Immune Responses to Hepatitis C Virus (HCV) Infection and the Prospects for an Effective HCV Vaccine or Immunotherapies

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Infection with hepatitis C virus (HCV) typically leads to persistent infection, with >170 million people estimated to be affected worldwide, putting them at risk for chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Importantly, 20%–30% of individuals are able to control the virus spontaneously, usually within 6 months of exposure. This suggests that HCV vaccines and immunotherapies are a distinct possibility. We discuss here the role of T cells in controlling HCV, the gaps in our understanding of protective HCV immunity, and the recent introduction of a HCV T-cell vaccine into clinical trials.

Keywords. hepatitis C virus; acute infection; T cells; protective immunity; vaccine.

The recent introduction of new hepatitis C virus (HCV)–specific antiviral agents and the prospects of even more powerful targeted therapies currently in development have suggested the possibility that HCV-associated liver disease might turn into a problem of the past [1]. This has raised the question of whether the development of HCV vaccines or immunotherapies remain worthy goals. However, it remains to be seen how many of the >170 millions people infected with HCV worldwide will have access to these extremely costly and sophisticated therapies. Also, if the new therapies cannot be used or are not effective in even a relatively small number of patients, the number of patients requiring alternative therapies will be substantial, owing to the sheer size of the HCV-infected population. Finally, injection drug users, who represent the major population experiencing new HCV infections in many countries, have a high risk of reinfection and thus might benefit most from interventions that can repeatedly prevent the establishment of chronic HCV infection [2]. Thus, it seems prudent to continue exploration of HCV immunotherapies and vaccines as additional weapons in the arsenal to fight HCV infection in all affected populations and in all regions of the world.

HCV AS A HUMAN MODEL TO ESTABLISH THE CORRELATES OF IMMUNE PROTECTION

The hope that HCV vaccines might be able to prevent chronic HCV infection stems from the observation that a proportion of infected persons, estimated as 10%–30%, can clear the virus spontaneously. This makes HCV infection quite unique among clinically relevant chronic viral infections in humans. Human immunodeficiency virus (HIV), cytomegalovirus, Epstein-Barr virus, and herpes simplex virus all establish chronic infection in every host, albeit with distinct clinical courses. Infection with hepatitis B virus (HBV) can also lead to both acute and chronic infection, but HBV infection becomes persistent almost exclusively in newborns and children, whereas almost all adults clear the virus [3]. In contrast, the outcome of HCV infection is only moderately impacted by age, with younger age being slightly beneficial, but overall...
the vast majority of HCV infections result in chronic infection in all age groups. Importantly, spontaneous clearance of HCV infection is usually observed within the initial 6 months of infection, highlighting the necessity of understanding the earliest immunological events. Cases of late HCV clearance, even years after infection, have been observed and should also be studied in more detail, since they might reveal pathways to therapeutic immune interventions. Interestingly, these late clearances have sometimes been linked to infection episodes with other pathogens [4]. Overall, these observations strongly suggest that the immune system can prevent chronic HCV infection and maybe even clear HCV after chronic infection has been firmly established. They also highlight the usefulness of HCV infection as a human model system to dissect the differences in the host response associated with a clearly defined dichotomous clinical outcome. A better definition of what exactly constitutes protective immunity in humans would be a major accomplishment with great relevance for other persistent infections.

EVIDENCE FOR THE ROLE OF T CELLS IN SPONTANEOUS HCV CLEARANCE

There are numerous lines of evidence that T cells play an important role in shaping the outcome of HCV infection. In the chimpanzee model, depletion of either CD4+ or CD8+ T cells after a self-limiting HCV infection and before reinfection with the same HCV clone prevented the expected viral clearance in this scenario [5, 6], although in the case of CD4+ T-cell depletion there was still partial control of the virus, as indicated by comparatively low viral titers.

There is also genetic evidence for the importance of T cells, with several class I and class II alleles associated positively and negatively with the outcome of HCV infection [7]. Interestingly, some of these alleles (foremost B27 and B57) are also protective in the context of HIV disease progression. In addition, the largest genome-wide association study (GWAS) to date, involving almost 1500 subjects, each with chronic and resolved HCV infection, established single-nucleotide polymorphisms in class II alleles as significantly associated with the natural outcome of infection, in addition to the previously described polymorphisms linked to IL28B (Duggal et al, Annals of Internal Medicine, in press).

