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Review

Plasmacytoid dendritic cells move down on the list of suspects: In search of the immune pathogenesis of chronic hepatitis C☆

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Chronic hepatitis C is a major public health problem. Despite numerous clinical studies in humans and experimental observations made in chimpanzees, hepatitis C pathogenesis remains poorly understood. Here, we review the clinical features of acute and chronic disease, and discuss the role of the immune system in the pathogenesis of disease. Many are aware of the dual role of T cells: responsibility for clearance of the virus during acute phase; and liver injury during chronic phase. Nonetheless, there is an emerging belief that failure to prime HCV-specific T cells is responsible for the failure to spontaneously clear the virus, and possibly, for the lack of response to pegylated-IFNα_{2a}/ribavirin therapy. We have focused on the latest suspects, plasmacytoid dendritic cells (pDCs), considered to be the professional type I IFNs producing cells. We review the somewhat contradictory data regarding the functional capacity of pDCs in chronic HCV patients and argue that, while lower in relative concentration as compared to healthy individuals, they are not defective in their ability to initiate an innate inflammatory response. Thus, instead of being the culprit, pDCs may in fact represent a novel therapeutic target in order to improve upon existing therapies for treating HCV patients.

According to the World Health Organization, HCV infection represents a major health concern with an estimated 180 million people infected i.e. 3% of the world's population, with 3–4 million new cases each year. Some regions (e.g. Egypt, Bolivia, Mongolia, Cameroon) are particularly affected with disease prevalence >10% of the population. Once individuals are infected, HCV rapidly accesses the liver, the dominant site of replication [1]. There are two general courses of HCV infection (Fig. 1). Approximately 20–40% of infections are generally benign, self-limited infections that clear within 6 months [2]. In fact, this may be an underestimate as there is evidence for viral transmission (based on detection of plasma viremia) with subsequent clearance in the absence of sero-conversion [3,4]. Still, it is believed that the majority of infected individuals (60–80%) develop chronic infections that result in accumulating levels of liver damage. HCV RNA typically becomes detectable in serum within 7–14 days following exposure, with viral levels peaking at 6–10 weeks and declining rapidly thereafter [5,6]. Of those who develop chronic hepatitis C, one-third will have a lifetime risk of developing liver cirrhosis and a significant number will develop hepatocellular carcinoma (HCC) (Fig. 1) [7]. HCV is responsible for more than half of all HCC cases and two-thirds of all liver transplants in the developed world. Chronically infected patients may undergo treatment with pegylated interferon and ribavirin, but treatment is difficult to tolerate and is effective in only 40–80% of patients, depending on the infecting genotype [8].

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Pathogenesis of chronic infection is not well-defined, but it is known that HCV replicates at an extremely high rate, ranging from 10^3 to 10^7 units per ml of serum with an estimated 10^{12} new viral particles produced each day [9]. While non-cytopathic, infection results in extensive liver damage; and based on histological observations, it is believed that liver injury is immune- rather than virus-mediated. The adaptive immune response, specifically T cell activation, while slow to start, is believed to be an important criterion for clearance during acute infection [10]. The breadth (diversity of the HCV-reactive T cell repertoire) and the magnitude (number of HCV-reactive T cells) are thought to be the determinants of clearance; though insufficient, T cell activation remains robust throughout the chronic phase. In turn, tissue damage within the liver results in the production of transforming growth factor β (TGFβ), which is considered to be one of the most important molecules in inflammation-induced fibrosis [11]. Long-term liver cirrhosis appears when the normal parenchyma is replaced by non-functional scar tissue, ultimately leading to liver insufficiency. Thus, the T cell response to HCV is a double-edge sword as cytotoxic T cells are essential to the destruction of infected hepatocytes, but over time, they destroy the liver.

