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Chemokine Responses to Hepatitis C Virus and Their Impact in Mediating the Treatment Responses of Antiviral Treatment

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1. Introduction

The hepatitis C virus (HCV) is a global health challenge with strong regional implications (Shepard et al, 2005). Currently, about 170 million people throughout the world are chronically HCV infected and it is the most important cause of liver disease worldwide. During the last 30 years the mode of transmission in industrial countries has changed from infection by medical use of contaminated blood products to infection by shared utensils by drug abusers. The incidence of HCV infection in Europe increased during the 1990`s (Rantala & van de Laar, 2008). It is unknown if this trend of increased incidence in Europe and worldwide has persisted after 2000.

Following acute HCV infection approximately 80 % of adults and between 50 to 60% of children develop chronic disease (Vogt et al, 1999). The reasons for the ineffective clearance of HCV virus is unknown, but most likely there are viral escape factors and host factors such as inappropriate immune based viral clearance. Progression of chronic HCV infection occurs in a proportion of infected subjects in a sequence via liver fibrosis to liver cirrhosis and finally death due either to liver failure or to hepatocellular carcinoma (HCC). The rate of progression is affected by various factors such as age at infection, gender, alcohol consumption, and co-infection particularly with human immunodeficiency virus (HIV), but also with hepatitis B virus (HBV) (Poynard et al, 2001). When compensated cirrhosis is established, the probability of decompensation is estimated to be 15-17 % within 2-3 years. The burden of expenses to health services due to HCV related disease has been predicted to be considerable in the future. In 2004, 23 % of all liver transplantations in Europe were related to HCV infection. Most likely the incidence of decompensated cirrhosis and HCC will increase substantially in the next few decades, due to the steady increase of HCV positive persons-at-risk (Lehman & Wilson, 2009).

Chronic HCV infection is treated with a combination of pegylated interferon (peg-IFN)-α, and the synthetic nucleoside analogue ribavirin. By this combination sustained virological response (SVR) is achieved in between 40 % and 50 % for genotype 1 and as high as

approximately 85 % for genotypes 2 and 3. Due to the limited success rate of this combined therapy approach, triple therapy options have been suggested. Thus, both protease inhibitors and polymerase inhibitors has been tested as addition to PEG-interferon and ribavirin. These drugs are not approved by the authorities as standard treatment since they are still under investigation. Depending of the efficacy of triple therapy, the future need for liver transplantation may be reduced, with a considerable impact on health expenditures.

Both viral and host factors are determinants for the spontaneous elimination or persistence of HCV. The high risk for chronic infection is most likely caused by a lack of a strong and specific immune response to viral antigens. On the other hand, an overly powerful immune response may lead to acute liver failure as seen in rare cases of hepatitis A and hepatitis B. The frequent mutations of HCV are challenging to the host immune response and results in a high risk for viral escape. In the recent years HCV research has been focused on the innate and adaptive response to the virus. Special attention has been put into the role of chemokines and their receptors which are responsible for recruitment of leukocytes from blood stream to the affected tissue. It has been proposed that this is one of the most critical immunological steps for an effective clearance of the virus. We have recently reported that in the antiviral treatment, SVR is dependent on a rapid (24 hours) chemokine response (Florholmen et al, 2011). This has initiated the present review of the immunological mechanisms against the HCV with a special emphasis on the chemokine response.

2. Aims

The first part of the chapter we will review the chemokine concept and its role in the HCV pathogenesis, their role in the innate and adaptive response to HCV leading to liver inflammation and liver fibrosis. The second part will concentrate on the chemokine response during antiviral treatment using interferon, ribavirin and the new nucleoside analogues.

3. Hepatitis C virus

HCV is a positive single stranded RNA virus with regions coding for structural peptides (an envelope, 9000 bases) and regions coding for non-structural (NS) peptides (1 – 5) (Myrmel et al, 2009). Eleven genotypes have so far been described and 6 are commonly diagnosed. The Genotypes 1, 4 and 6 respond to antiviral therapy (interferon (IFN) + ribavirin) with an SVR of 50 % and 85 %, respectively. The virus has a high production of estimated 1012 virions per day, with an average half-life of 2.7 h and a turn-over rate close to 99 %. The calculated annual mutation rate is in the order of 1.5–2.0 $x10^3$ nucleotide substitutions per site. Furthermore, the virus has no proofreading mechanisms. The naturally occurring mutations may thereby enhance resistance both to endogenous immune responses and to anti-HCV therapy. Mutations conferring resistance of hepatitis C virus to the new treatment agents, the NS3 protease inhibitors, have been described (Halfon & Locarnini, 2011). The various genotypes have not been associated to specific pathobiology. As described above, however, the pattern of genotype related resistance to therapy has been extensively documented. The molecular mechanisms of this resistance have been described to some extent. Of great interest is the interferon sensitivity-determining region (ISDR) in the non-structural NSR5 part of the virus genome. Amino acid substitutions in ISDR have been related to increased SVR of anti-HCV treatment (for review, see (Chayama & Hayes, 2011)).

