

THE IMMUNE SYSTEM AND HEPATITIS C VIRUS

by Stewart Cooper M.D.

INTRODUCTION

Humans, like all animals with a backbone, protect themselves from viral infections by deploying specialized cells, which engage and destroy most viruses that pose a threat. The specialized cells that work in concert to detect and eradicate foreign organisms are collectively called the immune system. The science of immunology studies how this highly evolved and efficient system works.

Because the immune system typically kills viruses and protects against infection it is curious that when hepatitis C virus (HCV) infects people, only a minority can clear it from their bodies. (For immunologists, this is far from an unimportant minority, which I will develop later, but at this point I would like the reader to firmly note that some people can indeed clear HCV infection naturally.) The majority experience persistent infection whereby the virus evades, subverts and/or weakens the immune system and survives for the life of the infected person. Such “chronic infection” often results in liver damage, which can lead to cirrhosis, liver failure, liver cancer, and – without liver transplantation – premature death. Approximately 5 million Americans and at least 200 million people worldwide are chronically infected, with a significant proportion developing progressive liver disease. This makes HCV the most common reason in the Western hemisphere why someone undergoes liver transplantation.

Clearly, the stakes for characterizing mechanisms of protective immunity as a gateway to developing a vaccine are high. The basic approach is to unravel the type of immunity that naturally overcomes HCV then to design vaccines and/or therapies aimed at stimulating and amplifying that immunity. Strategies could conceivably involve stimulating specific immune cell types, like “lymphocytes”, and/or blocking natural or HCV-mediated dampening influences—in essence firmly pushing the “on” button while blocking all attempts to trigger the “off” button. Here, my intent is to introduce the reader to aspects of the immune system that, at the time of writing—July 2006—seem central to this endeavor.

At the outset it is essential to recognize that some fundamental questions relating to the immunology of hepatitis C remain unanswered. For example: “How is HCV cleared from the body – a) naturally, and b) in response to Interferon-based therapy?” And, “Can the immune response itself contribute to the progression of liver disease?” In this chapter, intentional focus will be upon current immunological knowledge and those future research directions that show promise for translating into prevention and treatment of hepatitis C.

On a personal note, I hope this chapter will be of interest to a broad readership, including people currently infected by and recently exposed to HCV, and those living with and caring for people suffering this still much too “silent epidemic”. My aim is that attentive information seekers will:

- i) Understand the logical basis of immunological studies;
- ii) Derive a sense of where immunologists currently are in this arena of research;

- iii) Procure adequate knowledge to follow the gist and implications of immunological research as it unravels;
- iv) Be stimulated to ask questions and thereby contribute to the research endeavor.

Like all fields of medical research, this one will be greatly enriched and even accelerated by more public interest and participation. I believe the latter will be encouraged by better understanding of the key scientific questions and obstacles. My principal assignment is therefore to translate the concepts, discoveries and jargon of everyday science into something palatable for non-specialists. Any failure to achieve this rests solely with the author, who will always welcome questions, comments and suggestions. I will begin with a preamble to provide some context and basic grounding:

BACKGROUND INFORMATION

HCV persistence versus clearance

Persistence is unusual for viruses like HCV, which are made of a short single strand of RNA—a close but distinct chemical relative of DNA. The most infamous RNA viral “squatter”, HIV, can ultimately persist by slotting in among its host’s genes where it permanently avoids immune detection. This is a property that we do not believe HCV possesses. For HCV, viral and host factors that are responsible for persistence have yet to be satisfactorily explained. Clear, however, is that the fate of hepatitis C (self-limited versus chronic infection) is nearly always determined during the early, or “acute”, phase of infection – a period typically defined by the first 24 weeks (1). Although it can sometimes take even longer to clear, in the majority of cases when the immune response naturally expunges HCV, it accomplishes the charge during the first 6 months. Timeframes are important parameters in HCV infection. Because progression to advanced HCV-related liver disease (cirrhosis) takes years, natural clearance by the immune system averts disease progression and returns liver health. Early HCV infection has therefore become the focus of intense attention for some immunologists, including this author. We reason that if we understand the type of immunity that allows people with acute hepatitis C to clear infection, we can devise methods of stimulating the same type of protective immune response in others; that is, we can make a vaccine.

HCV clearance has been difficult to study

Most readers will probably not appreciate that acute hepatitis C has been deceptively difficult to study. For researchers, a frustrating curiosity is that most people have few or no symptoms of acute hepatitis (for example most do not develop jaundice—yellowing of the eyes, darkening of the urine and paleness in the stool) and do not consult doctors or other healthcare professionals. As a result, very few clinicians are likely to see even one case of acute hepatitis C per year. This paucity of clinically apparent cases has severely limited studies of the rate and predictors of viral clearance in natural HCV infection. During recent years we have therefore begun studying special groups of people: those who experience a high rate of new HCV infections.

Injection drug users experience a high rate of acute HCV infection

While modern blood-supply screening practices have reduced the number of new HCV infections in the West, cases continue to arise particularly among injection drug users (IDU). In recent years, diligent work by a few dedicated epidemiologists – scientists who analyze the

incidence, spread and control of diseases in populations – has defined study groups (called “cohorts”) of IDU who experience a high incidence of acute hepatitis C. These precious cohorts now provide unprecedented opportunity for immunologists and epidemiologists to collaboratively investigate acute HCV infection.

A smoldering immune response may underpin liver disease progression

While dissection of successful immune responses is of critical interest to aspiring HCV vaccine developers, the details of host immunity in people with chronic hepatitis C may provide clues for arresting the progression of liver scarring (officially termed “fibrosis”). For chronically infected people, interactions between the immune system and HCV-infected liver cells may determine the amount and rate of liver fibrosis and therefore the rate of liver disease progression. In this regard, the liver injury associated with persistent HCV may be similar to that incurred in chronic hepatitis B virus (HBV) infection. Like HBV, HCV probably causes little or no liver damage if ignored by host immune cells. When HCV stimulates but defeats waves of immune attack the immune system may actually cause the liver injury. Certain immune defense strategies—which may be genetically influenced—might be particularly damaging. A proportion of people with hepatitis C seem particularly prone to form scar (“fibrous”) tissue. A variety of other factors may further contribute to the cascade of events that leads to liver injury and scarring, such as ingestion of alcohol and, conceivably, stress and diet. To complicate the plot even further, each contributing factor may have immunological effects.