Regarding treatment of chronic HCV patients, recent advances include the introduction of pegylated interferons in combination with ribavirin, resulting in viral eradication in 54–66% of treatment-naïve patients [12,13]. A lack of response to anti-HCV treatment can generally be categorized as either a complete non-responder (where HCV RNA levels do not significantly decline by >2-$\log_{10}$ throughout therapy), or virological relapser (where HCV RNA becomes undetectable during treatment but is detected again after discontinuation of therapy). Non-responders, and to a lesser extent relapsers, are mainly males, over 40 years, either overweight or have fatty liver, extended fibrosis (F3) or cirrhosis and infected by genotypes 1 or 4. SVR, in contrast, is usually preceded by a rapid viral decline within the first weeks of the treatment, with viral loads undetectable 6 months after discontinuation of treatment. It is widely known that HCV genotype is perhaps the single most important factor in predicting a successful therapeutic outcome [12,14]. This is reflected in current treatment guidelines [15], which advocate a more aggressive approach to treatment in patients infected with 'difficult to treat' HCV genotype 1, where the probability of true non-response or virological relapse is somewhat greater than in patients infected with other HCV genotypes [12,16]. Importantly, the availability of an effective therapy has also provided an important clue concerning the characterization of immune-mediated clearance of HCV.

One additional point that bears mentioning is that at no point during disease pathogenesis are HCV patients thought to be immunosuppressed. Chronic HCV patients do not present to the clinic with evidence of opportunistic infections, a hallmark of an immunocompromised state. There is no higher rate of viral or bacterial infections in HCV-infected patients. Nor do they show evidence of poor response to vaccination (e.g. HBV vaccination). Thus, in considering the immune pathogenesis of the disease we insist that the clinical features of HCV infection do not fit with models in which global defects in immune responses are invoked (e.g. defective dendritic cells).

1. Overview of the immune correlates of spontaneous viral clearance

Much is now known about the host response to HCV infection and both chimpanzee and human studies have been instructive in determining which anti-viral mechanisms of the immune system correlate with clearance. There is a rapid IFN response during acute infection, however this does not seem to be predictive of spontaneous clearance. Similarly, HCV-specific humoral responses are commonly seen in HCV-infected chimpanzees and humans, however HCV-specific antibodies are not capable of conferring protection [17]. For reasons still unknown, they are late to emerge during acute infection with the average timing of sero-conversion being >40 days post-infection; and in chronic disease, the quasispecies diversity (in part due to the RNA polymerase being highly error-prone) results in the ability of HCV to evade humoral immune surveillance. In fact, a recent retrospective study of patient H illustrated the process of antigenic escape through the evolution and immune selection of viral glycoprotein variants, effectively outpacing the ability to generate neutralizing antibodies [18].

There is recent interest in NK cells, stimulated in part by the genetic study demonstrating that genes encoding the inhibitory NK cell receptor KIR2DL3 and HLA-C1 confer a pre-disposed ability to resolve HCV infection [19]. Functional studies on humans with distinct KIR/HLA genotypes support the notion that NK cells having a greater capacity to produce IFNγ translates into more efficient viral clearance [20].

As discussed above, more is known concerning the role of T cells during the acute phase of HCV infection, where it plays an important role in viral clearance. The role of T cells in chronic disease is less clear, in part due to the diversity of the viral proteome, a direct result of the immense quasispecies swarm. As for B cells, HCV is quite efficient at escaping the T cell immune and several mechanisms have been described [6]. This includes the evolution of escape mutations, both in the presented peptide epitope and in the consensus motifs required for its processing and presentation [21]. Some studies have focused on the notion of 'original antigen sin,' a belief

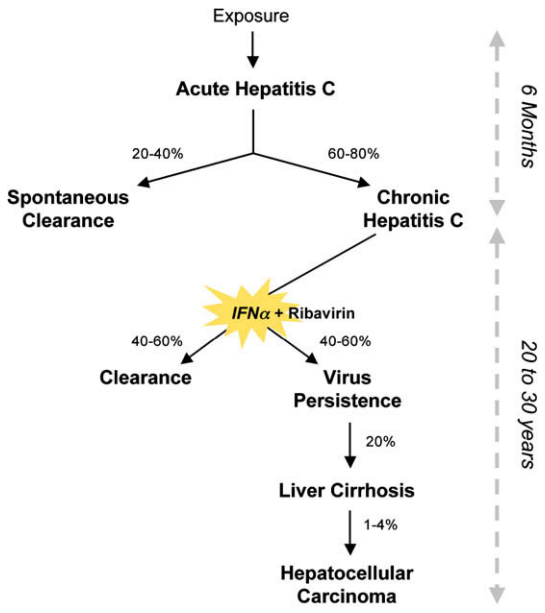

Fig. 1. HCV pathogenesis. Following exposure to the virus, acute HCV infection ensues and is asymptomatic in most of the cases. Infected individuals (60–80%) will not clear the virus and will develop a persistent infection that may last a lifetime, if untreated. Chronic infection triggers a chronic immune response and this persistent liver inflammatory liver is believed to be the cause of cirrhosis (20% of patients over the course of the disease) and can also result in the evolution of hepatocellular carcinoma (1–4% of individuals). Chronic HCV may be treated using a combination of Peg-IFNα_2 and Ribavirin, and depending on the viral genotype, response rates range from 40–80HCV.