4. Antiviral immune response

The immunological response to viral infection is a complex interplay between host tissue cells, the innate and adaptive immune responses. A series of mediators, systemic and paracrine, as well as cell-cell interaction will in most cases result in clearance of infection. Some viruses have developed strategies of immune evasion and can therefore establish chronic infections. In case of HCV infection, the resulting chronic inflammatory response is actually harmful to the host by driving development of fibrosis, cirrhosis, and liver failure or HCC.

Viral pathogens can enter the host in several ways, the mucosal membranes being the most frequently used. A few viruses mainly spread via direct inoculation in the bloodstream, HCV being a classic example. Each type of virus has its preferred host cell type based on specific homing mechanisms. The HCV tropism for hepatocytes and internalization process is partly characterised and involve cluster of differentiation (CD) 81 and Claudin-1 (Thorley et al, 2010).

Viral pathogens do not have metabolism and rely on modifying the host cell production apparatus to its own benefit. A range of defensive mechanisms has been developed in response to this strategy. The end result from these mechanisms is mostly death by lysis or apoptosis of the infected cell, while at the same time restricting spread of the infectious agent to neighbouring cells in the infected site. A short overview of the general immune response to viral infection with special emphasis on mechanisms related to the anti-HCV response will be given in the following.

Leukocyte trafficking is a very important feature of the immune system allowing for the immune cells to patrol the entire host organism and thereby detect any intruding microorganism, bacteria or virus. The ability to generate a rapid local response when an intruder has been detected is based on homing mechanisms which mainly are triggered by early response cytokines and chemokines. As it turns out in the case of HCV infection, chemokines may also be central in generating an effective immune response following pharmacological intervention, as a swift chemokine response early in the course of treatment can predict a sustained virological response.

4.1 Innate receptor systems

The innate immune defence consists of several specialized cell types like dendritic cells (DC's) granulocytes, natural killer (NK) cells and macrophages. A common trait for these cell types is the pattern recognition receptors (PRR's) consisting of both intracellular and transmembrane subtypes. The nucleotide oligomerization domain (NOD) receptors are intracellular and the toll like receptors (TLR's) are transmembrane receptors primarily directed towards the extracellular compartment. These innate receptors detect common motifs from pathogenic microorganisms including both bacteria and viruses. Upon triggering the receptor an intracellular pathway common to most of the TLR's involve myeloid differentiation primary response gene 88 (MyD88) and interleukin-1 receptor-associated kinase (IRAK) kinases leading to activation of NF-κ-B and transcription of pro-inflammatory cytokines.

The professional antigen presenting cell i.e. dendritic cell carry an array of pattern recognition receptors and these cells are crucial for the initiation of an adaptive response. All cell lines of the adaptive system must be stimulated by DC's in order to raise a response. The DC determines the profile of the adaptive system depending on its cytokine secretion pattern.

Most viruses have specific binding strategies for entry into host cells. This leads to a tropism of the virus rendering specific target cells its point of attack depending on the homing mechanism. Subsequently the virus particle is disassembled. At this point intracellular receptors may detect the pathogen and trigger production of early viral response cytokines – mainly type I interferons like IFN-α and -β. A possible trigger of IFN production can be double stranded RNA which has been found to stimulate type I interferons in vitro. Intracellular TLR3 is likely triggered by viral dsRNA.

4.1.1 Interferons

The family of interferons consists of three subgroups of mediators with high sequence homology. The first interferons were described by their physiological effects i.e. their ability to *interfere* with viral replication in cell cultures. The type I interferons is a group of five members: IFN -α, -β, -ω, -κ, and limitin. IFN-α and IFN-β can be secreted by practically all infected cells types following viral infection and the production of these cytokines is therefore not restricted to immune competent cells.

Type I IFN has a common receptor IFN-αβ-R which signals via the JAK-STAT pathway (JAK is short for Janus Kinase, and STAT is short for Signal Transducer and Activator of Transcription) towards the *Interferon stimulated response element* ISRE in the cell nucleus and induce transcription of several interferon inducible genes which in turn increase degradation of viral RNA and inhibit translation processes. The secreted interferon acts on both the secreting cell (autocrine stimulation) and neighbouring cells (paracrine stimulation) thus inhibiting local spread of the viral infection. Furthermore, interferons up-regulate major histocompatibility complex I (MHC-I) and thereby enhance the display of viral antigens to the adaptive effector cells (see below). Interferons also activate NK cells and thereby facilitate killing of infected cells. Thus, the entry of a virus in a cell induce production of interferons which in turn help protect neighbouring cells from infection but also facilitate killing of the infected cell by NK cells and/or antigen specific cytotoxic T cells.

