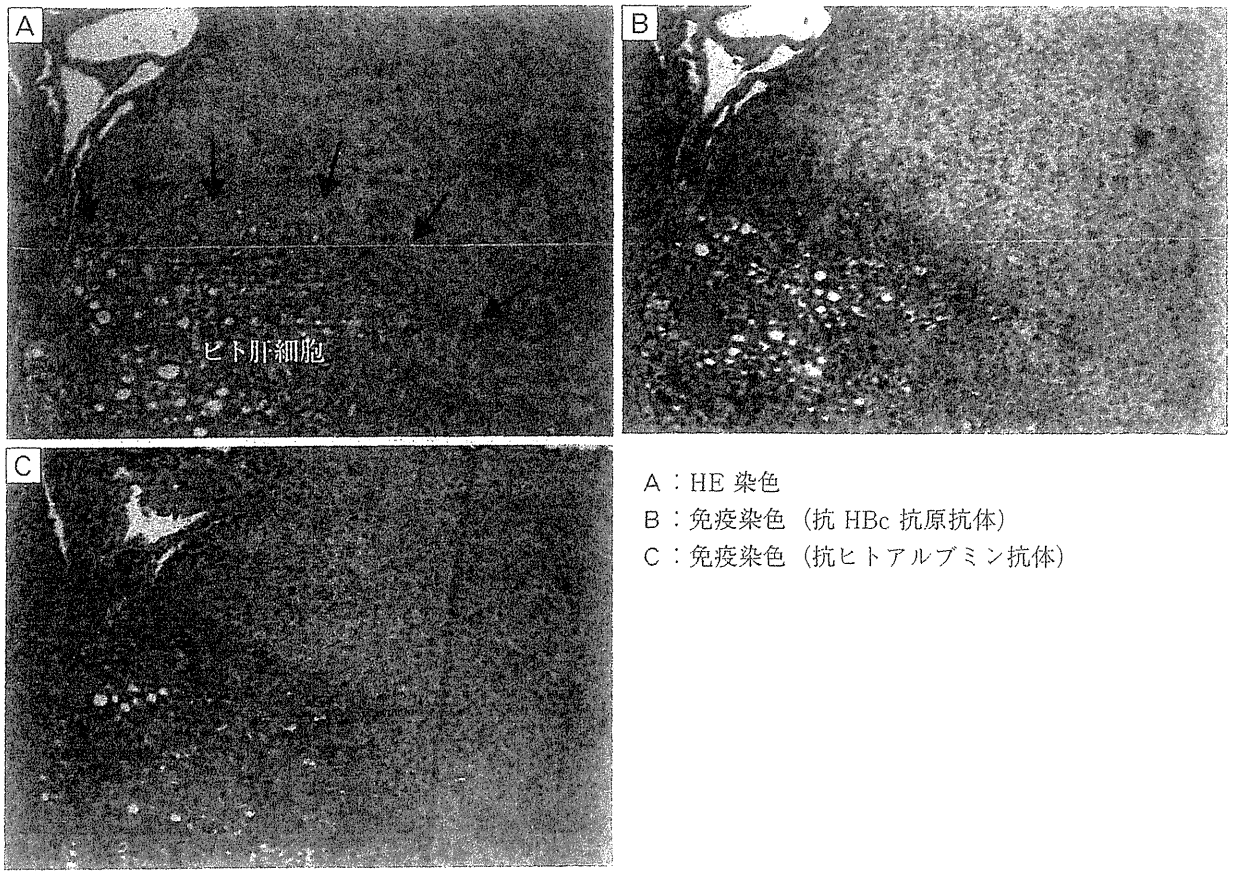
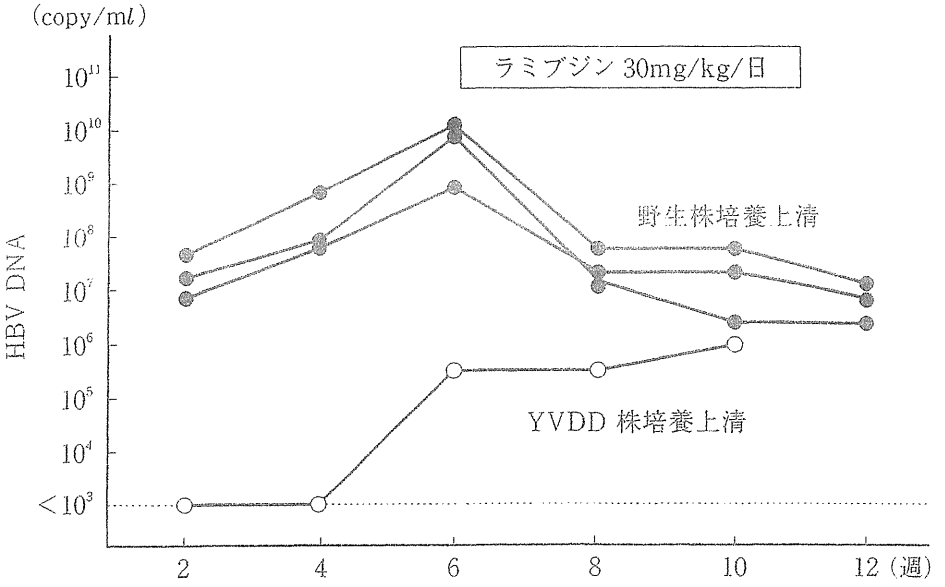
図3 HBV 感染マウスの肝組織



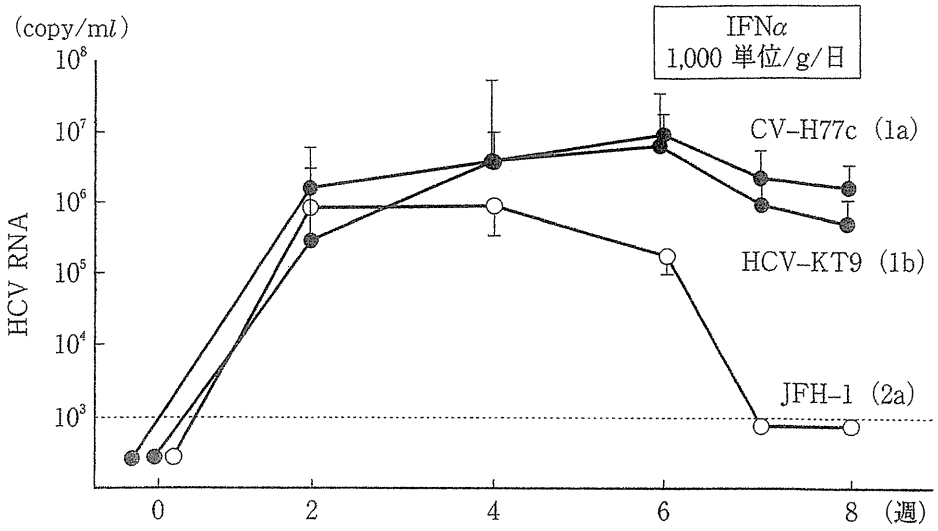
A：HE 染色
B：免疫染色（抗 HBc 抗原抗体）
C：免疫染色（抗ヒトアルブミン抗体）

図4 培養上清を用いたマウスへの HBV 感染



これらの培養上清をキメラマウスへ投与すると，野生株および YVDD 株ともに HBV 感染が確認され，人工的に作製した変異ウイルスに感染したマウスが作製される（図4）．これらHBV 感染マウスにラミブジンを経口投与すると，野生株感染マウスでは血中 HBV DNA の低下が認められたが，YVDD 株感染マウスでは低下を認めなかった（図4）．このことは，

図5　クローンを用いたマウスへのHCV感染



感染性クローンを用いたHBV感染マウスが薬剤の効果判定に有用であることを示している．この手法を用いて新規のラミブジン耐性株の評価も可能であり[3]，HBV遺伝子型間でのウイルス感染・増殖，肝線維化の違いも報告されている[4,5]．

C型肝炎ウイルス（HCV）感染マウス

ヒト肝細胞キメラマウスにHCV感染患者の血清を投与すると，マウス血中に高titerのHCV RNAが長期間検出される[6]．このHCV感染マウスにIFNを投与すると，マウス血中HCV RNAは低下する．これはHCV感染マウスが抗ウイルス薬の評価に有用であることを示しており，新規候補となる抗HCV薬の効果判定にも用いられている[7~10]．