that the existing pool of memory T cells present at the time of initial infection might influence the response to HCV [22]. This could favor viral clearance if the heterologous T cells are re-activated so as to generate a profound T cell activation during initial exposure to MHC complexes containing HCV peptide; but it may also be a disadvantage as cross-reactive low-affinity T cells could outcompete the generation of a more effective cohort of HCV-reactive T cells [23]. While this model is difficult to test, several lines of evidence would argue against its relevance to HCV immunity. First, prior exposure to influenza does not correlate with spontaneous resolution of HCV even through T cells reactive to HLA-A2.1/influenza $NA_{231–239}$ complexes cross-reacts with the immunodominant epitope HCV $NS3_{1073–1081}$ [24]. Second, recent data suggest that naïve T cells outcompete memory cells, not the other way around [25]. Finally, more careful analysis indicates that there exist relatively high precursor frequencies of naïve HCV-reactive T cells within the repertoire of presumed, unexposed individuals [26]. Currently, the field is concerned with the expression of the inhibitory receptor programmed death-1 (PD-1), a member of the B7 - CD28 superfamily known to abrogate T cell activation [27]. While PD-1 expression on HCV specific $CD8^+$ T cells was initially thought to be associated with cellular exhaustion [28], a recent study indicates that PD-1 expression level is not sufficient to predict infection outcome or to determine T cell func-

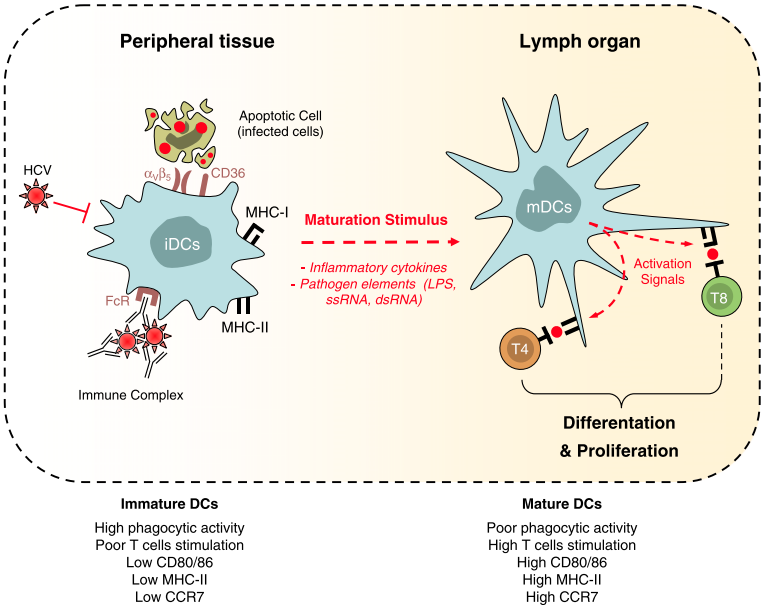

Fig. 2. Role for cDCs in inducing HCV-specific T cell immunity. In periphery, cDCs may capture HCV viral antigen by phagocytosis of infected apoptotic hepatocytes, endocytosis of immune complexes or by macropinocytosis of free virions. Antigenic elements are represented in red [*Note*. Based on their lack of Claudin-1 expression and the absence of miR-122 along with *in vitro* experimentation, we do not favor a role for direct infection.] During the maturation process, DCs may migrate to liver-draining lymph nodes and acquire a mature phenotype. They will also process antigen-derived peptides and present them on MHC-I and MHC-II molecules. MHC/peptides complexes associated with co-stimulatory molecules on DCs surface will trigger activation, differentiation and proliferation of antigen-specific CD4+ and CD8+ T cells. These activated T cells will go back to the site of infection to mediate the cellular immune response. In chronic HCV infection, these cells may be playing a role in chronic liver damage; but they are also critical for achieving viral clearance.