4.1.2 Natural killer cells

NK cells are part of the innate immune response, and have an important role in combating viral infections in the early phase until the specific adaptive cytotoxic response is raised. The NK cell is believed to distinguish infected from normal cells via an intricate process involving both stimulatory and inhibitory signalling. A set of immunoglobulin-like receptors (*K*iller cell *I*mmunoglobulin-like *R*eceptors: KIR's) and C-type lectins are involved in activation of the NK cell. A strong inhibitory signal is presentation of MHC-I on the cell surface which may be recognized by KIR's or CD94:NKG2. As part of the microbial survival strategy many viral infections inhibit MHC-I display in order to restrain presentation of antigens to the adaptive response. This strategy removes the inhibitory signal to NK cells and the infected cells display only activation signals to the NK cells and will be eliminated. Some viruses induce conformational change of MHC-I with the same result. Thus, the NK cell may be able to detect the infected cell even if it evades the adaptive response by cytotoxic CD8+ T cells (see below).

If stimulated by IFN-α or IFN-β the NK cell increase cytotoxic activity by a factor 20-100. The activated NK cell also secretes mediators important to direct the early response patterns in the tissue. The effector action of NK cells is completed by close binding to the infected cell

and may use different pathways including lysis of the cell membrane by perforins or triggering of apoptosis by interaction between Fas (CD95) and FasL (CD95L). The role of NK cells in HCV infection has been reviewed recently (Cheent & Khakoo, 2011).

4.1.3 Adaptive immune response

The adaptive immune response is antigen specific and can identify foreign antigens with great sensitivity and specificity. It consists of both humoral and cellular parts, of which especially the former can enhance the function of the innate response. Opsonising antibodies can boost the performance of innate phagocytes like neutrophil granulocytes and macrophages, and also enhance the function of an NK cell mediated cytotoxic response. The specific adaptive responses are modulated in phases: in the early response phase, activated cells undergo clonal expansion. This is followed by an effector phase where the strike against the microorganism is delivered. Finally the response is attenuated after elimination of the infectious agent – this phase is controlled by regulatory T cells (see below). In the process of down regulating the adaptive response, a small population of memory cells will remain dormant. These memory cells will be able to launch a swift and efficient adaptive response if the host should encounter the same agent at a later time.

A common feature of the adaptive immune system is that the cells are unable to generate a response without help of the innate system or other parts of the adaptive response. The T cell receptor only recognizes its epitope in the context of an MHC molecule in combination with co-stimulatory factors. Each of the adaptive cell populations are restricted by specific mechanisms. T helper (Th) cells must be stimulated by their antigen presented on MHC-II by antigen presenting cells (APC's). Cytotoxic T cells must be triggered by their epitope presented on MHC-I by the target cell. B cells bind their antigen on the B-cell receptor and internalize it for degradation and presentation on the surface by MHC-II. This allows for co-stimulation by contact with, and cytokine secretion from, Th cells with the same specificity. In this way, the B cell can also present antigens for stimulation of Th cells. The local cytokine milieu at the time of stimulation determines which effector profile the stimulated Th cell will have: IFN-γ and interleukin (IL)-12A: Th1; IL-4: Th2; transforming growth factor-β: Th3; IL-6 and TGF-β: Th17; IL-10: T regulatory-1 (Tr1).

In a viral infection, the adaptive immune response is triggered by presented antigens towards a classic Th1 profile enhancing a cytotoxic effector response. The cytotoxic CD8+ T cell is antigen specific in contrast to the NK cell, and stimulation of this cell line is primarily by Th1 cytokines like IFN-gamma and IL12A. The specificity rely on the T cell receptor recognition of the antigen as presented in the groove of a MHC-I molecule on the surface of the cell in question. Also, the cytotoxic T cell and the innate NK cell tend to mirror the Th profile in the immune response at hand, so these effector cells secrete cytokines and tend to enhance the milieu given by Th cells.

The humoral part of the adaptive response also enhances the phagocyte and cytotoxic responses by a mechanism called opsonisation. Innate immune cells like neutrophil granulocytes, macrophages and NK cells carry receptors for the stem of the antibody (the FC part). Antibodies bind their target in the binding sites, and can crosslink the target to FC receptors on the innate cell. This way a viral particle on the surface of cells can be "visualized" to innate cells. Antibodies in blood, mucosal membranes, and the extracellular space also neutralise viral particles by binding.