HCVは，遺伝子型間でIFNの感受性が異なることが知られている．すなわち，遺伝子型1型は2型に比べてIFN抵抗性である．また，同じ遺伝子型であっても患者によってIFNの治療効果が異なっている．これらの原因としてHCVのアミノ酸配列が重要と考えられており，実際にHCVのCore[11]，E2[12]，NS5A[13]領域のアミノ酸変異とIFN感受性の違いが報告されている．しかし，これらの変異がなぜIFN抵抗性と関与しているのかはいまだ明らかではない．種々のアミノ酸変異を持つHCV感染マウスの作製が可能となれば，これらの解明の足掛かりになると思われる．そこで我々は，遺伝子型1a型クローンであるCV-H77C[14]，遺伝子型1b型クローンであるHCV-KT9[15]，および遺伝子型2a型クローンであるJFH-1[16]を用いてマウスへの感染を行った．これらのcDNAを発現するプラスミドより*in vitro*トランスクリプション法にてHCV RNAを合成した．マウスへの遺伝子型1aおよび1b型HCVの感染は，合成したRNAをマウス肝臓内に直接注入して行った．一方，遺伝子型2a型のJFH-1は細胞内で感染性ウイルス粒子を作製することが報告されており[16]，合成RNAをHuh7細胞にトランスフェクション後，ウイルス粒子を含む培養上清をマウスに静脈内投与した．いずれの手法においても，投与2週後に血中HCV RNAは定量可能となり，合成RNAを用いた遺伝子型1a, 1bおよび2a型のHCV感染マウスが作製された（図5）[6,11]．これらウイルス感染マウスの血清をナイーブなマウスに投与するとHCV感染が確認され，マウス血中に感染性HCVが含まれていることが確認された．さらに，これらの感染マウスにIFNを連日投与すると，遺伝子型1a, 1b型の感染マウスでは投与2週後に血中HCV RNAはそれぞれ0.7

log，1.2 log 低下した．一方，遺伝子型 2a 型の感染マウスでは，投与1週後にすべて感度以下に低下した（図5）．これらの結果より，遺伝子型1型は2型に比べてIFN 抵抗性であることが確認された．

おわりに

ヒト肝細胞キメラマウスは，扱いが簡便であること，ウイルスの感染・複製がヒト肝細胞内で生じることなどの点より，優れた肝炎ウイルス感染モデルになりうると思われる．今後，肝炎ウイルス感染ヒト肝細胞キメラマウスを用いて，肝炎ウイルスの感染・増殖のメカニズムの解明，ウイルス性肝炎患者の病態解明，さらには新規抗ウイルス薬や感染予防薬の開発などに応用していきたいと考える．

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Hepatitis Virus–infected Animal Model by Using Human Hepatocyte Chimeric Mice

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動物モデルを用いたウイルス性肝炎研究　最前線

キメラマウス

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広島大学大学院医歯薬学総合研究科分子病態制御内科学／いまむら・みちお　つげ・まさたか　ちゃやま・かずあき

ヒト肝細胞キメラマウス●

肝炎ウイルスは，ヒトとチンパンジーにしか感染しない．そのため，生体を用いた肝炎ウイルス研究は困難であり，マウスなどの小動物を用いたより実践的な感染モデルが必要である．ヒト肝細胞キメラマウスは，肝臓が高度にヒト肝細胞に置換されたマウスであり，B型肝炎ウイルス(HBV)やC型肝炎ウイルス(HCV)の投与により置換されたヒト肝細胞への感染が可能である．

ヒト肝細胞キメラマウスは，マウスにヒト肝細胞を移植して作製する．免疫不全のSCIDマウスと，肝不全を有するurokinase-type plasminogen activator(uPA)トランスジェニックマウスを掛け合わせてuPA/SCIDマウスが作製される．このuPA/SCIDマウスの脾臓にヒト肝細胞を注入することでマウス肝臓の90%以上がヒトの肝細胞で置換されたヒト肝細胞キメラマウスが作製される(**図1**)[1]．

ヒト肝細胞キメラマウスを用いた肝炎ウイルス研究●

ヒト肝細胞キメラマウスにHBVあるいはHCV感染患者の血清を経静脈的に投与すると，10^6～10^8 copy/m*l*のウイルス血症が12週間以上継続する．HBVあるいはHCVを感染させたマウスに核酸アナログやインターフェロンなどの抗ウイルス薬を投与すると血中ウイルス量は低下することより，本マウスが抗ウイルス薬の効果判定

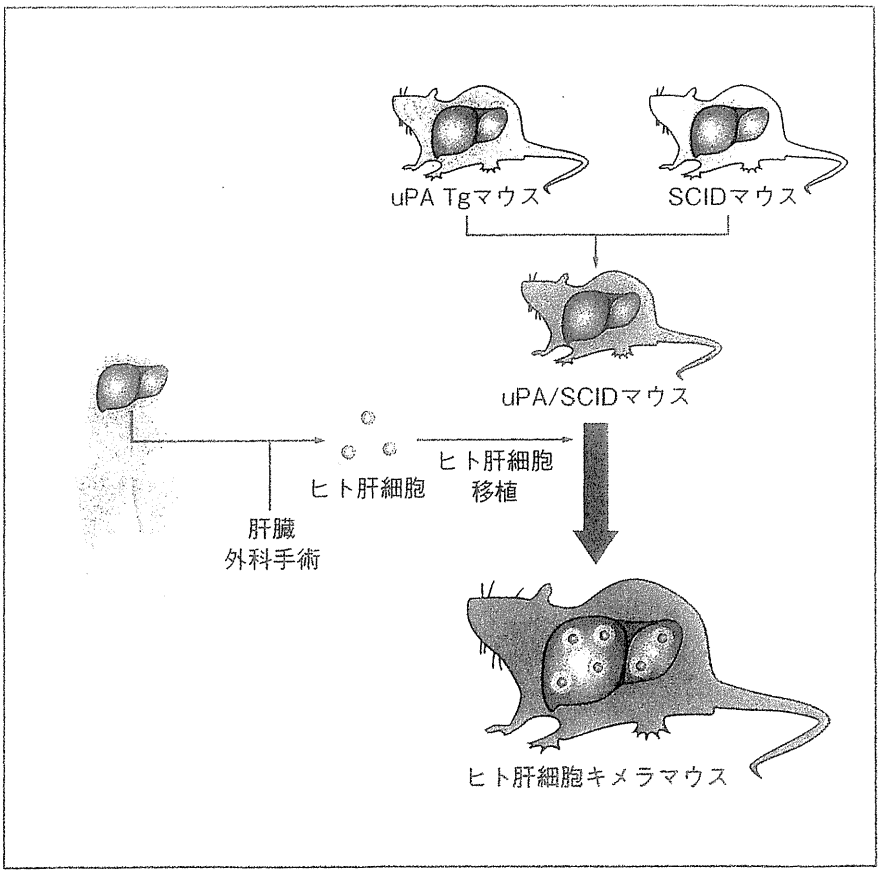

図1　uPA/SCIDマウスおよびヒト肝細胞を用いたキメラマウスの作製法
(茶山一彰：Hepatoday 10：3，2005，図1より引用)

ヒト肝細胞キメラマウスに肝炎ウイルス患者血清や合成ウイルス核酸を投与することにより，肝炎ウイルスの感染が可能となる．
肝炎ウイルス感染マウスは肝炎ウイルスの生物学的検討および抗ウイルス薬の効果判定に有用である．

に有用であると思われる[2,3]．本マウスに種々の抗ウイルス薬を組み合わせて投与し，より有効な治療法の開発が可能になり，また新規候補となる抗ウイルス薬や感染予防薬の効果判定にも有用である[4]．

近年，HCV の core 領域や NS5A 領域のアミノ酸変異とインターフェロン療法の治療効果の関係が明らかとなってきた．これらのアミノ酸変異がどのような原因でインターフェロンの効果と関係しているのか，あるいは難治性の HCV に対してどのような治療を行っていくべきかの検討は重要な課題である．そこで種々の HCV クローンから合成した全長 RNA をマウス肝臓内に直接投与することにより，モノクローナルな HCV 感染マウスを作製した[3,5]．さらに，最も課題となっている genotype 1b 型の HCV クローンにあらかじめ変異を導入することにより，種々の変異型 HCV 感染マウスの作製も可能である．これらマウスは HCV の変異と抗ウイルス薬の効果の関係の解明や，難治性 HCV に対する新規治療法の開発に有用であると思われる．

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Chapter Forty-Four

44

Natural killer cells and hepatitis C virus infection

Michael A. Nalesnik, Tatsuya Kanto

CHAPTER CONTENTS

What is of supreme importance in war is to attack the enemy's strategy.

Sun Tzu

If you want to understand the immune system, look to the viruses—they have been studying it for eons

Michael T. Lotze

ABSTRACT

Hepatitis C virus (HCV) infects more than 170 million people, 80% of whom develop chronic disease. Viral interference with host innate immune response, in particular natural killer (NK) cells, may set the stage for subsequent ineffective adaptive immune response and viral persistence.

Viral NS3/4a protease interferes with hepatocyte type I interferon (IFN) production via several mechanisms. NS5A protein may also interfere with this pathway via IL-8 upregulation. These and related changes, along with suboptimal dendritic cell (DC) response, possibly contributed to by IL-15 deficit, may impair NK cell activation. Viral E2 protein can directly engage NK cells via cellular CD81 and inhibit NK cell response to activation signals. HCV core protein upregulates hepatocyte HLA class I expression, serving as a likely deterrent of NK cell cytotoxicity. Core protein can also upregulate HLA-E on hepatocytes, and interaction of this molecule with inhibitory NKG2A receptors may downregulate NK cell activity.