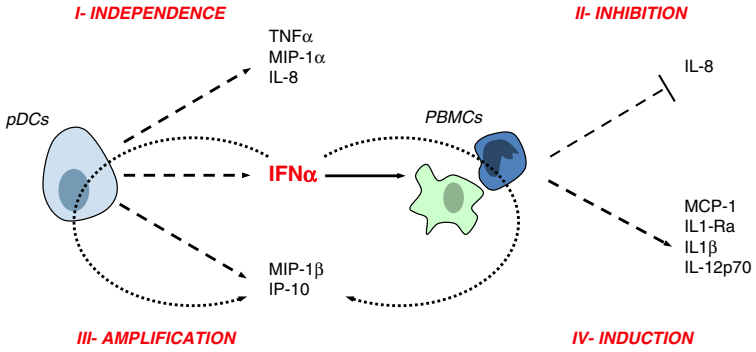

Fig. 3. Activation of pDCs induces four distinct chemokine/cytokine loops thus contributing to the initiation of an inflammatory response. Schematic representation of the four distinct cytokine loops that together help establish the pro-inflammatory response initiated by pDC activation. (I) In the first, activated pDCs secrete factors such as TNFα and MIP1α in a manner that is triggered by TLR engagement and independent of IFNAR stimulation. (II) IL-8 is the only molecule we identified that follows a second pattern of expression – it is secreted by pDCs in response to TLR engagement its production by monocytes is inhibited by IFNAR signaling. Interestingly pDCs are refractory to the inhibitory effects of IFNα suggesting that TLR7 and TLR9 induced IL-8 production follows a different signaling pathway from TNFα-mediated IL-8 stimulation. (III) The third class of molecules is secreted by pDCs in response to TLR engagement with their expression being enhanced by autocrine IFN. Interestingly, in the case of MIP1β and IP-10, pDC derived IFN may also induce other cell types to produce these chemokines in a manner that is apparently independent of direct TLR stimulation. (IV) In the fourth cytokine loop, illustrated by MCP-1 and also true for IL1Ra, IL1β and IL-12p70, the pDCs do not produce but instead induce the production of these molecules by other cell types. These results suggest a coordinated set of events that support recruitment of defined cells and the production of inflammatory analytes for the initiation of an afferent immune response.

tionality in HCV infection [29]. Finally, an effort is being made to determine the relevance of elevated levels of interferon-induced protein-10 (IP-10). Several studies have now reported that high plasma concentrations of