Liver damage is not universal

A central question is why liver inflammation converts to scar tissue in only some people. It will be important to work out the defense tactics deployed by host immune responses in these ill-fated battles, with the intent of distinguishing pathways that promote liver fibrosis. The eventual goal is to develop therapies aimed at blocking, dampening or diverting harmful interactions. Thus, in the chronic setting, the immune response might be more detrimental to the host than the virus. Passive co-habitation with HCV may prove more harmonious than uncommitted attempts at eviction.

Successful Interferon-based therapy may need a healthy immune system

An accumulating body of evidence suggests that in order to work, Interferon- α (IFN) and Ribavirin (RVN) – in combination the only therapy shown capable of eliminating HCV – probably need to boost a critical arm of the immune system. Therefore, studying the components of natural HCV immunity could also hold implications for understanding the mechanism of viral clearance in response to IFN and RVN. A possible role for the immune system in treatment-induced HCV clearance will be discussed later in the chapter.

Thorough study of the immunology of hepatitis C will undoubtedly require further collaboration between clinicians and scientists from different disciplines. In this article I will review what is known about the immune response to HCV and for reasons described above I will pay particular attention to the way in which the immune system engages the virus during the acute phase of infection. A coherent account is not possible without initially devoting some attention to the target and stimulus of the immune response in hepatitis C – the virus itself.

HEPATITIS C VIROLOGY FOR BEGINNERS

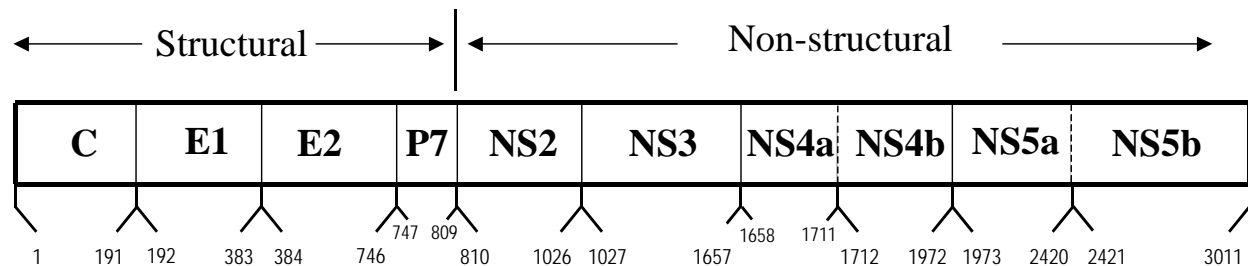
What is HCV made of?

For conceptual understanding of HCV immunity it is helpful to appreciate the basic structure of the virus. HCV is a relatively small virus whose genetic material is made from ribonucleic acid (RNA), not DNA—which is the building material of human genes (2). The entire genome of HCV is comprised by a single strand of merely ~9,600 units. (In comparison the average length of a single human gene is ~27,000 units). Each RNA genetic unit – officially called a “base” because of its chemical property (past high school students may recall “acids and bases”) – is comprised by the sugar, ribose, fused to a molecule of either Guanine (G), Adenine (A), Cytosine (C) or Uracil (U) arranged in specific sequence. The sugar, ribose and the base, Uracil, distinguish RNA from DNA.

Each sequence of 3 bases constitutes a code that usually specifies a particular amino acid: for example, “AUG” specifies the amino acid, Methionine. (Biologists therefore simply call each set of 3 bases a “codon”.) A series of codons therefore specifies a series of amino acids. Amino acids are the individual units that when strung together build proteins (in viruses, people and all living things). In nature, the vast majority of proteins are assembled from only 20 amino acids that are arranged in diverse permutations and combinations, as genetically instructed. The structure of a particular protein is determined by the specific sequence of amino acids that are strung together.

Figure 1. Schematic of the HCV Polyprotein

Letters indicate individual HCV proteins. Numbers indicate the amino acid coordinates of each protein. Shown here are the protein coordinates of HCV-1, the ‘prototype’ strain discovered by Choo and associates (2). C=Core, the



capsid protein. E=Envelope proteins, which participate in the viral outer structure – possibly with P7, a protein of unknown function. NS=Non-structural proteins. NS2 forms a protease in conjunction with NS3. NS3 possesses 3 different enzyme activities. NS4, like NS5, comprises two proteins, each denoted “a” and “b”.

HCV has ten major proteins (Figure 1). And the structure of all ten individual HCV proteins is determined by the specific sequence of bases in the HCV RNA.

HCV is a virus with a highly variable structure

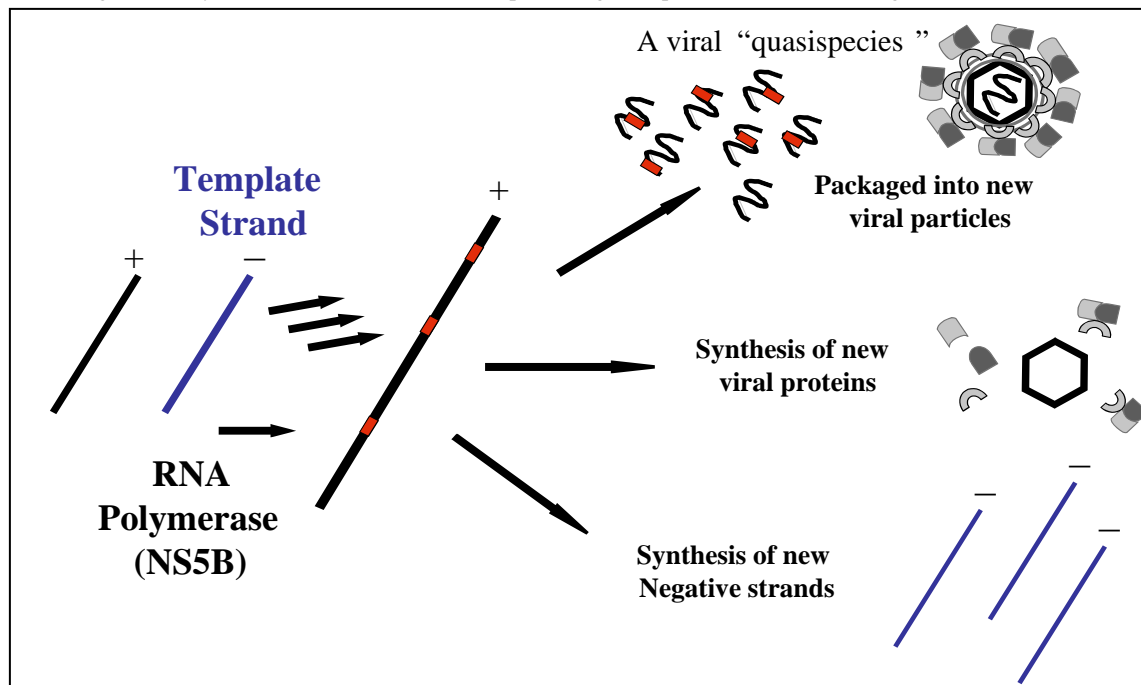
Remarkably, HCV seems to change its genetic and protein sequences with relative ease. This is an important concept and I will devote some time to it. The act of switching one genetic building block (a base) to another (for example, the base Adenine to Guanine) is called “mutation” and in general RNA viruses like HCV have much higher rates of mutation than their DNA counterparts. Please recall that HCV possesses a single strand of RNA. In a liver cell, this “positive sense” or “+” strand is copied into a complementary “-” strand, which acts as a template for making more