4.1.4 Regulatory T cells

The regulatory T cells include distinct subpopulations of which some are non-specific ($CD25^{high}$ natural T_{reg}) and others are antigen specific (Tr1 and Th3). A common trait for regulatory T cells is the expression of forkhead family transcription factor FOXP3. The natural T_{reg}'s are generated in the thymus and characterised by a high expression of CD25 (IL-2 receptor). Natural T_{reg}'s seem to act primarily by direct cell contact similar to the actions of NK cells. In contrast, the antigen specific regulatory T cells act by secretion of cytokines like IL-10 and TGF-beta. The regulatory cytokines and direct cell contact actions keep an important brake on the immune system in general, as an uncontrolled pro-inflammatory response can lead to serious pathology and even organ destruction. Thus, the function of regulatory T cells is to balance the response of pro-inflammatory immune cells in order to keep homeostasis and avoid excessive tissue damage as well as resolving inflammation when the infection has been eliminated. The balance between pro- and anti-inflammatory stimuli is delicate. The perfect immune response is swift, efficient, and causes a minimum of damage to host cells. Of course this is a compromise and the balance may tip in either direction. In HCV infection, an overly powerful response would lead to acute liver failure and death; it has been suggested that the development of cirrhosis in longstanding HCV infection is a result of an overly aggressive chronic inflammation (Larrubia et al, 2008).

4.2 Homing and chemotaxis

All of the cell types described above must be recruited to the site of infection in order to perform their part of the anti-microbial response. Though the adaptive humoral response and antibody production in most cases takes place in the regional lymph nodes, the B cells and Th cells must still be recruited and activated. The recruitment of leukocytes to the site of infection is an intricate process controlled by homing mechanisms. Some central mechanisms of leukocyte homing will be presented in the following.

Chemotaxis is a basic behaviour seen in bacteria, primitive organisms, and several cell types in the immune system. The definition of chemotaxis is that the cell in question moves towards a higher concentration of a given chemotactic compound. As the name implies, chemokines are chemotactic compounds and a cell releasing chemokines will attract the attention of nearby immune cells.

4.2.1 Chemokines

The chemokines are a family of highly homologous small proteins with a common *Greek key* structure. These mediators have a key role in the earliest phases of infection. They can be released by many cell types in response to infectious agents and to physical damage. Chemokines can recruit cells of both innate and adaptive lines to the site of infection.

The chemokines can be divided into two main subgroups: the CC group (at least 27 members named CCL1-28) with 2 adjacent cystein residues close to the amino-terminal, and the CXC group (at least 17 members named CXCL1-17) in which the two cystein residues are spaced by a single amino-acid. This structural difference is important because each subgroup has its own set of receptors. Some receptor cross-reaction within subgroup occurs, and each chemokine

may react with more than one of the receptors of the group. In addition to these main groups a few chemokines of C and CX3C group with their own receptor types have been described.

4.2.2 Chemokine receptors

The chemokine receptors have a common structure with a 7-transmembrane helix coupled to G-protein intracellular signalling. The subfamilies each have a set of chemokine receptors expressed on target cells. So far, ten CC receptors (CCR1-10) and seven CXC receptors (CXCR1-7) have been described. The system of chemokines and their receptors is quite complex and so far only partly described. However, at least theoretically, different chemokine secretion profiles combined with the receptor profiles of the target cells allow for close regulation of the homing process according to the infectious agent.

4.2.3 Chemokine effects

The chemokines trigger conformational change in the adhesion molecules (leukocyte integrins) on cell surface of leucocytes, thereby enabling a stable binding of the leukocyte to intercellular adhesion molecules (ICAM's) on the vessel wall. When the leukocyte is bound to the vessel wall it is able to squeeze between endothelial cells and enter the tissue. The cells first recruited are neutrophils, then later comes monocytes and immature dendritic cells. The chemokine activation also includes arming of the cells as effectors.

4.2.4 Homing

Upon chemokine activation endothelial cells present selectins and ICAM's on the luminal surface. Leukocytes tend to roll along the endothelial surface due to weak binding between endothelial selectin and leukocyte sialyl-Lewis^x (s-Le^x) blood group antigen. If the leukocyte integrin profile matches the ICAM a strong binding is established. This binding is enhanced further by conformational changes in the leukocyte integrin triggered by chemokine stimulation. When the cell is tightly bound, extravasation by diapedesis can be initiated. After extravasation, further movement along a chemotactic gradient to the site of infection follows.

The endothelial cells will be further activated by early response cytokines like tumor necrosis factor (TNF)-α. The chemokine activation of the neutrophil granulocyte will also stimulate the oxidative burst, which is a characteristic of the effector profile in this cell type.