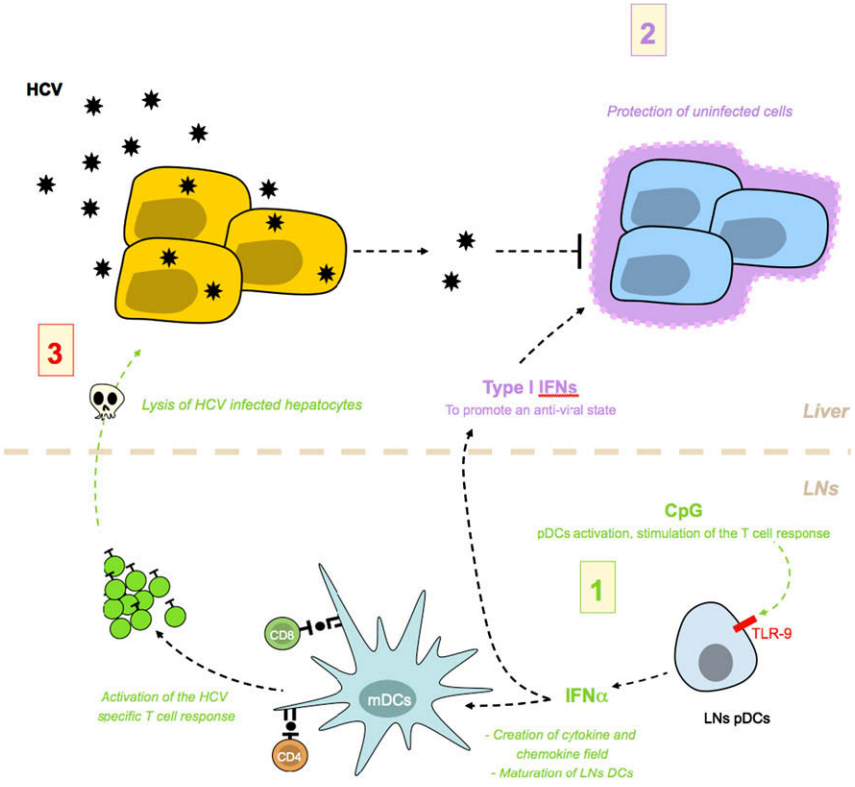

Fig. 4. Actions of pro-immune and anti-viral activity of pDC-derived endogenous IFNs. (1) CpG or other pDC agonists may be viable strategy for stimulating HCV specific T cell response in liver draining lymph nodes. Activated T cells will migrate to the liver and destroy HCV infected hepatocytes. (2) The endogenous IFN may also act to trigger anti-viral defense mechanisms within uninfected hepatocytes, inhibiting new infections and protecting from persistence of adaptive mutations. (3) Together, the dual action of pDC-derived IFN leads to better efficacy of anti-HCV therapy to achieve a definitive clearance of the virus. Advantages include the creation of a more robust cytokine/chemokine network and the avoidance of side effects that result from high doses of exogenous IFN.

IP-10 are a negative indicator in chronically infected patients, predicting failure to respond to therapy [30]. These data are somewhat paradoxical, as IP-10 is a pro-inflammatory chemokine, and is meant to attract T cells (as well as other leukocyte populations) to the liver. Current thought maintains that hormonal levels of IP-10 may in fact antagonize T cells from entering the liver, but additional work will be required to test this hypothesis.

Given the important role of T cells in HCV clearance, conventional dendritic cells (cDCs) have become the target of investigation, with the hypothesis that virus-mediated inhibition of cDC function might offer a possible explanation as to why some patients fail to mount an effective anti-viral immune response. Indeed, cDCs are critical for the priming of antigen-reactive T cells (Fig. 2) [31]. Initial studies focused on monocyte-derived cDCs, but quickly the field recognized the importance of studying cDCs *in situ*. Due to difficulties in gaining access to liver biopsies and the challenge of isolating cells from tissues, most have focused on circulating cells. Some studies have reported impaired allostimulatory function and defects in cDCs acquiring a mature or activated state [32–34]. In contrast, others have reported there to be no phenotypic or functional differences between cDCs isolated from chronically infected HCV patients *versus* those who have successfully cleared their virus [35]. Differences in isolation protocols, maturation cocktails or culturing conditions could all account for such differences, but the clinical evidence steers us away from such an explanation as there is no global impairment of the adaptive immune response in HCV infected individuals.

2. An important role for type I IFNs and a potential role for plasmacytoid dendritic cells

As mentioned, HCV typically reaches high serum titers within one week post-infection and studies of the innate immune response in chimpanzees have demonstrated that there is a profound host response. Transcriptional profiling of liver biopsies indicated induction if type I interferon (IFN) stimulated genes (ISGs) [36,37]. That said, it remains unclear what host sensors are responsible for the production of IFNα/β; which cell types are producing these innate anti-viral effectors; and why type I IFN responses in the liver do not correlate with clearance even though the virus is highly sensitive to IFNs in *in vitro* experiments [38]. Some studies have demonstrated a role for RIG-I in sensing HCV RNA, pointing to the hepatocytes themselves as a possible source of the IFNβ; however recent data that the HCV protein NS3-4A is capable of cleaving Cardif would suggest that the signaling pathways of intracellular host sensors for viral RNA are not active in infected cells [39]. Indeed, liver biopsies from HCV infected patients reveal aberrant localization of Cardif, consistent with it being inactive due its being cleaved from the mitochondrial membrane ([40] and personal communication, Jurg Tschopp). Furthermore, NS3-4A has been shown to inhibit IRF-3 phosphorylation, thus indicating that other host sensors (e.g. TLR3) are inactive in infected hepatocytes. In trying to account for the *in vivo* evidence of IFNα/β production during acute infection, one possibility is that HCV infection results in transient type I IFN production with a rapid shutdown after the virus has replicated and produced its non-structural proteins. These *in vitro* studies, however, do not fit with their being ISG expression during peak viremia [41,36] or with the data that shows endogenous IFNs being produced during the chronic phase of infection [42] . An alternative explanation, which integrates some of these findings, is that the type I IFNs are produced by non-infected hematopoetic cells.