+ strands. The process, however, is error prone, so that on average each daughter HCV RNA strand will possess at least one different base from its parent and siblings. This is depicted in Figure 2.

We estimate that there are approximately one trillion (10^{12} , outside the USA, one billion) replications each day. Because mutations occur more or less randomly, this huge number of replications implies that every single base in the HCV RNA strand can theoretically undergo mutation daily. In reality, many mutations will not lead to a fully functioning virus because they injure some aspect of the life cycle. Nevertheless, many capable mutant viruses emerge to face the host's immune defenses. The consequences are worth thinking about, which I will develop below. For detail seekers, excellent reviews can be found in (3, 4). The main point is that no one infected with HCV is infected with merely a single virus, but instead with a mixture of related viral sequences. Remarkably, the HCV sequences in a single person can exhibit greater percentage genetic difference than that distinguishing major mammalian species, such as human and chimpanzee. Scientists therefore describe these circulating families of close viral relatives as "quasispecies" – a term I introduce because it is often found in HCV literature (5). In essence each infected person harbors HCV quasispecies, not a single entity.

Figure 2. HCV Replication is Error-prone

Following viral entry into a liver cell, HCV starts reproducing. The positive-stranded HCV genome (+) makes a



complementary "negative strand" (-), shown in blue. New + strands, are synthesized by the viral enzyme, RNA polymerase using the negative strand as a template. Each "daughter" + strand is likely to contain at least one mutation compared with the parental strands. These new + strands are used to manufacture more negative strands and new viral proteins that fold around the + strands to make new virus particles. To reflect the differences in HCV RNA sequence even in single individuals, the infecting virus populations are referred to as quasispecies.

Genetic diversity results in diversity among the encoded proteins, which creates the basic material on which natural selection can operate when a population is placed under environmental pressure. This holds for all other living things including viruses. In comparison to humans and

other animal species, however, RNA viruses play out the evolutionary game by producing remarkable numbers of genetically diverse offspring in relatively brief timeframes. This creates rich opportunity for natural selection to operate quickly. Thus it is notable that many of the observed amino acid mutations in HCV proteins are clustered at sites targeted by the immune system, indicating they have not emerged merely by chance. Chance would not favor clustered mutations. We know that these mutations can disrupt the viral targets of immune attack – which in scientific jargon are called “epitopes” (6, 7). The important point is that if mutation alters the structure of an epitope, the mutant virus is liberated from that source of immune attack.

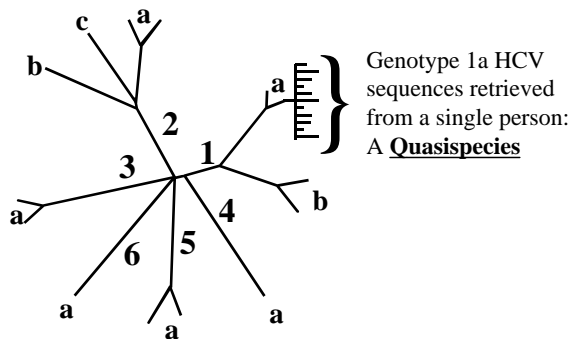
Natural selection works like this: mutations occur randomly, and when a certain mutation advantages its owner, that individual (officially called a “carrier”) is set apart from the pack. If the genetic mutation fails to injure the function or reproductive success of its carrier, the imbued privilege can be passed to its progeny. Extending the example of the HCV epitope mutation described above, viruses carrying such mutations will become more abundant in the host (in liver and blood) as their “fitness” outstrips that of other viruses neutered by the immune response. We are therefore able to readily observe them. This phenomenon is called “escape mutation” (another commonly used term) and represents evolution on the mini scale. Freed from immune attack, viruses with escape mutations can thrive.

As I have implied, examples of escape mutation by HCV have already been observed (6, 7) and are popularly invoked as a principal means of HCV persistence. Indeed, so talented is this “Changeling” virus that perhaps no HCV epitope can be guaranteed to remain permanently intact – a consideration that might confound vaccine development. It is important to emphasize, however, that the true extent by which mutation underlies HCV persistence remains uncertain. Because of the implications for vaccine design, some researchers are eager to measure the effect of HCV mutations on the overall efficiency of the immune response. The virus may, for example, experience more difficulty in surviving with some mutations than others. If a hierarchy of HCV mutations exists, discovery of epitopes that are problematic for the virus and therefore advantageous to the immune system, could significantly enhance prospects for vaccine development.

HCV mutation has resulted in “Genotypes and Subtypes”

The same genetic plasticity that results in HCV diversification has resulted in the evolution of even more divergent forms of HCV. The most profound HCV genetic divergence is observed between hepatitis C viruses from different human populations. Such comparisons reveal at least six major HCV genetic families, called “genotypes”, numbered 1→6 in their order of discovery (Figure 3). Genetic sequence variability has resulted in each genotype being further divided into “subtypes”, which are denoted by lower case letters: a, b, c etc. Hence, we routinely specify that someone is infected with HCV “genotype 1a” or “genotype 2b” etc. In the North American ethnic brew, HCV genotype 1 infection is most common with genotypes 1a and 1b occurring with approximately similar frequency – except in African Americans, among whom genotype 1a is predominant. Next most common are infections with genotypes 2 and 3 viruses. The “tree” shown in Figure 3 depicts the relationship between the HCV genotypes and subtypes.