In chronic HCV infection, both NK cells and DCs may produce increased IL-10, skewing the adaptive immune response towards Th2 type. In this condition, Cytotoxic $CD56^{dim}$ NK cells may be decreased, and cytokine-producing $CD56^{bright}$ NK cells may be increased. Population studies of polymorphisms affecting cytokine production or NK cell inhibitory receptor binding have shown associations with viral clearance, suggesting that these represent important factors of the host immune response.

Many current efforts towards control of HCV infection focus on antiviral agents or T-cell response. However, the virus itself seems to have expended a great deal of evolutionary effort in attempting to evade multiple aspects of the host innate immune response. A greater understanding of the role of NK cells may lead to interventions that facilitate early viral clearance and subsequently decrease the frequency of chronic infection.

KEY WORDS

Hepatitis C virus (HCV), Natural killer (NK) cells, Innate immune response

Introduction

It is currently estimated that about 170 million people, representing 3% of the world's population, are infected with hepatitis C virus (HCV) (1999). These individuals are at significant risk for the development of cirrhosis, the emergence of hepatocellular carcinoma and the possibilities of other complications such as cryoglobulinemia or renal disease. The incidence of infection is expected to rise in the immediate future, underscoring the fact that this virus represents a significant world public health problem.

HCV was identified in 1989 as a specific agent responsible for what had been known up to that time as non-A, non-B hepatitis (NANBH) (Choo et al., 1989). Its discovery entailed construction of a cDNA library from plasma derived from a chimpanzee with a high infectious titre of the then unknown virus. This library was used to express polypeptides that were in turn screened with serum from a NANBH patient. An assay derived from this approach showed that approximately 80% of patients with chronic transfusion-associated NANBH had antibody to HCV (Kuo et al., 1989).

As is the case with most viruses, a dynamic interplay exists between pathogen and host, with a variety of moves and countermoves by both partners. The success of the virus in this regard is reflected in the fact that approximately 80% of patients will fail to control the primary infection and go onto chronic disease (Bode et al., 2007). This chapter will briefly review the makeup and life cycle of HCV, outline some aspects of the host: virus interaction and discuss the role of natural killer (NK) cells in this venue.

Hepatitis C virus

Classification and viral genome

HCV is an enveloped RNA virus classified as a distinct genus Hepacivirus in the family Flaviviridae. It is divided into 6 genotypes and more than 50 subtypes based on genomic variability (Bukh et al., 1995). The virus has a single-stranded positive-sense genome of around 9600 nucleotides with a single open reading frame flanked on either end by conserved untranslated regions (UTRs).

The 5′ UTR is highly conserved and contains an internal ribosome entry site (IRES) that allows the virus to usurp the cell translation machinery (Otto and Puglisi, 2004). The efficiency of translation control varies among different genotypes and quasispecies (Honda et al., 1999; Laporte et al., 2000; Soler et al., 2002). The 3′ UTR is considered essential for viral replication (You and Rice, 2008) and has an additional role in enhancing the translation of viral RNA mediated by the 5′ UTR IRES (Song et al., 2006). It interacts with the cellular La and polypyrimidine tract-binding proteins (Domitrovich et al., 2005) and, in one study, was found to bind more than 70 host proteins (Harris et al., 2006).

The bulk of the genome is comprised of a single open reading frame that encodes a 3010 amino acid polypeptide that is cleaved in a cotranslational and posttranslational fashion to give rise to 10 viral proteins (core, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B) (Dubuisson, 2007). In addition, a frameshift involving the core region gives rise to an alternate reading frame protein known as F protein (Branch et al., 2005).

HCV proteins and structure

The mature HCV core protein ranges from 19 kDa to 21 kDa molecular weight and is thought to comprise the viral nucleocapsid. This RNA-binding protein is mainly found attached to the endoplasmic reticulum, in association with lipid droplets (Dubuisson, 2007). The protein is thought to have a role in hepatocyte steatosis and to render the host cell more susceptible to injury and carcinogenesis. Core protein contains epitopes recognized by both T cells and B cells (Barban et al., 2000; Beld et al., 1999; Jackson et al., 1999; Nattermann et al., 2005; Pirisi et al., 1995). A direct modulating effect on the T-cell response is likely. Yao et al. (2007) concluded that this protein led to an inhibition of T-cell function. Separately, Chung et al. (2001) suggest that it may cause an accelerated inflammatory response. HCV core protein acts as a ligand for toll-like receptor 2 (TLR-2) (Dolganiuc et al., 2004; Duesberg et al., 2002), and this may contribute to monocyte/macrophage activation in chronic infection (Dolganiuc et al., 2007). Ciccaglione et al. (2007) found that this protein was capable of inhibiting interferon (IFN) regulatory factor I (IRF-1) expression, leading to repression of subsequent target genes such as IL-12 and IL-15. It has been suggested that some of the effects attributed to the core protein may be due in part or in whole to an overlapping alternate reading frame protein expressed during natural HCV infection (Branch et al., 2005).

The glycoproteins E1 (gp31) and E2 (gp70) have been demonstrated on the surface of HCV virions (Kaito et al., 2006), confirming their role as major envelope glycoproteins. E1 and E2 form heterodimers and are found largely in the endoplasmic reticulum. E2 contains a hypervariable region HVR1 that can form quasispecies during the course of infection, possibly due to immune pressure leading to the development of escape mutants. E2 is thought to represent the main ligand of the mature virion for cell surface binding and has been found in vitro to link CD81 (tetraspanin) and SR-B1 (scavenger receptor class B type I), two putative cell

receptors for HCV (Heo et al., 2006). The interaction with CD81 is also thought to affect dendritic cell (DC) function during HCV infection (Nattermann et al., 2006; Zhao et al., 2007).

The p7 polypeptide is found either as a C-terminal portion of the E2 glycoprotein or as a separate protein (Major et al., 2001). In vitro studies show that it is membrane-localized and forms ion channels (StGelais et al., 2007) that can be inhibited by amantadine. This protein is necessary for infectivity in a chimpanzee model and has been found to be important for the efficient assembly and release of virions (Steinmann et al., 2007). The ability to enhance virus production varies among different isolates, suggesting that it may function as a virulence factor (Steinmann et al., 2007).

The NS2 protein is a membrane protein localized within the endoplasmic reticulum. A role in virus assembly and release has been proposed (Dubuisson, 2007), and a recent study found the protease domain of NS2 to be necessary for viral infectivity (Jones et al., 2007). NS2 has been shown to inhibit apoptosis mediated by the hepatocyte CIDE-B protein in vitro (Erdtmann et al., 2003).

The NS3 region is responsible for encoding a serine protease activity that cleaves the HCV polyprotein at multiple sites, in conjunction with a protein from the NS4 region. NS3 also encodes for RNA helicase and NTPase activity. Thoren et al. (2004) demonstrated that this protein was capable of inducing oxygen radical formation in mononuclear and polymorphonuclear phagocytes, which were then able to induce dysfunction or apoptosis in T cells, NK cells, and NK T cells. The NS3/4 protease also plays a role in the response of the virus to host type I IFN production (Hiscott et al., 2006).

The NS4 region encodes viral proteins NS4A and NS4B. NS4A is a cofactor that enhances the activity of the protease encoded by NS3. It also joins with viral proteins NS4B and NS5A to form a stable heterotrimer.

NS4B contains a nucleotide binding motif with GTPase activity that appears to be important for efficient RNA replication (Einav et al., 2004). It also induces a 'membranous web' comprised of vesicles in a membrane matrix that is thought to represent the viral replication complex (Egger et al., 2002). It may also contribute, along with NS3/4 A, to interference with hepatocyte type I IFN production (Tasaka et al., 2007).

The NS5 region encodes viral proteins NS5A and NS5B. NS5A is thought to play a role in replication of RNA and exists as hypophosphorylated and hyperphosphorylated forms. The hypophosphorylated form appears to support efficient viral replication in vitro, whereas the hyperphosphorylated form is inhibitory (Neddermann et al., 2004) Of interest, the mTOR inhibitor Rapamycin reduces the phosphorylation status of NS5A (Coito et al., 2004). NS5A has a separate domain referred to as the IFN sensitivity-determining region that inhibits the action of IFN-induced protein kinase PKR, an effector of IFN-induced antiviral activity (Gale et al., 1997).

NS5B protein contains the RNA-dependent RNA polymerase activity that is essential for viral replication (Luo et al., 2000), and it is likely that the interaction of this protein with the 3′ terminus of the viral genome leads to the synthesis of negative strand RNA. It may also serve to modulate the phosphorylation status of NS5A (McCormick et al., 2006). Choi et al. (2006) demonstrated that this protein may modulate TNFα signalling pathways.