Finally, there is a timely association between the induction of a T-cell response and control of viral replication. In both humans and chimpanzees, after weeks of usually high levels of HCV replication, control of HCV viremia is observed only after the emergence of functional T-cell responses [8]. Together these data highlight the importance of T cells for the control of HCV, although it remains an open question whether an effective T-cell response alone is sufficient for a self-limiting course of infection.

THE DIFFERENT PHASES OF EARLY HCV INFECTION

Only over the last decade have we begun to appreciate the clinical and immunological features of early HCV infection, mainly because of the significant difficulties in identifying subjects soon after exposure to HCV. Increased screening for subjects with acute HCV infection allows us now to draw a more dynamic and more differentiated image of the consecutive events leading to either spontaneous clearance of HCV or viral persistence, although major gaps in our knowledge still need to be filled. On the basis of clinical and immunological criteria, we suggest differentiating early HCV infection into 4 phases during the first year after infection, followed by a fifth phase that is stable chronic or resolved infection (Figure 1).

The initial phase starts with HCV exposure and is characterized by the rapid establishment of high viral titers in the blood, no biochemical evidence of liver damage, and the absence of adaptive immune responses by T cells or antibodies.
This phase inexplicably lasts longer than the total duration of many acute viral infections, at 6–8 weeks, as shown by Thimme et al in a group of hospital workers after needle-stick exposures [8]. This was confirmed by experiments in the chimpanzee model of HCV, where it was also possible to study intrahepatic samples, thus eliminating the possibility that local T-cell responses at the site of infection might have been generated earlier than those detected in the blood [9]. Clearly, the host is sensing the presence of HCV replication much earlier, as indicated by the observation of intrahepatic expression of interferon-stimulated genes and other indicators of an innate immune response, making the delay in adaptive responses even more puzzling [10]. It remains to be seen to what degree these innate immune responses are critical in setting the conditions for the adaptive immune response (ie, whether the success or failure of the T-cell response is already determined before it even appears). Why the adaptive response against HCV is just developing at a time when other viral infections have already been forgotten by a fully recovered host and how this phase shapes the outcome of HCV infection remain important scientific questions that warrant further investigation.

The second phase of HCV infection is initiated by the induction of adaptive immunity, with generation of virus-specific antibodies and quick expansion of HCV-specific T cells, typically followed by a rise in liver enzyme levels that indicates the destruction of infected hepatocytes [8]. Most infected individuals achieve at least partial control of viremia shortly after, whether or not they will be fully able to clear the virus later [11]. Only a minority of patients seems unable to exert any kind of control over HCV at any time point. During this critical period, many features of cellular immunity seem to be remarkably similar between individuals going on to control HCV and those developing persistent viremia, at least on the basis of limited available data (described further below).

The next phase lasts roughly from 12 to 24 weeks after infection. During this time, one can often observe substantial fluctuations of HCV viremia [11, 12], until the virus is finally cleared or has established persistent replication, usually at high levels. In parallel, differences in the T-cell response become increasingly apparent, with altered memory phenotypes and decreasing functional capabilities of the T cells in persistent as compared to resolving infection.

The beginning of the fourth phase, at 24 weeks after infection, marks the end of the true acute phase of HCV infection. At this point, almost all patients are and remain either viremic or HCV RNA negative, and the T-cell response is markedly stronger and more functional in subjects controlling the virus. Nevertheless, cellular immunity continues to evolve at this point, with, for example, continued contraction of the T-cell response in parallel to increased expression of the interleukin 7 receptor, a marker of T-cell memory, in resolved infection [13]. The magnitude and the detectable breadth of the T-cell response decline during this phase in all subjects [14], in contrast to HIV infection, in which T-cell dominance and the magnitude of the response continue to evolve for years. Following this phase, immune responses against HCV are also relatively difficult to detect in the blood, especially in persistent infection [15], although at least CD8+ T-cell responses remain easily detectable in infected livers [16].

**EARLY T-CELL RESPONSES AND OUTCOME OF HCV INFECTION**

Because it is so difficult to identify HCV infection very early after exposure, owing to the lack of specific symptoms, it has been quite challenging to study the earliest phase of the T-cell response in humans. Most immunological studies in acute HCV infection analyze predominantly samples from the second half of phase 3 and from phase 4, raising the possibility that immunological differences might be more of a consequence than a cause of infection outcome [10]. More work needs to be done on samples from phase 2 and early phase 3 to understand the events initiating the failure of the T-cell response associated with viral persistence. In addition, links have to be established between the innate immune responses already active in phase 1 and the timing and quality of the T-cell response that appears later.