Figure 3. Genetic diversification of the hepatitis C viruses



<u>Genotype 1->6 (population)</u>	<u>Subtype (a, b etc.) (population)</u>	<u>Quasispecies (individual)</u>
30% nucleotide difference	~20% nucleotide difference	1-5% nucleotide difference
Well-established diversity	More-recent diversity	Rapidly evolving diversity

Just as family trees depict the relatedness of human families, phylogenetic trees, such as the one shown here, depict relatedness between different genetic sequences. A computer program has compared corresponding positions of many HCV genetic sequences and represented the degree of correspondence graphically. All members of each of the 6 major branches (the genotypes, numbered 1-6) are defined by shared genetic characteristics. Genetic variability exists at three levels. HCV *genotypes* (numbered 1-6) represent the highest level of divergence and probably the most ancient splits among HCV sequences. Each of the 6 major genetic groups contain a series of more closely related *subtypes*, typically different from each other by ~20% compared with >30% between genotypes. Genotypes 1a, 1b and 3a are now widely distributed due to unscreened blood transfusion and shared injection drug use, and now represent the vast majority of infections in Western countries. Each person is infected with a broad range of viral variants referred to as *quasispecies*, which exhibit up to 5% sequence differences.

It is possible that the natural history of infection may differ between HCV genotypes in different ethnic groups, though little evidence in support of this possibility is yet available. Now well described, however, are differences in IFN-based treatment outcome according to HCV genotype and ethnicity (8). For example, genotype 1 causes most HCV infections in the West, where it is also the most difficult type of HCV to treat successfully. Furthermore, genotype 1 is significantly more treatment resistant in African Americans. Overall, genotypes 2 and 3 infections are the most treatment receptive, at least in Western populations, with genotype 2 being most susceptible. While treatment responsiveness is discussed in more detail in a different chapter, these differences remain largely unexplained and imply interplay between host and viral factors. We suspect that genetically determined differences in the immune response play an important role, but a discussion of this is beyond the scope of this article.

Finally, it should be stressed that non-mutational viral mechanisms are likely to also play a role in immune subterfuge (9-12). We suspect that HCV mutation may only be a factor in viral persistence – which requires further study. The following thoughtful reviews should at least partly satiate those detail-oriented readers with lingering hunger (3, 4, 13). Other potential viral mechanisms involved in immune subterfuge will be discussed in the relevant immunology sections.

THE IMMUNOLOGY OF HCV INFECTION

Like the body it protects, the human immune system has a physical and functional structure. Physically, it is made up of individual cells, receptors and chemicals. The immune system deploys many types of cell and a considerably greater number of chemical substances. “Receptors” are vitally important molecules embedded in the surface, or the inside, of a cell. Each receptor binds a specific chemical structure then signals either activation (“ON”) or inhibition (“OFF”) of various cellular functions. In essence, receptors allow cells to detect and respond to certain things happening in their outside environment. Functionally, the components of the immune system network to form a highly organized defense against invasion by abnormal cells and foreign organisms, such as viruses. It is an integrated system with in-built control and failsafe mechanisms. If some components fail to work, others are in place to provide backup – an arrangement that inevitably generates complexity. That our ubiquitous species has survived every infection it has encountered in every niche of this planet attests to the sophistication and efficiency of the human immune system.

An approach that continues to serve students of immunology well is to categorize the immune system into two principal arms, called the “innate” and the “adaptive” systems. Typically, these are described as separate entities which defend in sequence: the innate system first. As I will soon explain, in reality these two systems are interactive and interdependent.

INNATE IMMUNITY

The innate immune system provides front-line defenses. It has a long list of chemical components, many of which are unstudied in the context of HCV infection and hence unnecessary to catalogue here. Chemicals released inside infected cells that suppress viral replication may be the most ancient form of “innate immunity”. Interferons are in this category, can be effective inhibitors of viral replication and can suppress HCV in many people when used therapeutically. Why such innate immune mechanisms commonly fail to prevent HCV establishing a foothold is deeply interesting but as yet relatively unstudied. I will therefore devote most attention here to the cellular arm of the innate immune system. Cellular components of likely importance in influencing the outcome of HCV infection are called antigen-presenting cells (APCs) and the lymphocytes of innate immunity are called natural killer (NK) cells.

Antigen Presenting Cells

Antigen presenting cells serve to activate effector lymphocytes (NK and T cells) – the immune system’s front-line cells that attack foreign invaders. Sympathetic with the lament that “the immune system has too many cells”, even APCs are not a single type of cell. Antigen presenting cells include so-called “dendritic cells” (DC) and “macrophages” (Φ). (Often mentioned in the literature, “Kupffer cells” are a type of Φ found in the liver). A third type of cell, called a “B cell”, is also officially categorized as an APC, though the tenacious reader will later learn that B cells, like modern folk, multitask; being also the cells that make antibodies. In summary, the three major categories of APC are dendritic cells, macrophages and B cells.

Different APC may serve to activate effector lymphocytes at different points in the immune response, and perhaps in different places. That is, there seems to be a subdivision of labor among APC. The way in which APC activate T cells (the effector lymphocytes of adaptive immunity) is

relatively well worked out but we have recently learned that DC (the most proficient APC) also activate NK cells – the effector lymphocytes of the innate immune system (14-17). The mechanism of NK cell activation by DC, however, remains incompletely deciphered. An up to date review can be found in (18).

Dendritic cells initiate new immune responses

Dendritic cells were originally discovered in mice during the 1970s by Drs. Zanjil Cohn and Ralph Steinman at the Rockefeller Institute, New York (19). We now recognize a particular expertise of DC is their ability to kick-start a new immune response. Unlike Φ and B cells, DC continuously express high levels of so called, “co-stimulatory molecules”, which are inserted into their cell membranes. Co-stimulatory molecules are important because they are required for activating naïve T cells – rookies that have never previously engaged a foreign invader. Even DC come in different varieties. It now appears that there are distinct lineages of DC, each developing from a shared parental cell-type in response to local micro-environmental conditions. In turn, each DC subset stimulates different lineages of T cells such as “Th1”, “Th2” and “Treg” cells. (These T cell subsets will be discussed in the section on Adaptive Immunity, but please note here that we believe strong responses by the Th1-type of T cells are required for HCV clearance.) Like military field commanders, DC can therefore play a pivotal role in regulating an immune attack (20), by dictating the type of local forces deployed. Dendritic cells may thus play an important role in determining the fate of HCV infection – a possibility that immunologists are beginning to consider (21).