The HCV virion has a spherical appearance and is approximately 50nm in diameter. It is enveloped, and, in the peripheral blood, is found in probable association with LDL, and in another form either free or complexed with immunoglobulin, as determined by sucrose gradient centrifugation (Hijikata et al., 1993; Kanto et al., 1995). It is thought that association with LDL may protect against antibody neutralization.

Life cycle of HCV

Until recently, there was not an effective cell culture system for HCV (Lindenbach et al., 2005; Yi et al., 2006). Thus, most studies were performed using either standard mammalian cell expression in vitro or in vivo with chimpanzees, as the chimpanzee is the only animal model that mimics most but not all aspects of the human infection. The development of permissive cell culture models has allowed progress in the study of the viral life cycle and has facilitated the testing of drugs and neutralizing antibodies against HCV.

HCV generally is spread through parenteral routes and reaches the liver via the bloodstream. Virions may circulate as free particles or as particles bound to immunoglobulins, low density or very low density lipoproteins (Nielsen et al., 2006). The viral surface protein E2 can bind cellular CD81 (tetraspanin) as well as scavenger receptor class B type I (SR-BI) (Zeisel et al., 2007), both of which are found on hepatocytes and appear to function in viral entry. The LDL receptor (Nahmias et al., 2006) and the lectins L-SIGN and DC-SIGN (Lozach et al., 2004) may also facilitate entry of the virus into the hepatocyte. Further entry is dependant upon the presence of claudin-1, 6 or 9 (Meertens et al., 2008), and the virion is endocytosed into clathrin-coated pits. Viral surface membrane and endosomal fusion occur in the context of acidification, and the nucleocapsid is released into the cytoplasm where uncoating occurs. The positive strand RNA initiates translation by means of an internal ribosomal entry site (IRES) that is located in the 5′ noncoding region and binds the 40S ribosome. This leads to the formation of a single polyprotein that is processed into individual

peptides in a cotranslational and posttranslational fashion, using both viral and cellular protease activity.

A replication complex then arises from a combination of viral nonstructural proteins and cellular material. Viral NS4B, NS5A, NS5B and the NS3 helicase-NTPase domain are known to be important components of this structure, and the cellular substrate is referred to as a 'membranous web', which is a perinuclear vesiculomembranous aggregate thought to be derived from the endoplasmic reticulum. At this site, active RNA synthesis occurs.

The assembly and release of mature virions is not completely understood. Assembly likely occurs in proximity to the membranous web, and secretion may be dependant upon the ion channels formed by the p7 protein. Gastaminza et al. (2008) concluded from their studies that the virus hijacks the host machinery for assembly, maturation, degradation and secretion of VLDL, thereby explaining in part the tropism for hepatocytes.

In the liver, in situ hybridization shows up to 50% of hepatocytes to contain HCV in infection (Pal et al., 2006). Antigenic expression detected by immunohistochemistry is reported in 5% or less of hepatocytes, and in lesser numbers of biliary epithelial cells and sinusoidal lining cells (Nouri-Aria et al., 1995), although this result may be artifactually low due to antigen instability in formalin-fixed tissues. Occasional mononuclear cells also may express HCV antigens (Nouri-Aria et al., 1995). Using RT-PCR, HCV has also been detected in lymph nodes, pancreas, bone marrow, spleen, thyroid, brain and adrenal gland (Forton et al., 2004; Lerat et al., 1998). It is not known whether the virus replicates in haematopoietic cells.

Host response to HCV infection

Innate immune response to HCV

The cell immune response is important in light of the fact that the virus is largely noncytopathogenic, and mechanisms external to the infected cell are required for viral elimination. The integrated host response to HCV infection is comprised of innate and adaptive components of the immune system, with each arm modulating the kinetics of the other to some extent. In addition, there is a separate crosstalk between host and virus, as each tries to gain advantage and establish a favourable equilibrium. This initial set of interactions, in which NK cells play an important role (Khakoo et al., 2004), is crucial in determining the outcome of the infection (Gale and Foy, 2005). In approximately 20% of acutely infected patients, this process generates a strong T-cell response that leads to spontaneous resolution of infection. More often than not, however, the virus gains a marginal advantage that permits its survival at the cost of chronic hepatitis and attendant complications for the host. This discussion will focus on aspects of the innate immune response with emphasis on those features impinging upon the role of NK cells. Several recent reviews have addressed the host response to this infection with emphasis on the T-cell response (Blackburn and Wherry, 2007; Bowen and Walker, 2005; Ishii and Koziel, 2008; Li et al., 2008; Manigold and Racanelli, 2007; Neumann-Haefelin and Thimme, 2007; Neumann-Haefelin et al., 2007; Rehermann, 2007; Semmo and Klenerman, 2007).

Hepatocyte infection, IFN production and HCV countermeasures

IFN-α production indirectly activates NK cells via its effect on DCs, which provides one of many areas in which the host:virus struggle is played out. Following infection, type I IFNs are elaborated by multiple cell types, likely beginning with hepatocytes. This can be considered as the immediate early innate immune response. Li et al. (2005) showed the existence of TLR-3 as well as a retinoic acid-inducible gene (RIG) pathways in cultured hepatocytes, suggesting that these are potential in vivo mechanisms for type I IFN production by liver cells. Upon engagement of dsRNA by cell surface or intracytoplasmic membrane-bound TLR-3, binding to the adaptor protein TRIF (Toll/interleukin-1 receptor domain-containing adaptor protein inducing IFN-β) occurs, which ultimately leads to the activation of the transcription factors NFκB and IRF-3 (IFN regulatory factor-3). Similarly, intracytoplasmic RIG-1 recognition of dsRNA leads to IRF-3 activation via a separate pathway, as well as to formation of the active form of the transcription factor AP-1. These products in turn induce IFN-β gene transcription, and this protein is produced and secreted by the cell (Bode et al., 2007).

IFN-β exerts its effect in an autocrine or paracrine fashion by binding the cell surface type I IFN-α/β receptor. This engages the Jak/STAT pathway to lead to the production of the IFN-stimulated gene factor 3 (ISGF3) complex, which translocates to the nucleus and acts to enhance transcription of a family of IFN-stimulated genes. These include IRF-7, which, in conjunction with IRF-3, upregulates IFN-α transcription, thereby propagating a positive feedback mechanism to magnify the antiviral effect (Bode et al., 2007).

Reflecting the importance of this process in establishing an environment conducive to control of virus infection, HCV has evolved a number of potential strategies to combat the host offensive. Foy et al. (2003), using Huh7 hepatoma cells, demonstrated that the viral protease NS3/4 was capable of blocking phosphorylation and activity of IRF-3, resulting

in enhanced viral replication in vitro. The viral NS3/4 A protein is also able to interfere directly with both TLR-3 and RIG-1-mediated signal transduction pathways. In the former case, this involves proteolysis of the adaptor protein TRIF (Li et al., 2005) and, in the latter case, cleavage of the mitochondrial tethered adaptor protein CARDIF (caspase recruitment domain adaptor-inducing IFN-β, also known as IPS-1, MAVS or VISA) (Foy et al., 2005; Hiscott et al., 2006). Tasaka et al. (2007) also found a role for viral NS4B in the inhibition of the RIG-1 pathway. Abe et al. (2007) reported that HCV NS5A can interfere with TLR-dependent cytokine production in mouse macrophage cell lines by interacting with the death domain of the adaptor molecule MyD88. This study is of particular interest since the interaction of HCV proteins with TLR pathways represents a potentially significant and largely unexplored mechanism by which the virus may evade host immune surveillance. HCV may also interfere with the Jak/STAT signal transduction pathway, possibly by effects of the viral core protein on STAT-1, SOCS-3 (suppressor of cytokine signalling-3) and ISGF3. The controversies and implications surrounding these interactions have been recently reviewed (Bode et al., 2007). Despite these efforts by HCV, studies in the chimpanzee model show high levels of intrahepatic IFN-α production during early infection (Thimme et al., 2002). This is not associated with a concomitant decrease in HCV genomic levels, suggesting that resistance to, rather than interference with, elevated IFN-α level may be more important for viral survival.

NK cells and HCV infection

Introductory comments

Prior to a detailed discussion of NK cells in the context of HCV infection, a few general comments are in order. First, regional differences in the distribution of NK cells need to be stressed. Golden-Mason and Rosen (2006) point out that intrahepatic mononuclear cells contain a higher percentage of NK cells than is seen in the peripheral blood. Within the liver, NK cells comprise 20–30% of mononuclear cells and may account for up to half of intrahepatic lymphocytes, as compared to representing 10–15% of peripheral blood mononuclear cells. This suggests that HCV would need to have evolved specific mechanisms for long-term survival within an environment rich in NK cells (Golden-Mason and Rosen, 2006).

A number of published studies have compared the more easily obtained peripheral blood NK cell frequencies in HCV-infected patients versus uninfected patients. Such studies have led to sometimes conflicting results, raising the possibility that rapid phenotypic or functional changes of NK cells are occurring in this compartment in vivo. Examination of liver-infiltrating NK cells is technically more demanding but may provide more precise and otherwise unobtainable insights into the role of these cells in HCV infection.