So far, the clearest difference that has been observed early in HCV infection is that broadly directed and vigorous CD4+ T-cell proliferative responses are required for self-limiting HCV infection [17]. Individuals resolving HCV infection spontaneously display such responses early, regardless of the level of viral replication at that moment. In contrast, in subjects with persisting infection, CD4+ T-cell proliferative responses are mostly absent, although they can be observed in some subjects with complete temporary control of HCV [17]. This dichotomy in the CD4+ T-cell proliferative response has been confirmed in subsequent studies [18], but importantly this functional defect should not be interpreted as an indication that HCV-specific CD4+ T cells are not primed in subjects progressing to chronic HCV infection. We have recently shown that these cells are actually present in almost all subjects during the earliest phase of the adaptive immune response and are often vigorous and multispecific [18]. However, these responses collapse rapidly with persistent infection and, at 6 months after infection, are virtually never detectable in the blood of patients with HCV viremia. The mechanisms inducing this rapidly emerging dichotomy in the CD4+ T-cell response, just weeks after T-cell responses appear, could be the key correlates of protection from chronic HCV infection. It will be important to identify what causes this rapid collapse of the CD4+ T-cell response but also to define...
the functional and transcriptional profiles of these cells before they disappear. Inhibiting or modulating signals from the early CD4+ T-cell response could influence other arms of the immune response beyond the time when these cells have already disappeared.

The characterization of the earliest events in the CD8+ T-cell response are also awaiting better definition, especially since in subjects recruited early after infection one does not observe the significant differences in the breadth or the magnitude of the CD8+ T-cell response that can be observed later, after the outcome of infection has been established [15]. Clearly, most subjects studied in phase 2 usually have rather robust and broadly directed HCV-specific CD8+ T-cell responses, including those developing chronic viremia [14]. HCV is able to circumvent immune pressure from these CD8+ T-cell responses, such as through the development of viral variants (known as "viral escape mutations") that are not recognized by the CD8+ T-cell response [19, 20] or through upregulation of inhibitory T-cell receptors, leading to impairment of T-cell functions (known as T-cell exhaustion) [21]. Both mechanisms are definitely operational in chronic HCV infection. What remains to be determined is the relative importance of each mechanism and the exact time points when they begin to be operational. Until then, it is hard to tell whether these viral evasion mechanisms are causing viral persistence or whether they just add to T-cell failure once the T-cell response is unable to control HCV for other reasons. It also remains to be seen whether the failure of the CD8+ T-cell response is based on CD8+ T-cell intrinsic mechanisms or whether it is mainly a consequence of the even quicker collapse of the CD4+ T-cell response in viral persistence.

A T-CELL VACCINE TO PREVENT CHRONIC HCV INFECTION?

Given our current understanding of HCV immunology, designing an HCV vaccine approach rationally is not a trivial undertaking. Not only do we not know the exact requirements for immune protection, but the extreme diversity of circulating HCV strains (even bigger than the diversity observed for HIV type 1) make the choice of vaccine antigens extremely challenging, be it for antibody- or T-cell–based immunization. There is little evidence from the natural history of HCV that sterilizing immunity can be achieved, although the recent isolation of broadly neutralizing HCV antibodies raises the possibility of a vaccine that completely prevents HCV infection [22]. This is similar to the situation for HIV infection, in which antibody-based vaccine approaches have also reentered center stage recently [23]. However, more advanced at this point is the development of a vaccine mimicking the immune response that allows at least 20% of infected subjects to clear the virus after infection spontaneously and, thus, prevent the

sequentae of chronic viral hepatitis. It seems obvious that a broad and, importantly, long-lasting CD4+ and CD8+ T-cell response is required to control HCV, but many additional details remain unclear, such as whether certain HCV regions should be preferentially targeted or whether the T-cell responses induced by a vaccine need to possess a certain phenotype or specific functional qualities. It is also not clear whether T cells alone are sufficient or whether other arms of the immune response are additionally required to establish full control of HCV.