4.2.5 Chemokines in the adaptive immune response

Certain chemokine receptors are expressed in certain immune profile cells. Thus a Th1 chemokine receptor set can be defined: CCR5 and CXCR3; while CCR3, CCR4 and CCR8 are linked to Th2 responses (Larrubia et al, 2008). Therefore, in a viral infection a certain subset of chemokines are especially interesting, as the Th1 response is considered the adequate and efficient response type. Ligands for the CXCR3 (Interferon gamma induced protein 10 (IP-10), Monokine induced by gamma interferon (Mig), Interferon-inducible T-cell alpha chemoattractant (I-TAC)) and CCR5 (Regulated on Activation, Normal T Expressed and Secreted (RANTES), macrophage inflammatory protein (MIP)-1-alpha, and MIP-1-beta) are theoretically crucial for the initiation of response and resolution of infection. Indeed, a frame shift mutation on the CCR5 receptor increases susceptibility to HCV infection (Woitas et al,

2002). In effect, both theoretical and experimental data support the crucial role of the chemokine response for mounting an efficient resolution of the viral infection.

4.3 Special immunobiological features of the liver

The liver is an immunotolerant organ with constitutive high expression of IL-10 and TGF-β (Crispe et al, 2006; Manigold & Racanelli, 2007). The sinusoids are inhabited by a special type of 'pit cells'; large granular lymphocytes of the NK cell trait. Data from mouse studies indicate that the NK cells of the liver tend to secrete more regulatory cytokines and less pro-inflammatory cytokines than their peripheral counterparts (Cheent & Khakoo, 2011; Lassen et al, 2010). When the HCV virus enters this environment, a proper immune response must be launched, and to this end a massive recruitment of different types of immune cells is needed. Cells of the innate immune system such as dendritic cells and NK cells are important for the initial response and stimulation of a proper adaptive Th1 response including antigen specific T helper cells, cytotoxic T cells and B cells as well as inducible regulatory T cells. All of these cell types must be recruited from the circulation and to this end an array of chemotactic signals are activated.

Chemokines have local effects on endothelium activating processes for trans-endothelial migration, and the leucocytes also have chemokine receptors activating leukocytes rolling and binding of selectins to integrin receptors. Thus chemokines have an important role in infection response allowing extravasation of leucocytes to the site of infection. As the liver has some inherent immunetolerance as mentioned above, this recruitment of external cells is especially important. Furthermore, considering the treatment of HCV with peg-IFN-α and Ribavirin the therapy may be efficient by altering the profile and composition of inflammatory cells in the liver. In this respect, the leukocyte recruitment seems have a key role in resolution of the infection.

4.3.1 Immune response to HCV infection

HCV has parenchymal liver cells as primary target utilizing CD81, claudin-1, and possibly the LDL receptor. After binding of to a target cell, the viral particle is internalized and disassembled in the cytoplasm. HCV virus has developed strategies to evade some of the basic antiviral mechanisms described above. The early IFN-β response can be blunted by cleaving adaptor proteins necessary for activating IFN transcription, and can also inhibit the JAK-STAT pathway thus inhibiting the intracellular effector events after stimulation by IFN-α.

Relatively recently a new series of λ-interferons have been described. These include the highly homologous IL-28A (IFN-λ-2), IL-28B (IFN-λ-3) and IL-29 (IFN-λ-1). Especially IL-28B has turned out to be interesting in regard to HCV infection. It seems that a firm IL-28B response is necessary for viral clearing, and that CC genotype in the rs12979860 single nucleotide polymorphism (SNP) (The Duke) in the promoter of the IL-28B gene is associated with a higher rate of spontaneous resolution of infection and also can predict response to treatment with peg-IFN-α (Langhans et al, 2011).

4.3.2 Chemokines in the context of HCV infection

Considering the immunotolerant milieu of the liver, an efficient immune response against a pathogen like HCV must be based on a considerable influx of fresh immune cells. In this

respect, the chemokine response is crucial and may be one of the main factors that determine if the infection becomes chronic or is spontaneously resolved. One of the effects of peg-IFN-α therapy is to increase the pro-inflammatory response including the chemokine response allowing for fresh Th1 cells, B cells, NK cells, and dendritic cells to engage the virus. As such, the chemokine response can be seen as a common marker for a step-up in the immune response in initiation of treatment. Whether observed chemokine responses are directly triggered by the peg-IFN-α or result from a general increase in immunologic activity in the liver remains to be determined. However, as a biological marker of sustained virological response, the early rise in chemokine activity is interesting.