A synopsis of the following discussion of NK cells and HCV infection is presented in Figure 44.1 and in Table 44.1.

A second comment relates to the issue of NK cell subpopulations. NK cells contain at least two different subpopulations according to their degree of CD56 expression, and these subpopulations differ in function as well as phenotype. There is speculation that CD56-defined subsets may play distinct roles in the pathogenesis of HCV-induced liver disease, such as in inflammation or fibrosis (Lin et al., 2004; Morishima et al., 2006). Several recent reports have demonstrated that, in chronic HCV infection, the frequency of $CD56^{dim}$ NK cells is reduced, whereas numbers of $CD56^{bright}$ NK cells are increased (Golden-Mason et al., 2008; Meier et al., 2005; Morishima et al., 2006). Future investigation may be necessary to elucidate the extent to which previously reported functional impairment of NK cells in HCV infection can be ascribed to such alterations in subset populations.

NK cell receptors and HCV infection

Effects of NK cell CD81 engagement by HCV

CD81 is a widely expressed cell surface protein that that has been mentioned previously as a cellular coreceptor for ligation of HCV to the hepatocyte. This receptor is also present on most if not all cells of the immune system. It is a member of the tetraspanin family, whose constituents share the presence of four transmembrane domains that contain small and large extracellular loops (Levy and Shoham, 2005), the latter comprising the binding site for the viral E2 protein (Drummer et al., 2002). CD81 and related proteins are thought to integrate extracellular, cytoplasmic and intramembranous components into a 'tetraspanin web' with diverse functions dependant upon context.

In the case of NK cells, the E2:CD81 interaction results in downregulation of NK cell response to activation signals from CD16, NKG2D, IL-2, IL-12, IL-15 or β1 integrin (Crotta et al., 2006; Li et al., 2004; Tseng and Klimpel, 2002). This inhibition includes a reduction of IFN-γ production, decreased release of cytolytic granules and diminished proliferative activity. Li et al. (2004) showed that NK cells cocultured with HCV replicon-containing hepatic cells secreted IFN-γ that in turn upregulated hepatic cell STAT-1 and IFN-α production, resulting in marked inhibition of HCV RNA expression. These effects could be inhibited by cross-linking CD81 by specific antibody or by antibody to IFN-γ.

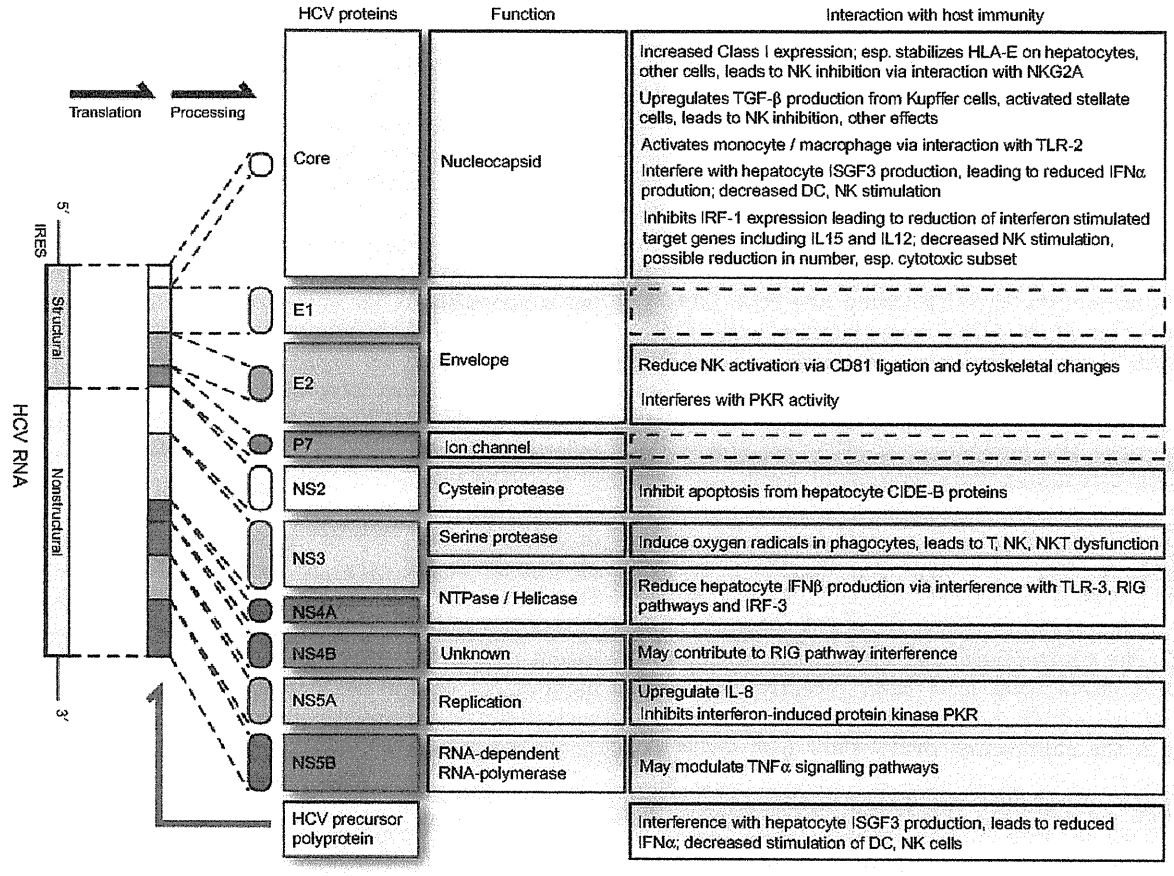

Figure 44.1 • Schematic view of HCV genomes and protein products showing associated interactions with host immunity. The HCV genome is divided into structural and nonstructural areas on the left, with the rightward direction corresponding to representations of the precursor polyprotein and individual viral proteins, respectively. Brief depictions of protein functions within the setting of the viral life cycle are followed by tabulation of probable interactions with the host immune system. The interactions are largely restricted to those involving the innate immune system. See text for additional details.

These authors concluded that NK cells, which were not directly cytotoxic to the infected hepatic cells, were potentially capable of inhibiting HCV replication via an IFN-γ-dependent pathway that was subject to viral interference via the HCV-E2:CD81 interaction. Agrati et al. (2002) suggested that CD81-associated inhibition of NK cell function might thereby contribute to a lack of viral clearance with progression to chronic infection.

Crotta et al. (2006) demonstrated that CD81 cross-linking by antibody or HCV E2 protein resulted in cytoskeletal rearrangement in NK cells as well as in T cells, as based on morphological alterations and enhanced F-actin capping. Whereas these cytoskeletal changes enhanced the response of T cells to CD3-induced TNF-α production, they decreased the CD16-mediated generation of IFN-γ and TNF-α by resting or activated NK cells. These authors were able to decrease the response of T cells and increase the response of NK cells to these stimuli by preincubation of cells with low-dose actin polymerization inhibitors. This led them to conclude that CD81 engagement induces cytoskeletal rearrangement in both NK cells and T cells, but that this process has opposite effects, leading to inhibition of NK cell responses and stimulation of T-cell responses. Since this phenomenon also extended to the inhibition of NK cell response to IL-12, they inferred that the inhibition was independent of KIR involvement. Tseng and Klimpel (2002) showed that the cross-linking of CD81 on NK cells by viral E2 inhibited both NK cell cytotoxicity and NK cell IFN-γ production, suggesting that this may be an important mechanism by which the virus can shift the balance of the early innate host immune response.

Table 44.1 Additional factors potentially influencing NK cell function during HCV infection

Mechanism	Effect
Weak affinity inhibitory allotype of HLA-Cw1 and KIR2DL3, potentially allowing relatively stronger stimulatory KIR interactions	Some evidence for protective antiviral effect
Strong affinity stimulatory allotype of HLA-B Bw4 and KIR3DS1	Protective in one study; increased chronic inflammation in another study
Increased inhibitory NKG2A expression in HCV NK cells	Facilitates inhibitory interaction of NK cells with HLA-E upregulated on other cells, esp. HCV-infected hepatocytes; may also lead to increased NK IL-10 and TGF-β causing defective DC interaction
Increased affinity MICA/B allotypes as ligands for activating NK NKG2D receptor	Potential role in facilitating HCV clearance
Decreased IL-15 as potential cause of decreased DC MICA/B expression	Decreased ability to stimulate NK cells; can be overcome in vitro with IL-15
Lower expression of NKp30 and NKp46 on HCV NK cells (in some studies)	Results differ among studies; possible inhibitory mediator of NK cell function in this setting
Increased NK IL-10 production upon stimulation	Potential skewing of adaptive immune response to Th2 phenotype

Interactions of NK cell receptors with HLA molecules as expressed in HCV infection

HLA class Ia levels and NK cells

Herzer et al. (2003) used recombinant adenovirus constructs to express the HCV core protein in several hepatocyte cell lines and showed upregulation of MHC class I expression on the surfaces of HepG2 cells but not on Hep3B or Huh7 cell lines. These latter cell lines lack wild-type p53, which is present in HepG2 cells, and reconstitution of these cells with wild-type p53 led to an increase in HLA class I in the setting of core protein expression. Transporter associated with surface processing-1 (TAP-1) protein, which is p53 responsive, was also upregulated. The increased expression of class I molecules led to a significant reduction of NK cell-mediated cytotoxicity as assessed in 48-hour chromium release assay, and the authors concluded that this was a likely mechanism of viral evasion against NK cell cytotoxicity in vivo.

