes is expected to stop. It is not clear for how long the antibody response can continue to adapt to the virus population. As the disease progresses, the antibody response is more likely to fail to adapt to the virus population. This could also contribute to the reduced rate of evolution seen in HCV-infected patients with severe liver disease.

It is an interesting observation that accumulation of antigenic diversity and the development of disease do not correlate with a significant increase in virus load in the model. Upon development of symptoms, virus load may be relatively small: while CTLs induce pathology in the model, they can suppress viraemia to a certain degree at the same time. Variations in virus load observed between patients is thus unlikely to be due to differences in the amount of antigenic diversity and disease status but to differences in other host parameters. Hence, the model suggests that there is no obvious correlation between pathology and virus load. This is consistent with clinical data (de Araujo et al., 2002; Manzin et al., 1997; Pontisson et al., 1999; Puoti et al., 1999).

Finally, it is important to note that a simplified scenario was used, as antigenic variation was included only in the context of antibody responses but not in the context of CTL responses. Once the weak CTL responses become more prevalent following virus evolution, escape from CTL responses is also very likely to occur. This can enhance virus replication and tissue pathology further. However, it is not essential for the shift in balance between antibody and CTL and is hence not essential for the initial occurrence of liver pathology.

Conclusion

In this paper, a theoretical framework that explores the role of virus evolution for disease progression in HCV infection is put forward. The models point to the importance of the balance of immune responses for determining whether a patient remains asymptomatic or whether pathology is observed. Virus evolution can shift the balance of immune responses from a state where antibody responses prevent the development of liver pathology to a state where antibody responses are weakened relative to the CTLs. This can result in an increase in CTL-induced liver destruction and, therefore, pathology. Experiments can now be performed to test this theoretical framework. Chimpanzees should be infected with a homogeneous virus inoculum as well as with an inoculum that contains antigenic diversity in antibody epitopes. This should shed more light on whether diversity helps the initial establishment of persistent infection or not. Animals that develop persistent infection should then be monitored closely (for virus, antibodies, CTL levels and pathology) to assess whether the diverse inoculum gives rise to faster progression and to determine how it influences the balance between CTL and antibody responses over time.

This work has broader implications regarding the role of lytic and non-lytic immune responses in the development of pathology in persistent human infections. An important example is human immunodeficiency virus, where virus evolution could shift the balance of immune responses to a state that favours destruction of the CD4 T cell population.
and thus the development of AIDS. This will be explored in a separate paper.

REFERENCES


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