5. Treatment of hepatitis C

As described in the Introduction, peg-IFN-α in combination with the nucleoside analogue ribavirin is the standard treatment of HCV infection. Upcoming new drugs are albumin-INF-α, and nucleoside analogues or protease inhibitors, and nucleoside analogue/non-nucleoside analogue polymerase inhibitors. In general, these agents act via inhibitory mechanisms on the HCV gene to reduce the viral replication. These new anti HCV drugs exert their effects directly on the virus replication: protein kinase inhibitors on NS3A/B and polymerase inhibitors on NS5A/B (for review, see (Vezali et al, 2011)).

5.1 IFN-α

The mechanisms of action of peg-IFN-α are through indirect activating of the immune system and a direct antiviral mechanism at the interferon-sensitive sites of the HCV inhibiting the transcription. These mechanisms of action are rather complex and beyond the scope of this presentation. Briefly, peg-INF-α triggers a cascade of intracellular events including activations of IFN-inducible genes and increased synthesis of IFN-induced proteins (Katze et al, 2002). These proteins such as RNA-dependent protein kinase inhibit intracellular virus replication by a RNA-degrading mechanism. Peg-IFN-α also inhibits the viral replication indirectly via an immune response and most likely via activation of immune cells. These are complex mechanisms such as increased MHC-I expression and activation of immune cells with cytokine secretion. Finally, peg-IFN-α also induces an immuno-modulation in the favour of a Th1 response and an inhibition of a Th2 response (see fig 1, for review, see (Vezali et al, 2011)).

5.2 Ribavirin

The exact mode of action of ribavirin is unknown. As ribavirin alone does not inhibit the virus replication, a synergistic action together with IFN-α has been proposed. The proposed mechanisms of actions of ribavirin are: 1. an indirect host change of Th profile from a Th2 to a Th1 profile. 2. A direct inhibitory effect on the NS5B encoded RNA dependent RNA polymerase (for review, see (Lau et al, 2002)) (figure 1)

6. Chemokines in antiviral therapy

As described above, the chemokines play a pivotal role in the chemotactic immune response to HCV by acting via their specific receptors on immune active cells. The role of chemokines in the antiviral treatment is so far only incompletely understood. Of special interest for the

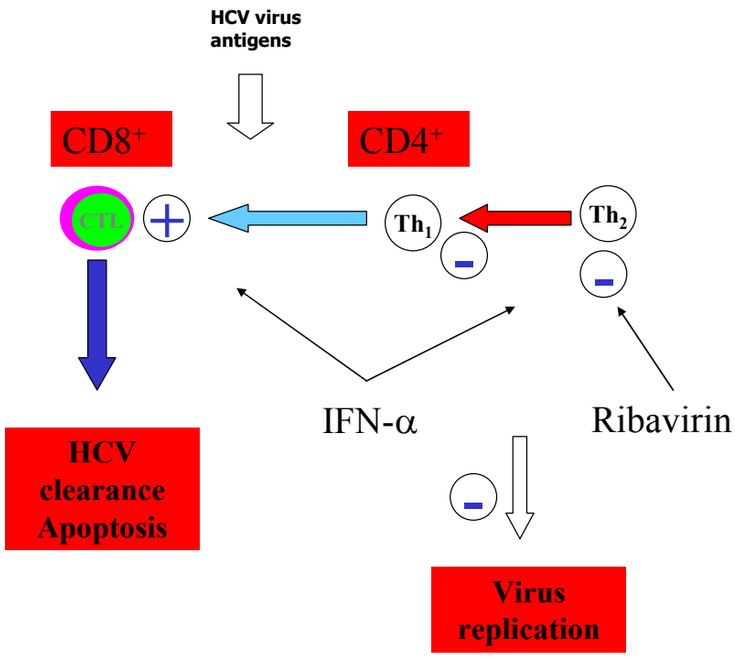


Fig. 1. Targets for antiviral therapy.

hepatic immunity is the CC chemokines macrophage inflammatory protein (MIP) -α (CCL3), MIP-1β (CCL4) and Regulated on Activation, Normal T Expressed and Secreted (RANTES) (CCL5). These chemokines are expressed by the portal vessel endothelium and recruit macrophages and lymphocytes into the liver (Ahlenstiel et al, 2004; Kusano et al, 2000). In the following we present the role of chemokines at baseline and as an early predictor of antiviral responses and clearance of the virus.