Class Ia HLA and killer cell immunoglobulin-like receptors (KIR)

Killer cell immunoglobulin-like (KIR) receptors are clonally expressed in a stochastic fashion on NK cells. They may be stimulatory, with a short cytoplasmic tail and a charged transmembrane domain that allows association with signalling proteins, or inhibitory with a long cytoplasmic tail that contains an immunoreceptor tyrosine-based inhibitory motif (ITIM). They most often recognize class I HLA-C molecules and bind in a manner that overlaps but differs from T-cell receptor binding (Boyington et al., 2000). Different KIR bind with different affinities, and it seems likely that inhibitory KIR engage class I HLA more strongly than do stimulatory KIR (Vales-Gomez et al., 1998).This opens the possibility that different KIR haplotypes may influence the courses of various diseases, including infection with HCV. To date, epidemiologic studies have produced conflicting results.

Khakoo et al. (2004) examined the KIR and HLA-C status in 685 individuals with persistent HCV infection and 352 individuals with resolved HCV infection. Within this population, they focused on those with HLA-C allotypes containing asparagine at residue 80 (HLA-Cw group 1 alleles), which serve as ligands for the inhibitory KIRs, KIR2DL2 and KIR2DL3. Of these receptors, KIR2DL3 binds with weaker affinity. They found a protective effect in individuals who were homozygous for this HLA-C allotype and also homozygous for KIR2DL3. This protective effect was evident in those who were infected with HCV by accidental needle stick or during the course of intravenous drug abuse but not in those who were infected by blood transfusion. They hypothesized that the inhibitory effect of the relatively weakly binding KIR2DL3 could more easily be overcome by other nonvariable activating NK cell receptors. Further, they suggested that this protective effect could be overwhelmed by a massive viral inoculum, explaining the loss of protection in those who received the presumed larger viral dose during blood transfusion.

Rauch et al. (2007) performed a similar study in 142 patients with chronic HCV infection and 33 with resolved HCV infection. These individuals were part of the Swiss HIV Cohort Study and as such obtained HCV primarily as a consequence of intravenous drug abuse. These investigators were unable to find any association

between the status of KIR genotype and HLA-C ligand in their population. They felt that a lack of statistical power was unlikely but left open the possibility that the HIV positive status of their population, which differed from that of the previously cited study, may have had an effect on the results.

More recently, Romero et al. (2008) examined KIR receptor distribution and HLA class II alleles in a population of 121 intravenous drug users with chronic HCV infection and 39 others with spontaneous viral clearance. They eliminated the possibility of genetic stratification in this Puerto-Rican American cohort by analysis of autosomal short tandem repeat markers. They were able to confirm the association among homozygous KIR2DL3 status, homozygous HLA-Cw group 1 alleles and spontaneous viral clearance, and found an additional association of KIR2DL3, DRB1*1201 and spontaneous clearance. They tabulated all prior studies to date in their report and concluded that additional population studies were necessary.

A weak protective effect was also previously suggested for the combination of HLA-B Bw4 and the stimulatory KIR3DS1 (Khakoo et al., 2004). In contrast, Paladino et al. (2007) found an increased frequency of this combination in HCV-positive individuals who had progressed to cirrhosis, leading them to suggest that the presence of higher cytotoxic activity might actually be associated with progression of HCV. Lopez-Vazquez et al. (2007) in summarizing studies to date also concluded that evaluation of other large cohorts of patients with HCV infection is needed to confirm the possibility of an association between the interaction of KIR and HLA with disease progression.

HLA class Ib and NK cells

HLA-E interactions with NKG2A and NKG2C receptors HLA-E is a widely expressed member of the nonpolymorphic MHC class Ib molecules that contains a nonamer peptide binding motif that typically contains derivatives of signal peptides from other class I molecules and can present other epitopes as well (Rodgers and Cook, 2005). HLA-E can bind CD94-NKG2A (inhibitory) or CD94-NKG2C (activating) on NK cells. Nattermann et al. (2005) found that the hydrophobic peptide YLLPRRGPRL, representing an HLA-A2-restricted and known T-cell epitope derived from amino acid positions 35–44 of the HCV core protein, was able to stabilize surface HLA-E expression in an HLA-E-transfected K562 cell line. Chromium release assay showed inhibition of NK cell cytotoxicity against the HLA-E transfected HCV peptide 35- to 44-loaded K562 cells. No inhibition occurred when transfected cells were preincubated with an irrelevant peptide or when HLA-E-negative K562 target cells were preincubated with the HCV core peptide sequence. Inhibition was abolished in the presence of antibodies to either CD94 or NKG2A, implicating this receptor complex in the process.

As in vivo correlates, these investigators found increased inhibitory NKG2A expression on circulating NK cells from patients with chronic HCV infection as contrasted with those without infection (Nattermann et al., 2006). No difference was found in levels of NKG2C expression; however, reduced levels of the NK cell stimulatory receptors NKp30 and NKp46 were found in the cells of the hepatitis patients. Liver biopsy specimens from patients with chronic HCV infection demonstrated enhanced HLA-E expression in $CD68^+$ macrophages/Kupffer cells, $CD31^+$ sinusoidal endothelial cells, $CD83^+$ DCs, $CD14^+$ monocytes and hepatocytes. In the latter case, HLA-E expression was higher in hepatocytes expressing HCV core protein. These studies suggest that this T-cell epitope may also contribute to chronic viral infection by virtue of its synchronous inhibition of NK cell activity (Golden-Mason and Rosen, 2006).

Similar results were found in a study employing freshly isolated circulating NK cells from patients with chronic HCV (De Maria et al., 2007). NK cell cytotoxicity against FO1 melanoma or Daudi Burkitt lymphoma target cells were similar to those obtained from uninfected donors. However, HCV NK cells showed a significant reduction in cytolytic activity when HepG2 hepatocellular carcinoma target cells were used. These investigators also implicated the HLA-E: CD94/NKG2A ligand receptor interaction in this phenomenon and demonstrated increased expression of NKG2A on HCV NK cells compared to uninfected control cells.

Related studies (Jinushi et al., 2004) demonstrated that NKG2A ligation on NK cells is also associated with defective signals for DC maturation, discussed later.

MICA/B and NKG2D receptor MHC class I chain-related sequence (MIC) genes are thought to represent phylogenetically old members of MHC class Ib molecules (Rodgers and Cook, 2005). The two proteins in this group, MICA and MICB (MICA/B), are polymorphic, with approximately 60 and 25 known alleles, respectively; do not present peptides or associate with β-2 microglobulin; and serve as ligands for the activating NKG2D receptor on NK cells (as well as macrophages, CD8+ T cells, γδ T cells, and NKT cells) (Bauer et al., 1999; Stastny, 2006; Yokoyama and Plougastel, 2003).

Similar to the situation with KIR, the binding affinities of MICA/B to NKG2D appear to vary among different allotypes, which has been suggested as a potential influence on the threshold of NK cell activation (Steinle et al., 2001). Karacki et al. examined MICA polymorphisms in 442 individuals with chronic HCV and 228 others who cleared the virus. They found a statistically significant association with the presumed high affinity MICA*015, which occurred twice as often in patients who cleared HCV than in those with persistent disease.

The allele occurred in only 5.6% of their patient population, and no other significant associations were uncovered, leading them to conclude that MICA polymorphisms did not play a significant role in facilitating HCV clearance. However, the association was found in a small number of Black patients, as it was too rare in Whites to analyse. It would appear that further related studies in this defined patient subpopulation may provide additional information.

Studies addressing defective DC MICA upregulation leading to reduced NK cell activation (Jinushi et al., 2003a) are discussed in more detail in the upcoming section addressing NK: DC crosstalk.

Other NK cell receptors in HCV infection

Nattermann et al. (2006) found that patients with chronic HCV infection and viraemia had reduced levels of the natural cytotoxicity receptors NKp46 and NKp30 in circulating NK cells compared to healthy uninfected individuals. Further, patients who had cleared the virus following therapy with pegylated IFN-α and ribavirin exhibited levels of these receptors similar to those of uninfected controls. Although the two receptors were expressed in a proportionate fashion, there was no correlation between the level of expression and circulating viral genomic load in those patients who had viraemia. These investigators also examined receptor expression in intrahepatic cells by flow cytometry following mechanical disruption of tissue samples. At this site, they also found a lower expression of NKp30 and NKp46 in HCV patients compared to those with other liver diseases. In a redirected killing assay using antibodies against natural cytotoxicity receptors and an FcR^+ target cell line, decreased cytotoxicity was seen in HCV NK cells. These authors suggested that the combination of reduced natural cytotoxicity receptors along with increased inhibitory NKG2A expression on HCV NK cells contributed to impaired function of these cells in patients with chronic HCV infection.