Based on the recent advances in defining the source of type I IFN, many have proposed plasmacytoid dendritic cells (pDCs) as a prime suspect for producing IFN in chronic HCV patients; and we and others have been actively evaluating a role for these cells. pDCs are considered the natural IFN-producing cells, present in peripheral blood and capable of producing 100–1000 times more IFNα than other cell types when exposed to several viruses or bacteria [43,44]. Human pDCs are now well characterized and their ability to produce high amount of type I IFNs has earned them a place as a principal player in innate anti-viral immune responses. Human pDCs express TLR-7 and 9 [45], which recognize ssRNA and dsDNA, respectively, making them poised to respond to infectious pathogens. Whether pDCs also use cytosolic sensors to trigger type I IFN production has not been fully evaluated, but it seems as if they do not express RIG-I. Careful analysis has shown that upon activation, pDCs devote 60% of their global transcriptional activity to type I IFNs production [46] and the secretion of type I IFNs may be observed in less than 4 h after stimulation. In healthy individuals, pDCs are found in the blood and lymph organs and are believed to be absent from tissues in the stable state. Upon activation or during inflammatory responses, pDCs may migrate, both to the T cell area of lymph organs and also into the inflamed tissue parenchyma [47,48]. Regarding migration to lymph tissue, it is interesting to note that trafficking is directed to LNs that drain sites of inflammation [49] and that the path they travel differs from the one used by cDCs. In contrast to resting cDCs, which reside in tissues and migrate to the T cell area of local lymph nodes via the afferent lymphatics [31], pDCs migrate via the high endothelial venules (HEV) [50] involving L-selectin and CCR7

[49,51]. The mechanism of trafficking into the tissue remains less well defined, but may involve CXCR3, the receptor for IP-10 and two other related, interferon-induced chemokines, I-TAC and MIG. Once pDCs reach their destination, they are thought to stimulate aspects of both the innate and the adaptive immune response.

pDC derived-IFN may participate in a direct antiviral response, however increasing evidence suggests that it is through activation of other effector arms of the immune system that IFNα/β mediates HCV clearance. Indeed, both NK and $CD8^+$ T cells are regulated by type I IFNs [52]. Type I IFNs have been shown to directly activate NK cells to enhance their cytotoxic activity [53] and also induce IL-15 production [54], which plays a critical role in proliferation and maintenance of NK cells. Concerning CTLs, a recent study has shown that $CD8^+$ T cells lacking the IFNα/β receptor (IFNAR) are impaired in their ability to expand and differentiate into effector CTLs in the context of a viral infection [55]. Just as important may be the ability of pDCs to produce type I IFNs within lymphoid tissue, serving as an endogenous adjuvant for cDCs and provoking an enhanced production of IL-12p35 – the limiting subunit in IL-12p70, important for the differentiation of $CD4^+$ T cells toward the Th1 effector lineage and the priming of $CD8^+$ T cells. Whether IFNs also promote maturation of immature cDCs may turn out to be species-specific – this seems to be true in mouse models as shown in both *in vitro* and *in vivo* studies [56–58]; however human DCs do not behave in a similar manner. In addition to the production of IFNs, pDCs may directly interact with cDCs, engaging CD40 thus providing a distinct mechanism of cDC activation [59].

Our recent work has contributed in this area of study by providing a first-generation multi-analyte profile (MAP) of how pDCs serve to bridge innate and adaptive immune responses in the context of systemic viral or bacterial infections [60]. Taking advantage of high-quality data coming from medium-throughput proteomic tools such as Luminex xMAP technology, we have carried out an in-depth analysis of the cytokines and chemokines secreted when activated pDCs interact with other innate cells within the immune system (Fig. 3). Interestingly, we identified four distinct cytokine loops by which pDCs contribute to the initiation of an inflammatory response: (i) molecules secreted by the pDC itself and independent of IFN production; (ii) molecules secreted by the pDC and inhibited by paracrine IFN; (iii) molecules secreted by the pDC and amplified by paracrine IFN; and (iv) molecules not produced by pDCs but triggered by paracrine IFN. These cytokine/chemokine loops are shown here and have helped to provide a foundation for understanding the functional status of pDCs in different disease states.