6.1 Chemokines at baseline

In one small sample sized study baseline levels before anti-HCV treatment serum levels of MIP-1β could predict a significant effect on SVR, but not eotaxin, MIP-1α, RANTES (CCL5) and IL-8 (Yoneda et al, 2011). Serum levels of MIP-3α (Yamauchi et al, 2002) have also been associated with a positive prognostic response. Moreover, increase of CXCR3 expressing CD8+ cells during treatment has been associated with achievement of viral control (Larrubia et al, 2007). Of interest was that a substitution in the ISDR was associated with response to treatment. In contrast, another study showed that baseline IL-8 level was inversely related to the response to therapy i.e. the higher IL-8 levels, the lower chance of SVR (Akbar et al, 2011). In a broad screening study of baseline CCL and CXCL chemokines, only CXCL10 was significantly associated to lack of SVR (Moura et al, 2011). In another study high CXCL10 gene expression during treatment (Sixtos-Alonso et

al, 2011) and plasma level (Moura et al, 2011) were negative predictors of SVR. Finally, in two other studies baseline levels of IP10 were associated with a negative prognostic response to treatment with peg-IFN-α and ribavirin (Butera et al, 2005; Lagging et al, 2006). Interestingly, as the CCL 3-5 are produced in the portal vascular endothelium while IP-10 is produced mainly in sinusoidal endothelium and hepatocytes surrounding lobular inflammation (Zeremski et al, 2007).

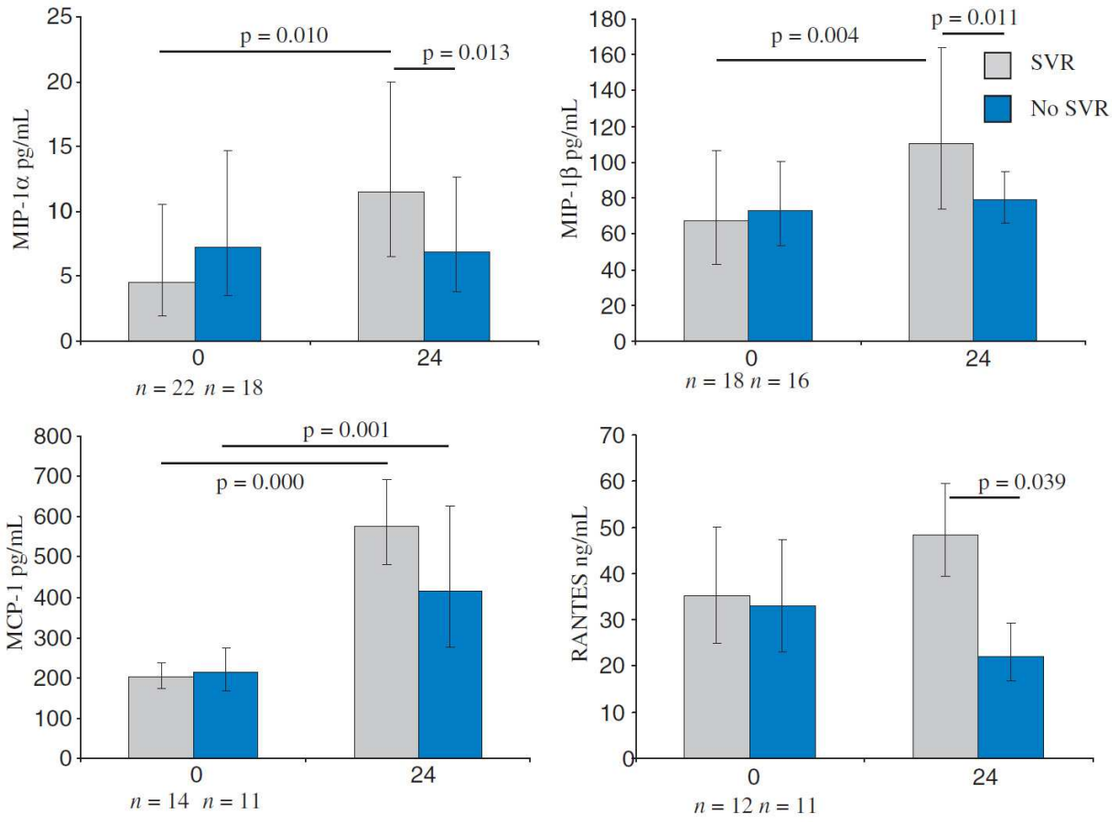


Fig. 2 Early serum chemokine responses to the treatment of chronic HCV infection. (Florholmen et al, 2011)

It is hard to interpret these apparent contradictional results of how chemokine levels can predict the response to treatment. It is of interest to note that the chemokines predicting an effective viral clearance are the CC-chemokines expressed by the portal vessel endothelium. The other chemokines predicting a lack of effect of antiviral treatment are recruited from other sources. These chemokines reflect an apparent state of viral resistance, but further studies are needed to reveal the mechanisms of action.