In contrast, De Maria et al. (2007) reported that circulating NK cells from patients with chronic HCV infection showed increased expression of the stimulatory natural cytotoxicity receptors NKp46 and NKp30 compared to uninfected adults. This finding was unexpected, as prior studies in other conditions such as HIV infection or acute myelogenous leukaemia had shown reduced levels of these receptors, providing a rationale for positing a functional deficit of the NK cell compartment in chronic disease conditions.

HCV NK cells were not activated as shown by a lack of increased HLA-DR expression, and no correlation was found between natural cytotoxicity receptor expression density and level of viraemia. NK cell cytotoxicity was intact using FO1 melanoma target cells and could be partially inhibited by antibodies to NKp30, NKp46 or NKG2D. Cross-linking of NK CD81, which was expressed at levels comparable to healthy individuals, had no effect on cytotoxicity.

It is possible that differences in patient populations, circulating NK cell subpopulations, or methodological details may have contributed to disparate findings between these groups. Significantly, both groups of investigators concluded that NK cells play an important role in chronic viral persistence. Additional studies to further clarify this role are needed.

Interactions and crosstalk between NK cells and DC in the presence of HCV

Most studies of DCs in the setting of HCV infection have been performed in vitro or are based on chronic infection, and the relevance to the acute phase of the innate response may remain to be proven.

In response to IFN-α, or other specific inflammatory cytokines, DCs typically undergo a maturation process that includes upregulation of class MHC molecules, costimulatory molecules and production of cytokines. Myeloid DCs ($BDCA1^+$, $CD11c^+$, $CD83^+$, $CD33^+$, $HLA\text{-}DR^{bright}$, $CD14^-$) produce IL-12, IL-10, IL-18 (Kaser et al., 2004), TNFα and IFN-β. Plasmacytoid DCs ($BDCA2^+$, $BDCA4^+$, $HLA\text{-}DR^{bright}$, $CD123^{bright}$, $CD11c^-$, $CD33^-$) preferentially produce IFN-α. The cytokines IFN-α, IL-12 and IL-18 are all capable of activating NK cells.

Jinushi et al. (2003b) showed that IFN α-induced upregulation of HLA class Ib MICA/B expression on monocyte-derived DCs was able to activate resting NK cells, enhance NK cell cytotoxicity against K562 cells and increase NK cell production of IFN-γ. This was shown to require direct cell:cell contact and to be dependant upon MICA/B:NKG2D ligation. However, DCs isolated from patients with chronic HCV infection showed impaired modulation of DC MICA/B expression in response to IFN-α, as well as a decreased ability to stimulate NK cells in this circumstance. Further work by this group showed that the DC defect was related to impaired IL-15 production. Using an in vitro coculture system, this group demonstrated that the defect could be overcome with exogenous IL-15 (Jinushi et al., 2003b) but not with administration of IFN-α (Jinushi et al., 2003a). They postulated that an autocrine/paracrine loop involving IL-15 and type I IFNs subserves the ability of DCs to upregulate MICA/B and thereby activate resting NK cells, and that this pathway is blocked by an unknown mechanism in HCV infection. Perhaps related to this is the finding of Ciccaglione et al. (2007) that HCV core protein inhibits IRF-1, thereby reducing IL-15 transcription.

The actual in vivo situation is likely more complex, as Golden-Mason et al. (2004) demonstrated by showing increased IL-15 concentrations from HCV-infected

tissues, which they attributed to production by infiltrating monocytes and resident Kupffer cells. In contrast, Meier et al. (2005) found reduced levels of IL-15 in serum from patients with chronic HCV infection. Additional studies to unravel the variables likely responsible for these superficially contrasting results are needed.

Decreased IFN-α production by circulating plasmacytoid DCs has been reported in chronic HCV infection (Dolganiuc et al., 2006; Yonkers et al., 2007). Lai et al. (2007) examined these cells from fresh HCV-infected livers and showed a relative reduction in this cell type when compared to uninfected but inflamed control livers (Lai et al., 2007). In HCV infection, these cells showed higher BDCA-2 expression. These investigators found reduced numbers of IFN α-producing cells in HCV livers and suggested that this may be related to the fact that BDCA-2 ligation inhibits IFN-α/-β production in plasmacytoid DCs and increases IL-12 secretion.

A reciprocal interaction whereby NK cells from patients with chronic HCV infection may inhibit activation of DCs has also been demonstrated (Jinushi et al., 2004). In these studies, NK cells derived from uninfected donors and cocultured with liver epithelial cells were capable of inducing maturation and activation of DCs. This did not require direct contact between NK cells and DCs, as the maturation effect could be reproduced with conditioned medium from prior coculture of NK cells with Hep3B cells. In contrast, when NK cells from chronic HCV patients were used, no activation of DCs occurred. Rather, the HCV–NK cells elaborated IL-10 and TGF-β, and showed higher levels of the inhibitory receptor complex CD94/NKG2A than did normal NK cells. The investigators hypothesized that the effect was dependant upon ligation of NKG2A by hepatoma cell HLA-E. Blockade of NKG2A restored the ability of HCV–NK cells to activate DCs, concomitant with a reduction in IL-10 and TGF-β production. Further, these treated HCV–NK cells were able to stimulate DCs to produce Th1-polarized $CD4^+$ T cells (Jinushi et al., 2004).

Recently, Ebihara et al. (2008) used direct in vitro infection with the JFH1 HCV strain to address the interactions between NK cells and DCs. They were unable to directly infect monocyte-derived DCs but rather found that double-stranded viral RNA (dsRNA) was introduced into these cells via phagocytosis of apoptotic debris from the infected hepatocytes, and that this colocalized with TLR-3 within DC phagosomes. Following subsequent maturation, these cells secreted IL-6 and IFN-β and were able to activate NK cells in a manner dependant upon DC–NK cell contact. This led them to suggest that activation of NK cells via soluble factors such as type I IFN and IL-15 may only have a subsidiary role in HCV infection.

In a study of freshly separated NK cells derived from patients with chronic HCV infection, De Maria et al. (2007) showed that these cells produced increased amounts of IL-10 in addition to IFN-γ upon stimulation. They speculated that if these cells entered the liver, crosstalk with resident DCs might serve to skew the adaptive immune response to allow viral persistence.

Additional cytokine and chemokine studies relevant to the role of NK cells in HCV infection

IL-10

In the investigations of De Maria et al. (2007), challenge of HCV NK cells with FO1 melanoma cells also resulted in IFN-γ production at levels comparable to control cells, along with increased IL-10 production relative to normal NK cells. The authors suggested that the natural cytotoxicity receptors may play a role in the increased IL-10 production, with implications for Th2 skewing and viral persistence. This position finds support in the study of Knapp et al. (2003) who found that HCV patients with the promoter GG genotype at the IL-10 (−1082), which is associated with higher levels of this cytokine, were more likely to have persistent infection than those without this genotype. Kanto et al. (2004) found that myeloid and plasmacytoid DCs from patients with chronic HCV infection primed increased numbers of IL-10 producing cells relative to controls. Subsequent studies by Gelderblom et al. (2007) examined cytokine production using monocyte-derived DCs from patients with chronic HCV infection and found elevated IL-10 but not IL-12p70 secretion by these cells. Both teams also found reduced IFN-α production by DCs in chronic HCV-infected patients (Gelderblom et al., 2007; Kanto et al., 2004).

As noted earlier, HCV NK cells were capable of producing IFN-γ at normal levels in vitro. Meier et al. (2005) also found normal production of IFN-γ by HCV NK cells in response to stimulation with IL-12 plus IL-18. However, these investigators also noted a reduction of circulating NK cells in these patients and suggested that in vivo availability of IFN-γ derived from these cells may be limited.

IL-15

IL-15, discussed earlier in the context of DC–NK cell crosstalk, plays a role in the development, function and sustenance of NK cells (Becknell and Caligiuri, 2005). Golden-Mason et al. (2004) examined intrahepatic IL-15 levels using a combined approach of RT-PCR, enzyme linked immunosorbent assay and immunohistochemistry. They found a significant increase of this cytokine in HCV-infected liver samples and localized this to Kupffer cells and infiltrating monocytes. They also demonstrated that 80% of NK cells expressed the IL-2/IL-15 receptor β chain (CD 122), which led them to suggest that expression of this cytokine helped to shape the intrahepatic lymphoid population and also

likely played an additional role in the host response to HCV infection.