3. Plasmacytoid DCs in HCV disease pathogenesis: Friend or Foe?

The observations concerning the role of type I IFN in facilitating cDCs to prime $CD8^+$ T cells has led many to consider the possibility that HCV inhibits pDC function, thereby blocking endogenous IFN production. As it is difficult to reconcile this data with the fact that individuals chronically infected with HCV are not immunocompromised and that they have high levels of endogenous IFN [42], we evaluated the phenotypic measures and functional activity of patient pDCs as compared to healthy controls and HCV patients that had successfully cleared their virus (sustained virologic responders or SVR). We found no obvious defect in circulating pDCs. This conclusion was based on studies using both TLR7 and TLR9 agonists, assessing the ability of patient pDCs to: upregulate activation markers and homing receptors upon pDC activation; produce a broad array of cytokines and chemokines; and create an inflammatory network via their direct effects on other cell populations within the peripheral blood. While our results are in agreement with the study of Piccioli et al. [61], several groups have reported subversion of pDC in chronic HCV patients [34,62,63]. One important consideration is that we tested cytokine and chemokine production on a per-pDC basis [60,64]. This likely accounts for the differences reported by Szabo et al., who monitored IFN production within total PBMCs and did not account for the fact that there are 2–3× fewer pDCs in the patients with chronic HCV as compared to their normal control population [63]. Moreover, in some of the reported studies, pDCs were purified from PBMCs utilizing anti-BDCA-2 antibodies – importantly, BDCA-2 engagement is known to affect pDC function and might have confounded some of the findings [65]. It is less clear why Murakami et al. and Kanto et al. observed impaired pDC function though it may be a result of different culturing conditions used, as pDC survival *ex vivo* is quite poor in the absence of exogenous growth factors or adequate TLR stimulation.

In addition to immunologic measures of pDC function, there has been interest in defining whether there exists extra-hepatic sites of HCV replication. Indeed, demonstration that pDCs are infected by HCV might help support the notion of immune subversion. With the recent advances in the field concerning the generation of replication competent HCV and the ability to engineer reporter viruses, it has become easier to directly test this hypothesis. Using recombinant viruses engineered to express renilla luciferase and a highly sensitive measure of infection, our studies suggested that there is no direct infection of pDCs. Given the lack of an intrinsic defect in chronically infected HCV patient pDCs, and the ability of circulating cells to respond appropriately to TLR stimulation, this result is not very surpris-

ing. Moreover, it is clear that pDCs (as well as cDCs for that matter), lack the expression of claudin-1, one of the co-receptors for HCV entry into target cells [66,60].

4. Plasmacytoid DCs as a viable drug target for chronic HCV patients

These observations open up the possibility for pDCs to be harnessed for their therapeutic potential (Fig. 4). Many *in vivo* experiments using mouse models have explored how CpG could potentiate the cellular response to specific antigens [67–69], presumably acting via pDC stimulation. There are limitations in these systems, however, due to the fact that CpG will directly activate cDCs, in turn triggering maturation and T cell priming [69]. Moreover, there is evidence for direct activation of T cells in mice treated with CpG [70].

In contrast to mice, human cDCs do not express TLR-9. This makes it difficult to translate findings in experimental models to humans. Nonetheless, it is believed pDCs will be able to create an inflammatory milieu that will facilitate an enhance cellular immune response. Use of pDC agonists may in fact offer an alternative to the use of exogenous rIFNα_2. It is worthy of mention that this approach may have three important advantages over conventional therapy: (i) it facilitates delivery of the IFN stimulation to the lymph node micro-environment. This is achieved by harnessing the biology of activated pDCs, which upregulate CCR7 and traffic to the site of T cell priming. In this way it offers a second potential benefit – (ii) by concentrating the IFNα production to the lymphoid organs, it may be possible to achieve similar activity with lower systemic levels of IFNα. Thus, the stimulation of the hypothalamic–pituitary–adrenal axis may be less significant, helping to avoid some of the more severe side effects of therapy such as mood disorders. (iii) This strategy also capitalizes on two waves of chemokine/cytokine production initiated by activated pDCs with the secretion of several known (e.g. TNFα and CCL3) and possibly many additional undefined analytes that are produced in a manner that is independent of IFNα/β [71,60].