Because the local environment can influence the type of DC that develop, we are interested to learn more about DC in the human liver – the seat of HCV infection. As yet, information about DC in the liver remains relatively scant. Understanding the biology of the liver’s DC and their likely role in governing immune responses against HCV should fill an important gap in current knowledge. In particular, unraveling the biology of the liver’s version of a DC subset called “Plasmacytoid DC” (22), which promote Th1 cells, might shed greatest light on the earliest determinants of HCV clearance. Readers wondering about the relevance of DC research for people with hepatitis C should recognize that DC are potential targets for vaccines aimed at eliminating HCV – and perhaps other persistent viral diseases.

Dendritic cells possess antique receptors for detecting viruses

Like other APCs, DC take up foreign organisms, probably including HCV (21, 23). Like the most fearsome immigration officers, DC detect foreigners, take them into custody (uptake the organisms into the cell interior) then process them in a variety of merciless ways. The following are two principal examples of how viruses are “processed” (the official term) by DC and the immune response subsequently activated:

1. Dendritic cells possess “pattern recognition receptors” (PRR) that bind highly conserved structural motifs in the genetic material of different viruses and thereby distinguish the type of material (RNA or DNA) the virus is made from. Different PRR recognize distinct motifs, and which PRR is bound determines what the response of the APC will be.

Single stranded RNA viruses, like HCV, are bound by a brand of PRR unwieldily named Toll-Like Receptors (TLR) (24), perhaps especially TLR-7, 8 & 9 (humans have ten TLR) (25). TLR

are themselves remarkably conserved molecules; their close relatives, “Toll receptors,” were originally discovered in flies. When molecules are retained over long evolutionary time (the common ancestor of humans and flies is estimated to have lived more than 1 billion years ago) it implies that they are so exceptionally useful that species cannot survive without them. As with PRR in general, which TLR is bound determines the type of response. TLR binding stimulates APCs to release chemicals, called “cytokines”. Cytokines are small molecules that typically activate other immune cells. (A convenient description of individual cytokines can be found at <http://www.copewithcytokines.de/cope.cgi> and a thought provoking review in (26)). This is the way by which stimulated APCs recruit the help of cells specialized in active combat (collectively called “effectors”). For students of trench warfare, cytokines are the immune system’s “runners”, providing local and long distance communications. Therefore, using TLR, DC identify the nature of the invading organism (virus or bacterium and if virus, RNA versus DNA etc.) and turn on a response that is usually appropriate for repelling that type of invader.

Cytokines released following TLR binding by viral RNA include Interferon-alpha (IFN- α) – the same chemical we use to treat hepatitis C – together with a variety of “Interleukins” that stimulate activation of effector lymphocytes including NK and T cells. Notably, a variety of DC, called pDC2, seem particularly important as major producers of IFN- α and other Interferons, which we believe are essential for natural clearance of HCV (27). Importantly, the profile of cytokines released during this earliest encounter with a virus (possibly even within the first hours) may set the stage for the type, strength and eventual success of a person’s immune response.

It is important to note that the amount and type of cytokine released by APCs during an antiviral immune response can differentially promote the function of NK cells, T cells and B cells (in their mode as producers of antibodies). In the response against HCV, the lymphocytes that are recruited and the vigor of those responses almost certainly dictate whether HCV will be cleared or persist. Later on I will develop this concept further. Possible, however, is that HCV is already waging trickery during this earliest phase of infection, by undertaking strategies for deviating the immune response away from a path that would otherwise lead to its removal from the host (28).

A spate of recent studies have suggested that by the time chronic infection is established, DC numbers are reduced and their ability to produce beneficial cytokines diminished. See, for example (29). Precisely how the virus pulls this off, and at what stage of infection, currently remains uncertain. These central questions will be most accurately addressed by carefully studying DC during the course of acute HCV infection.

2. Antigen presenting cells not only detect the genetic material of internalized viruses, they also pay attention to the wrappings. Viral proteins, and probably other bits, are ‘chopped’ into small fragments then transported back to the cell surface where they are firmly held and paraded by dedicated molecular scaffolds (more officially called “antigen receptors”). In this immunological equivalent of a gamekeeper’s gibbet, these dismembered corpses are closely inspected by marauding lymphocytes, some of which will recognize their presence, become activated and embark upon the immune system’s version of “ethnic cleansing”. For NK lymphocytes the details of this recognition mechanism have not been fully worked out, but we know that interaction of NK cells with DC leads to activation, whereby the NK cell itself releases a variety

of cytokines and small packets of lethal chemicals (30). In the immunological squad dispatched to challenge HCV, NK cells are emerging as ‘very probable’ players. This area of research is likely to mature quickly and I will therefore provide the reader with an “Essential Guide” to relevant aspects of NK cell function.

NK Cells

These large lymphocytes kill infected cells during the very early stages of viral infection (for a general review see (31)). NK cells not only participate promptly in anti-viral responses, they also promote other cellular immune responses. Unlike T lymphocytes, their relatives in the adaptive immune system, NK cells are always ready for action. Within minutes of activation they release an assortment of potent chemicals. Pre-packaged combinations of “Perforin” and “Granzymes”, a cocktail of lethal proteins, are lobbed onto the outer membranes (the cell’s equivalent of skin) of any virus-containing DC with which NK cells have forged specific contact. Perforin punches pores in the DC membrane that allow granzymes to enter and cause the cell to commit a form of ritual suicide called “apoptosis” (a programmed sequence leading to cell death).

Activated NK cells are not silent assassins; they stir things up like the most accomplished agitators. Already highly prepared, they quickly manufacture and release a set of cytokines, including Interferons (IFN- α , IFN- γ), Interleukin 12 (IL-12) and tumor necrosis factor alpha (TNF- α) (31, 32), which activate and attract other cells, including cells of the adaptive immune system – the subject of the next section. As will be discussed, the cytokines released by activated NK cells closely resemble those produced by a type of T cell called a “T helper 1” (Th1) cell, which promotes antiviral immunity by a far more selective virus-killing lymphocyte, called a “cytotoxic T cell”. Hence NK cells not only serve the innate immune system, they act to bridge innate and adaptive immunity. This is an important concept. It therefore seems reasonable to assume that the efficiency of NK cell activation in response to a particular virus may be a critical factor determining whether the virus is eliminated or able to persist. And this may be especially pertinent in early HCV infection, when the balance between clearance or persistence could be finely suspended.