As noted previously, Meier et al. (2005) reported that patients with chronic HCV infection had reduced levels of circulating IL-15. They were able to promote NK cell survival in vitro with this cytokine and found that it had a preferential effect on survival of $CD56^{dim}$ cells relative to $CD56^{bright}$ cells. This corresponded to their in vivo finding of a greater proportionate decrease in the $CD56^{dim}$ versus the $CD56^{bright}$ NK cell subpopulation in peripheral blood of chronically infected patients. This translates to a shift of the ratio of cytotoxic ($CD56^{dim}$) to cytokine producing ($CD56^{bright}$) NK cell subpopulations. These investigators suggested that IL-15 might be considered as a form of adjuvant immunotherapy in these patients. The implications of NK cell subset differences in the reinterpretation of earlier studies focusing only on NK cell function were considered earlier.

IL-21

Similar in some regards to IL-15, IL-21 is a pleiotropic cytokine that is produced by activated $CD4^+$ T cells and NK T cells; enhances proliferation, activity, and survival of NK cells; and has differential effects on NK cell subsets (Skak et al., 2008; Wendt et al., 2007). To date, no studies have addressed the role of this potentially important cytokine in acute or chronic HCV infection.

TGF-β

Among other functions, transforming growth factor (TGF)-β exerts an inhibitory effect upon NK cells. This cytokine is elaborated primarily by Kupffer cells and activated stellate cells in the liver. Using HepG2 hepatoblastoma cells, Taniguchi et al. (2004) reported that HCV core protein was capable of upregulating TGF-β production directly within these cells, raising the possibility that a similar pathway may also occur in natural infection. Kimura et al. (2006) found the −509CC genotype of the TGF-β1 gene promoter to be associated with a higher clearance rate of HCV. This polymorphism is associated with lower promoter activity, concordant with the concept of TGF-β as a factor favouring viral persistence in this setting.

IL-8

IL-8 (CXCL8) is chemotactic for neutrophils but also has a variety of other effects. Khabar et al. (1997) showed that IL-8 was capable of interfering with the IFN-α pathway. The significance of this pathway for NK cell function was discussed earlier. In vitro exposure of human umbilical vein endothelial cells to HCV-like particles resulted in upregulation of IL-8 production by these cells (Balasubramanian et al., 2005). Polyak et al. (2001) found that the viral protein NS5A alone was capable of inducing IL-8 production and that this activity correlated to the results of an in vitro bioassay to detect interference with the antiviral effects of IFN-α. Thus, NS5A may interfere with the IFN-α pathway by two mechanisms: induction of IL-8 production and interference with the function of the IFN-induced double-stranded RNA-activated protein kinase (PKR) (Polyak et al., 2001).

The report by Asselah et al. (2005) provides potential insights into the timing of IL-8 changes. This group used real-time RT-PCR of 240 selected genes and examined expression levels in normal livers and livers from patients with chronic HCV infection. The latter were subdivided into varying stages of progressive fibrosis as defined using the METAVIR scoring system (Bedossa and Poynard, 1996). No difference was seen in IL-8 mRNA expression when livers with early fibrosis were compared to normal samples. However, IL-8 was significantly upregulated in livers from patients with more advanced fibrosis relative to those with only mild fibrosis, suggesting an increased role for this cytokine in more advanced disease. In contrast, livers from HCV patients with only slight fibrosis showed significant upregulation in a number of type II IFN inducible genes relative to livers from normal controls.

Other chemokines

Chemokines, or chemotactic cytokines, can evoke a number of proinflammatory effects. The production of type I IFNs consequent to HCV infection induces upregulation of the chemokine MIP-1α (macrophage inflammatory protein 1-α) from Kupffer cells (Ahmad and Alvarez, 2004) or endothelial cells, although reports vary regarding cell type (Zeremski et al., 2007). This attracts and leads to locally increased numbers of NK cells (as well as T cells, monocytes and immature DCs) mainly via the CC chemokine receptor 5 (CCR-5). IFN-γ produced by NK cells in turn stimulates hepatic sinusoidal endothelial cells to produce several additional chemokines, including MIG (monokine induced by IFN-γ, CXCL-9), IP-10 (IFN-γ-inducible protein 10, CXCL-10) and I-TAC (IFN inducible T cell α chemoattractant) (Zeremski et al., 2007). These chemokines attract activated Th1 cells that express the surface receptors CCR-5 and CXCR-3 (CXC chemokine receptor 3) (Zeremski et al., 2007), thereby providing a bridge between innate and adaptive immunity (Ahmad and Alvarez, 2004). Enrichment of intrahepatic T cells bearing these receptors has been demonstrated in patients with chronic HCV infection (Apolinario et al., 2002). IP-10 levels have been associated with the degree of lobular inflammation in these patients (Harvey et al., 2003), and this has been proposed as one of several predictive markers for both rapid and for sustained viral responses (Romero et al., 2006).

Zeremski et al. (2007) recently reviewed this topic and suggested that chemokine generation may play an important role in both viral clearance and in the propagation of chronic inflammation in this infection. In their model, early chemokine production ultimately leading to strong T cell mediated antiviral effector cells is desirable and is a likely correlate of the spontaneous resolution that occurs in 20% of acutely infected patients. In contrast, persistence of chemokine generation in the remainder of patients who generate an ineffective cell mediated response may lead to the continued and nonspecific attraction of inflammatory cells, causing continued necrosis and eventually leading to cirrhosis in the face of viral persistence.

Current therapy of HCV infection

Combined treatment with pegylated IFN-α and ribavirin remains the mainstay of therapy for patients with HCV infection. The regimen is given for 6 or 12 months. Sustained virological response is seen in approximately 55% of treated patients; 10–25% of patients have a transient response with relapse following cessation of therapy, and the remainder are nonresponders. These unsatisfactory results are further qualified by the fact that a number of patients are not eligible for or cannot tolerate therapy and are not included in these figures.

Response to therapy is manifested as a rapid decrease in circulating viral genomic levels, which is interpreted as a suppression of viral replication, followed by a slower decline thought to be related to elimination of infected cells. Treatment during the acute phase of infection appears to be more effective, leading to a reduced frequency of chronic HCV infection from the expected 80% to approximately 10%. Feld and Hoofnagle (2005) suggest that this may indicate that resistance to therapeutic IFN-α might be an acquired phenomenon that arises during the chronic phase, pointing out the need to dissect the host:viral interactions from a temporal perspective.

In one microarray study of liver biopsy samples (Chen et al., 2005), upregulation of IFN-responsive genes prior to therapy was associated with nonresponder status. This suggests (Feld and Hoofnagle, 2005) that an IFN response already in place in these patients is unable to effectively manage the infection and that additional stimulation limited to this pathway is futile. It also highlights the complexity of the host:viral immune interaction and further underscores the fact that the phenotype responsible for loss of viral control likely varies among patient subpopulations.

A major current effort is being directed towards the development of small molecule inhibitors of HCV enzymes. The major viral targets include the NS3/4 A protease and the NS5B polymerase. Several of these agents are in clinical trials and are the subject of recent reviews (De Francesco and Migliaccio, 2005; Harrison, 2007; Pawlotsky et al., 2007). Despite optimism in this area, Pawlotsky et al. (2007) point out that problems with lower antiviral efficacy in vivo compared to in vitro studies, unfavourable toxicity profiles and the development of viral resistance suggest that current therapies will remain as standard care for some time. These predictions, while presently true, are always subject to change.

Current success, however limited, with IFN-α-based regimens does indicate that stimulatory immune modulation at the level of the innate immune system may lead to either transient or prolonged viral remission. Agonists of TLR-7 and TLR-9 are currently in phase 1 clinical trials. Engagement of these receptors, normally found on plasmacytoid DCs, leads to increased IFN-α production, maturation of DCs and stimulation of NK cells.

Direct stimulation of NK cells is a potential avenue of therapy that may reduce the HCV burden in an additive or perhaps synergistic manner when combined with other therapies directed towards stimulation of adaptive immunity or against components of the viral life cycle. Based on studies of mechanisms contributing to NK cell inhibition in chronic HCV, the use of IFN-γ or IL-15 has been suggested as a possibility. Other possible approaches, such as interference with HCV E2: CD81 interaction on NK cells, stimulation of natural cytotoxicity receptors, reduction of IL-10, TGF-β, or IL-8 activity, among others, can be inferred from the earlier discussion of disordered NK cell physiology during HCV infection. Indeed, Golden-Mason and Rosen (2006) hypothesized that the NK cell is the primary target upon which HCV formulates its immune evasion strategy and that the defective crosstalk between NK cells and DCs underlies the observed T-cell defects in this disorder. However, the warning of Zeremski et al. (2007) must also be remembered: What constitutes an effective immune response in acute viral hepatitis does not necessarily imply that a similar response is desirable in later stages. A detailed understanding of the differential effects of NK cells at varying time points during the course of infection must underlie any future efforts to manipulate these powerful cells for the benefit of the host.

Acknowledgement

The authors gratefully acknowledge Mitsuru Sakakibara, M.D., for the design and implementation of Figure 44.1 in this chapter.

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