One such clinical trial was carried out by Coley Pharmaceuticals to evaluate the efficiency of CpG as a treatment for chronic HCV. A phase 1b trial using CpG 10101 was conducted in chronically infected HCV patients and the results have been recently published [72]. They reported low levels of type I IFNs present in the plasma, but a strong IFN signature (based on IP-10 production and 2′5′-OAS expression in PBMCs). Most notably, CpG treatment was associated with a dose-dependent decrease in viral load. These results are interesting as low plasma concentration of type I IFNα yielded a clinical response, suggesting that indeed it may be possible to segregate the pro-immune effects from the neuro-endocrine effects generally associated with rIFNα therapy. CpG treatment was also associated with a global activation of leucocytes. Activation of T cells, B cells, NK cells and pDCs were observed with a coincident reduction in the number circulating cells (also an indicator of activation) [73].

5. Plasmacytoid DCs remain on the list of suspects

While our studies concluded that pDCs isolated from patients with chronic HCV infection are phenotypically and functionally normal; and we are enthusiastic about pDC as a potential drug target, there remains some concern in using this approach and there may be some new data to argue that pDCs are not yet off the hook.

One consistent observation across most (if not all) of the published studies is that the relative percentage of circulating pDCs per total PBMCs is decreased in chronically infected HCV patients [64,60,33]. While this was also the case in patients who had achieved SVR [64,60] as well as individuals with non-viral liver disease [64], it begs the question as to whether the pDC numbers are decreased due to poor production, increased death, or differentiation and migration of the cells into sites of inflammation and/or lymphoid organs. There is no data regarding the first two proposals, but two reports have offered data concerning pDC trafficking, suggesting this is not the cause for lower numbers of circulating pDCs. Lai et al. evaluated liver DCs, comparing cDC and pDC populations from chronic HCV patients and individuals with non-viral liver disease. In contrast to the cDCs, which were more numerous and phenotypically and functionally mature, pDCs were present at lower frequency and expressed higher levels of BDCA-2 (a C-type lectin that negatively regulates IFNα/β production). Tang et al. studied the liver draining lymph nodes of chronic HCV patients, and compared to normal individuals, there was no marked increase in pDC number. One caveat that applies to both studies is that pDC differentiation/activation in settings of chronic inflammation remains poorly defined and surface expression of lineage markers may be altered in HCV infected patients.

There is also the concern that pDCs studied *ex vivo* do not recapitulate the effect of viral antigens on pDC function. While both reports that have utilized replication competent HCV to study pDC infection conclude that they are not susceptible, there remains the possibility that engagement of surface receptors by E1-E2 or soluble core might alter their function. pDCs express CD81 [74], which has been shown to engage HCV envelope [75,76]. In addition, circulating pDCs express C1qR (unpublished data), and while it has

not yet been formally demonstrated in pDCs, core binding to C1qR in several other cell types has been reported to be counter-inflammatory [77]. This may also have an impact on the use of pDCs as a drug target. In fact, one recent study suggested that HCV engagement of receptors on pDCs results in the inhibition of TLR9. While it remains to be dissected at a molecular level, and additional information is required to define how TLR9 but not TLR7 signaling is affected, this observation may impact the potential use of CpG in the treatment of HCV patients. For these types of studies we must also identify strategies to deal with the diversity of viral antigens within the quasispecies swarm as we already know, for example, that specific core variants can influence the immune system in unique ways [78].

6. Critical unknowns

Part of the confusion in defining the role of pDCs in HCV pathogenesis is that many questions remain about the mechanism by which type I IFNs mediate viral clearance. We will have to understand more about the endogenous IFNs produced during both the acute and chronic phases of infection: which cell(s) produce it; do type I IFNs mediate spontaneous clearance and if so why do they fail to limit HCV replication in chronic phase; and how are endogenous sources different from exogenous IFNs? We also believe that the working model for understanding the immune pathogenesis of HCV should be re-evaluated. HCV reactive T cells are present in chronically infected patients. In fact they are likely the effector cells responsible for the persistent liver damage. As such, pDCs may be involved in their activation and instead of looking for pDC dysfunction; perhaps they are hyper-activated as a result of a chronic infection and inflammation.

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