The above emphasizes the value of NK cells in innate immune defense. At this point, however, I would like the reader to reflect upon a potential problem faced by nature in allowing NK cells to evolve. (Their presence in jawed cartilaginous fish suggests that NK cells first arose more than 350 million years ago.) The advantage of having cells capable of such potent effects “ready to go” in our organs and blood could result in rapid catastrophe if they were to activate by mistake – a self-destructive condition referred to as “horror autotoxicus” by the 1908 Nobel Laureate, Dr. Paul Ehrlich (33). Since seminal experiments by Karre and colleagues in 1986 (34) several teams of scientists have shown that nature’s elegant solution involves regulation of NK cell activation using an intricate system of cell-surface receptors. NK cells integrate signals from arrays of activating and inhibitory receptors, but inhibitory receptors – which dampen cellular activation – play the dominant role. Nature has chosen safety. Therefore, as one would predict, every NK cell has at least one inhibitory receptor. Without one, by default, the NK cell will activate. By the same principle, if an NK cell’s inhibitory receptor(s) is not engaged, the NK cell will activate.

A thorough discussion of NK cell receptor biology is beyond this chapter’s brief, but more diehard readers can seek satiety in a recent review by one of the field’s pioneers (31). Of

relevance for HCV infection, however, an Anglo-American collaborative study led by Drs. Salim Khakoo and Mary Carrington recently provided first evidence that certain NK cell inhibitory receptors significantly influence the chance of clearing acute hepatitis C (35). This striking discovery holds potentially exciting prospects for future HCV therapies, and for readers craving more insight I will provide the necessary framework.

The Control of NK Cell Activation by Receptors for HLA Class I Molecules

NK lymphocytes sample the health of the body's tissues by briefly touching cells – all of which are of course potential targets for NK cells if something goes awry, such as a virus infection. Each touch is carefully contrived; the contrivance mediated by the aforementioned NK cell receptor system. (Readers have already learned that in the immune system, “receptor” is a general term encompassing many different types of signaling molecules. At this point it will be helpful to know that the molecular partners specifically bound by receptors can also be collectively described, and are called “ligands”. Simply put, receptors bind ligands.)

Binding to cells by the inhibitory NK cell receptors (iNKR) is accomplished by engaging ligands called HLA class I (“one”) molecules, which are embedded in the surface of virtually every normal cell in the body. HLA molecules happen to also be the things that create your “tissue type”, and therefore the things that transplant surgeons want to match as closely as possible before installing someone else's organs into people whose own organs have failed (the liver is uniquely different in this regard – but that is incompletely understood and would require a separate chapter). The ‘matching problem’ surrounding HLA class I molecules arises because the HLA genes, and the proteins they encode, are so remarkably variable that very few individuals will be identical. Extreme variability at a population level is the hallmark of the three so called “classical” HLA class I genes, HLA-A, HLA-B and HLA-C, which are nestled closely together on chromosome number 6. As of October 2004, 338 versions (officially called “alleles”) of HLA-A genes, 617 versions of HLA-B and 179 versions of HLA-C have been found in people around the world (IMGT Database: <http://www.ebi.ac.uk/imgt/>). Please note that each genetically normal person can have a maximum of only two versions of each HLA gene.

Each cell in the body harbors two copies of chromosome 6 – one inherited from each parent. Each person therefore carries up to six different HLA class I genes (2xHLA-A + 2xHLA-B + 2xHLA-C), since there are three (1xHLA-A, -B, -C) on each chromosome 6. Genes encode proteins – the entities that perform biological functions. Therefore, a person can express 3-6 HLA class I proteins. A major function of HLA class I proteins is to bind short pieces of processed viral proteins (“peptides”—of about 8-11 amino acids in length) and to display them at the cell surface where they can be detected and engaged by T lymphocytes (36).

The most variable parts of HLA genes encode the regions of HLA proteins that physically contact viral peptides. Therefore, different HLA proteins have a strong tendency to bind different sets of viral peptides. We believe that the adaptive immune response benefits greatly from this HLA class I genetic and protein diversity, which imbues ability, particularly at a population level, to bind huge numbers of viral peptides. The work that spawned these discoveries started in the 1970s (37, 38), eventually earning Drs. Peter Doherty and Rolf Zinkernagel the 1996 Nobel Prize in Medicine, well before the discovery that HLA class I

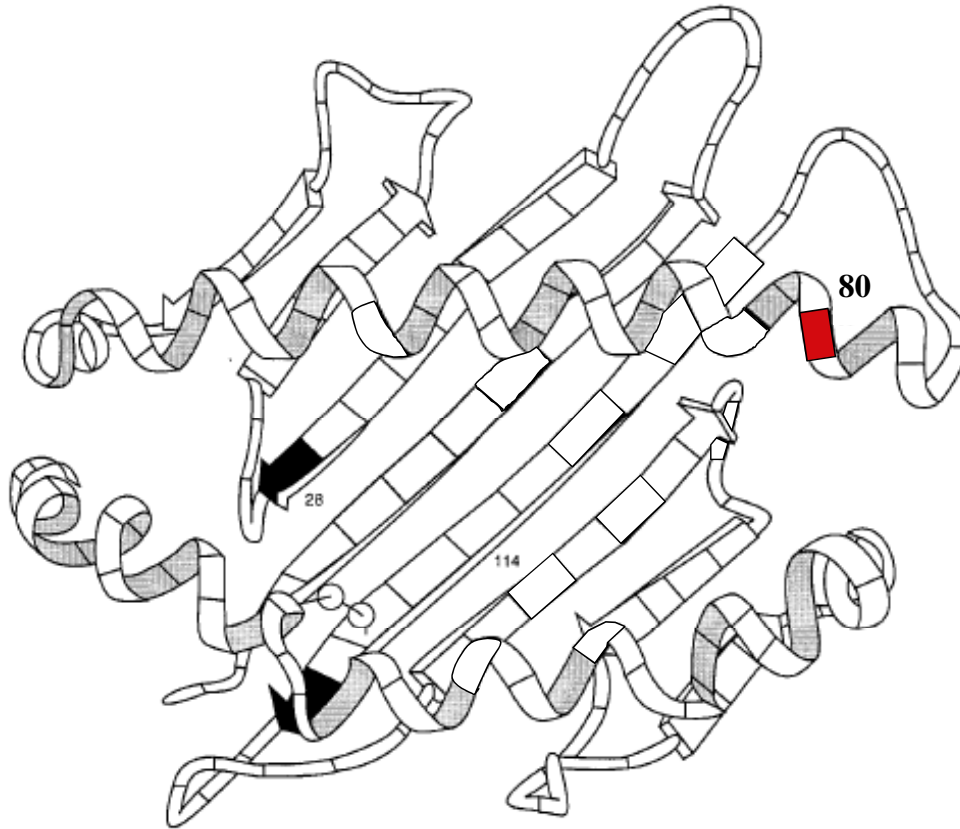
molecules possessed a second major function – in innate immunity – as mediators of NK cell inhibition (34).