With the recent initiation of the first trial of a prophylactic HCV T-cell vaccine in humans, we have the hope that it will achieve its primary end point of protection from chronic infection. We can also expect to learn many important facts about how T-cell immunity relates to control of HCV infection, even if the primary goals of the trial were not achieved. This National Institutes of Health–sponsored vaccine trial is taking place at 2 sites in the United States (Johns Hopkins University, led by Dr Andrea Cox, and the University of California–San Francisco, led by Dr Kim Page) in populations of HCV-negative drug users, who are at extremely high risk of exposure to HCV and who are followed prospectively in short intervals [11, 24]. The immunization is modeled after a series of experiments in chimpanzees [25] and humans [26] that demonstrated impressive immunogenicity, as detailed below.

This HCV vaccination approach is based on a prime-boost immunization schedule that, in its latest iteration for the ongoing clinical trial, consists of priming with a replication-deficient chimpanzee adenovirus vector, AdCh3. This vector contains the HCV antigens NS3, NS4, and NS5A/B, derived from an HCV GT1b prototype sequence [26]. The same antigens are later delivered with modified vaccinia Ankara (MVA) as the vector for a boost at week 8. An adenoviral chimpanzee vector was selected with the hope of preventing the issue of preexisting neutralizing antibody responses that are found against many of the adenoviral strains that typically infect humans [27], since neutralization of the vaccine vector impedes immunogenicity.

This approach had been initially tested in the chimpanzee model of HCV infection, although with different adenovirus vectors and with a DNA boost, demonstrating striking immunogenicity [25]. Induction of vigorous and broadly directed T-cell responses after vaccination was associated with rapid clearance of HCV infection in all animals, although the necessarily small number of animals and the typically high rate of spontaneous clearance in this model do not allow true prediction of the protection rate. In the first trial in healthy humans, the overall immunogenicity of the vaccine was also excellent, both with the AD6 vector that had been tested in the chimpanzee experiment, as well as with AdCh3, the vector now used in the prophylactic vaccine studies [26]. All subjects primed functional HCV-specific responses. In quite a few
individuals, responses were at a magnitude that is rarely seen in natural infection, even during an acutely resolving infection. Both CD4+ and CD8+ T-cell responses were broadly directed in most vaccinees and were able to exert multiple functions, such as cytokine production and cytotoxicity. Boosting with the other adenovirus vector, however, was not that efficient, most likely because of cross-reactive antibodies that were induced during primary immunization. For this reason, the current trial in drug users at high risk for HCV infection instead boosts with MVA. The other area of concern is how broadly protective the vaccine can be. While some of the HCV genotype 1b–induced T cells also recognized genotype 1a and 3 sequences, the genotype 1b–induced responses are clearly not broadly cross-reactive with other HCV genotypes. It seems important to continue thinking about improved ways of delivering HCV antigens that can elicit immune responses against a majority of circulating HCV variants.

The results of this study will help us answer some important questions that are difficult to address otherwise. If the key to HCV control is in the outcome of the race between viral replication and the initiation of the immune response, the vaccine should be effective. Similarly, if the magnitude of the response matters, the advantage should be with the vaccine. We should also learn the degree to which the breadth of the response matters, since only two-thirds of the HCV genome is used as antigen and not all healthy subjects primed a highly multispecific T-cell response. We also do not know yet how long the vaccine-induced responses will persist and what frequency of the T-cell response is required to provide a quick recall response in the case of HCV exposure. The ongoing clinical trial will allow carefully controlled immunological studies and thus will give us invaluable insights into the relationship between T-cell responses and HCV.

CONCLUSIONS

Since the discovery of HCV in 1989, we have learned many details about the host immune response to HCV infection and the mechanisms used by HCV to circumvent or escape immunity. Yet, despite HCV infection offering the unique opportunity to compare protective versus failing immune responses against the same virus, we have not yet clearly defined the correlates of protection or the initial causal mechanism for the failure of the immune response against HCV in the majority of people. Well-curated clinical cohorts of patients with acute HCV infection, together with recent technological breakthroughs in studying T cells, hold significant promise that better definitions of protective immunity are on the horizon. In addition, data from the ongoing clinical trial of a highly immunogenic HCV T-cell vaccine will close important gaps in our understanding of HCV immunology that could not be addressed otherwise. The impact of these studies could reach far beyond HCV infection alone, since the clinically distinct outcomes of HCV infection allow better definition of the difference between protective and inefficient immunity in humans in general.

Notes

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