6.2 Early chemokine response

It is expected that an initial strong immune response is critical for a successful viral clearance in the anti HCV treatment. As far we know only one study have investigated early (24 hrs) chemokine responses during anti-HCV treatment. An early response of MIP-1α, MIP-1β, and RANTES may predict a sustained virological treatment response. MCP-1 was significantly increased but could not discriminate between SVRs and non-SVRs (Fig. 2) (Florholmen et al, 2011). However, the receiver-operator characteristic (ROC) analyses for MIP-1α, MIP-1β shows that alone, these chemokines are not suitable for clinical decisions like termination of therapy due to probable non-response (Fig. 3). Therefore, this study indicates that an early response of chemokines can be critical for an effective virus clearance during the anti-HCV treatment.

The chemokine studies mentioned above have to be interpreted with some caution both due to small sample sizes and that none of them were designed for prognostics and stratified for confounding factors.

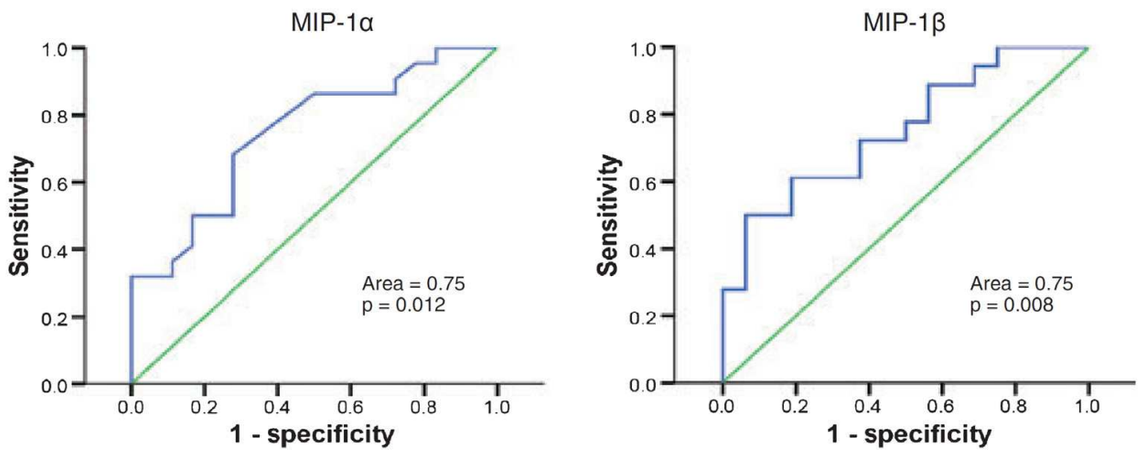


Fig. 3. ROC analysis of early chemokine response as predictor of sustained virological response (Florholmen et al, 2011).

7. New antiviral agents

The new antiviral agents based on inhibition of proteases and polymerases exert their effects on the various NS regions of the HCV genome described above. Ribavirin also has effects on an indirect mechanism of host T-cell mediation with a change from a Th2 to a Th1 profile. So far there is no evidence that the new antiviral drugs have direct suppressive effects on the HCV. Experiences from treatment of HIV show that there is a need of combination of two or more therapeutical molecules to prevent development of resistant HCV strains. So far there is an increase of SVR from 40-50 % to 75 % going from duo-therapy to triple-therapy of

patients with HCV genotype 1. It would be of great interest to know if the new triple-therapy is dependent on an additional chemokine-based viral clearance for an effective treatment response. Therefore, we are waiting for further studies.

8. Concluding remarks

Chemokines seem to play a pivotal role in the immune response to HCV both to induce a spontaneous clearance during an active infection but also during the immunotherapy with peg-IFN-α and ribavirin. The mechanisms of action of chemokines are complex and still far from being fully understood. The understanding of both the successful treatment and the apparent resistance mechanisms with a virus escape from the chemokines and other immune factors is still incomplete. Most of the CC chemokines seem to play an important role in the anti virus attack. However, for other chemokines including some CXC-chemokines, increased secretions represent an apparent state of antiviral resistance to therapy. This paradox is so far poorly understood, but different compartments of chemokine production for the CC and the CXC chemokines may be a clue. It seems that the CC chemokines located in the portal vein may play a pivotal role for en effective clearance of the HCV, and the early chemokine response during antiviral treatment may be used as prognostic biomarkers. However, most of all there is a need of future studies relating viral kinetics to the chemokine responses in vivo, experimental in vitro models may contribute to a more comprehensible understanding of the role of chemokines in HCV infection.

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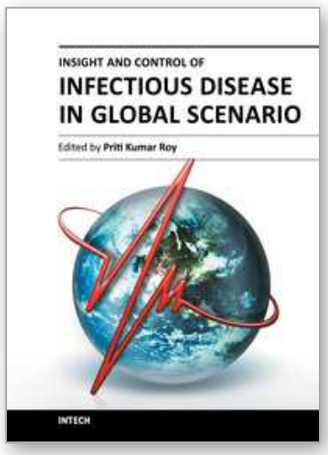
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