T cells are equipped to embrace HLA structural diversity by possessing a huge number of dedicated receptors (“T cell receptors” or TCR). On the other hand, HLA diversity poses a potential headache for NK cells, which possess only a limited number of inhibitory receptors. How could relatively few inhibitory receptors reliably detect so many different HLA structures? The solution, like many in nature, is extremely elegant and results from the simplicity of an NK cell’s primary concern: “are HLA class I molecules present?” If not, then something is wrong with that cell and it should be destroyed (many viruses and cancers, for example, prevent HLA class I molecules from appearing at the cell surface). If HLA class I molecules are not present, inhibitory receptors will not be engaged and NK cells will, by default, activate. Now clear is that over evolutionary time, inhibitory NKR have rigorously frisked HLA class I molecules to identify discrete spots that do not change, or that change in only limited and predictable ways. Because Dr. Khakoo’s recent Science paper indicated that joint possession of an inhibitory NKR, called KIR2DL3, with its HLA-C ligand increases the chance of clearing HCV, I will devote all remaining attention to this system.

According to inheritance, NK cells deploy up to three types of inhibitory receptor whose dedicated task is to engage self (your own) HLA-C molecules. All three inhibitory receptors are members of the same molecular family, given the tongue-twisting name “killer-cell immunoglobulin-like receptors” – shortened to “KIR”. More specifically, the inhibitory KIR that engage HLA-C molecules are called “KIR2DL”, and, the three types are logically named, KIR2DL1, KIR2DL2 and KIR2DL3. These three inhibitory receptors effectively collapse all known HLA-C molecules into two types. Of the 340-odd amino acids in most cell membrane-bound HLA-C molecules all three KIR2DL receptors focus interest on amino acid number 80 in the HLA-C protein sequence (Figure 4). In all known HLA-C molecules, position 80 is occupied by either the amino acid asparagine or lysine. There is a single letter code used to denote amino acids and by a befitting twist of fate, the single letter code for asparagine is “N” and for lysine, “K”! Notably, this proclivity for only two possibilities at position 80 extends to all known chimpanzee equivalents of HLA-C molecules, despite our species’ divergence times of close to six million years. We therefore refer to HLA-C molecules possessing asparagine (N) at position 80 as “HLA-C1-type” and those with lysine as “HLA-C2-type”.

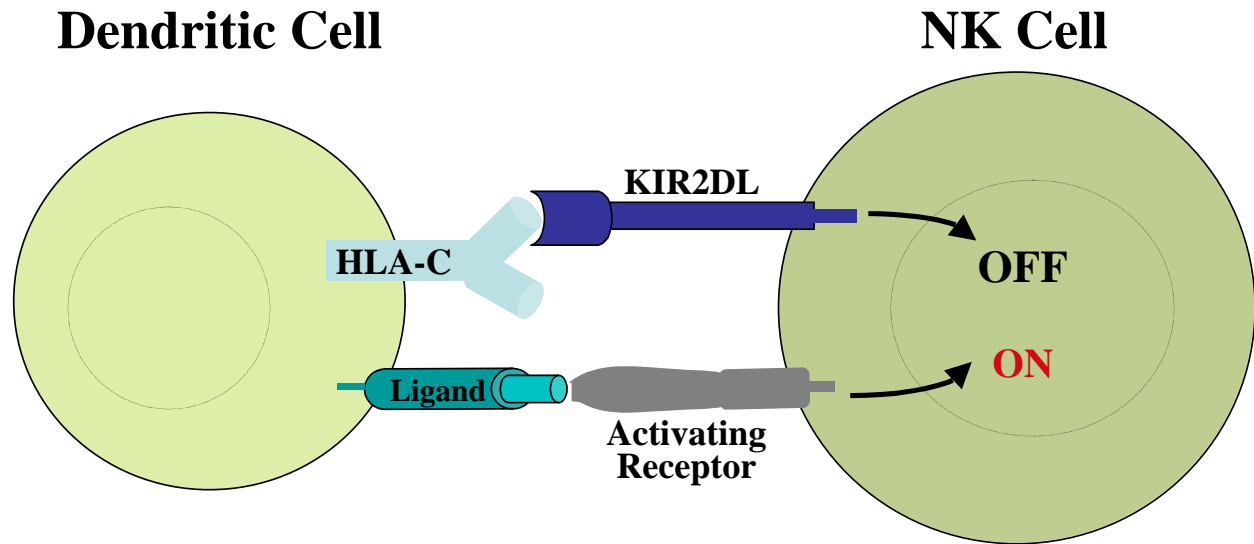
Figure 4.

A. Ribbon Diagram of the HLA-C Protein Structure Showing the NK cell ‘Control Zone’



The ribbon diagram is based upon the known crystal structure of HLA class I molecules (36) and depicts part of an HLA-C molecule viewed from above. The part shown is held outside of the cell by the remainder of the structure which forms a subframe that traverses the cell membrane (not shown). Two spirals of amino acids form the respective lips of a mouth whose base is formed by parallel strands of amino acids (39). Some amino acids are numbered to show their order in the protein sequence. Conspicuously, position 80 (shown in red) – either the amino acid Asparagine or Lysine in all HLA-C molecules – is located on the outer edge of the upper spiral, easily available for interaction with KIR2DL receptors on patrolling NK cells (see Panel B). Typically, self or viral peptides fill the groove (oriented horizontally across the page) and may influence, but not prevent, KIR2DL binding to position 80.

B. Control of NK cell Activation by KIR2DL



Triggering of an NK cell to kill and release cytokines is determined by the integration of activating and inhibitory signals delivered into the NK cell by various receptors. KIR2DL receptors (dark blue) deliver inhibitory (“OFF”) signals after engaging appropriate HLA-C molecules.

As shown in Table 1, KIR2DL1 is the receptor for HLA-C2 (K80) types and KIR2DL2 and KIR2DL3 each engage HLA-C1 (N80) molecules. Now for some apparently important subtlety. It seems that the strength of inhibitory signals delivered by each KIR2DL is not equal. The hierarchy of inhibitory potency is shown, also in Table 1.

TABLE 1. KIR2DL Receptors and HLA-C Ligands

INHIBITORY RECEPTOR	HLA-C Ligand	Position 80	NK Inhibitory Potency
KIR2DL1	C2	Lysine (K)	Strong
KIR2DL2	C1	Asparagine (N)	Intermediate
KIR2DL3	C1	Asparagine (N)	Weak

KIR2DL1 most potently inhibits NK cells carrying this receptor and KIR2DL3 inhibits its carriers least. Clearly in this scheme, if people have two versions of an HLA-C2 type (one inherited from each parent – a state called “homozygous”) and their NK cells carry KIR2DL1, their NK cells will be most resistant to activation. On the other hand, in people who only inherit HLA-C1 types (“HLA-C1 homozygotes”) KIR2DL1 is functionally redundant – it is there but does nothing. People carrying only HLA-C1 types therefore rely on either KIR2DL2 or KIR2DL3 for HLA-C mediated NK cell inhibition. And because KIR2DL3 delivers weakest inhibition, NK cells carrying only KIR2DL3 should activate most readily. In fact, it turns out that nature has made this latter situation possible and it seems that such individuals, who are homozygous both for KIR2DL3 and HLA-C1, are privileged in the battle against HCV infection: among the 1037 people studied by Khakoo et al., they were significantly more likely to clear acute HCV infection (35). In the NK world, these (KIR2DL3-HLA-C1 homozygotes) might be the Type A personalities; genetically ‘wired’ to be most highly resistant to viral infections.

Does NK cell receptor biology hold implications for people with hepatitis C?

We have suspected that vigorous early immune interaction is necessary for HCV clearance (1) but these recent findings suggest that vigor during even the very earliest interaction may influence the fate of HCV infection. Furthermore, that inhibitory signaling by KIR2DL-HLA-C has been so emphatically implicated offers some intriguing possibilities for new treatments. For example, if individuals with KIR2DL3-HLA-C1 are so gifted, could NK cells in people with the other KIR2DL-HLA-C combinations be manipulated to become more KIR2DL3-HLA-C1-like? – that is, rendered more excitable? The answer is, possibly. One strategy might be to administer agents (like monoclonal antibodies) that bind and block KIR2DL1 and KIR2DL2. The intent would be to diminish the inhibitory signals delivered by each receptor and convert these equivalents of couch-potato NK cells into gym flies. (The concept of inhibiting an inhibitor proves difficult for many students; I hope readers will now understand that for an NK cell this means NK activation will occur more readily.)

Dr. Khakoo's study implied that sole possession of KIR2DL3 and HLA-C1 molecules wrought enhanced clearance of HCV during acute infection. However, receptor manipulations of the type discussed in the last paragraph could conceivably improve chances of HCV clearance in people undergoing treatment for chronic hepatitis C. Increased local release of the so called "Th1 cytokines" (IFN- γ , TNF- α , IL-12) by liver NK cells seems likely to augment the type of T cell response that we now believe is important for treatment-induced clearance of chronic hepatitis C.

Another potentially positive twist arises from the fact that the HLA-C-specific KIR2DL inhibitory receptor system has also been found on some T cells, including some that have been shown to engage HCV. We suspect that, as on NK cells, KIR2DL will dampen activation of T cells and make them less vigorous (40). Thus the same therapeutic manipulations suggested for NK cells might make at least some HCV-targeting T cells also work more efficiently.

NK Caveat Emptor

Before letting enthusiasm overwhelm caution I should point out that the above Anglo-American study indicated advantage in carrying KIR2DL3 and HLA-C1 only in people presumed to have contracted HCV in relatively low dose – for example by needle-sharing among injection drug users. This is now the typical acquisition route for new HCV infections in the West so the caveat certainly does not weaken the importance of the Anglo-American team's observation. However, it suggests that other mechanisms for defeating host immunity are afoot when HCV is acquired in high dose, for example following transfusion of HCV infected blood products. Among the possibilities, NK cells may still be involved.

Many things other than HLA class I receptors litter the surface of NK cells. Among these is a common molecule, called CD81, which has been shown by Dr. Sergio Abrignani's research team at Chiron Corporation to bind the HCV envelope (outer coat) proteins (41). Recently, another group of Chiron researchers led by Dr. Nick Valiante made the interesting discovery that when HCV binds and sticks together adjacent CD81 molecules in the NK cell surface – the official term is "cross-linking" – all aspects of NK cell activation (killing and cytokine production) are prevented (42). We might therefore anticipate that this HCV-triggered off-switch for NK cells would be most firmly thrown in the presence of a large number of HCV particles. If this mechanism indeed plays a significant role, might advantage be swayed back in the host's favor if

we blocked both the HCV envelope proteins and the NK cell KIR2DL inhibitory receptors as part of a therapeutic regimen? Such questions can keep immunologists awake at night.

The Intracellular Response to HCV Infection

As noted above, the reaction of a single cell to limit damage from viral infection probably represents one of the earliest types of (innate) immune response. Following pioneering studies by Dr. Markus Heim and colleagues in Basel, Switzerland (43), recent work has suggested that HCV interferes with a principal signaling pathway used by IFN- α and IFN- β (the so-called type 1 interferons partly discussed above in the context of Toll-like receptor activation) to transmit messages into the nucleus of an infected cell. Type 1 interferons are among the most potent cytokines and are produced in most cell types, including hepatocytes.

Scientists who work on signal transmission inside cells (usually called “transduction”) have been as inventive with their shorthand as particle physicists; hence the specific IFN signaling pathway inhibited by HCV is called JAK-STAT. In longhand this respectively converts to Janus family of tyrosine kinases, and signal transducers and activators of transcription (STATs). JAKs add phosphate molecules (in chemical notation PO₄) to STATs (there are two: STAT1 and STAT2) and thereby activate them. Adding phosphate groups is a biological trick for energizing molecules – somewhat akin to the double espressos imbibed by the scientists who study such systems. Enzymes that energize molecules by adding phosphate groups are generically called Kinases – a name with two roots: a Greek word for moving (*kinetikos*) and, by convention, all enzymes end in *ase*.

In general, an understanding of overarching principles is more indelible than attempts to memorize snippets of detail. Thus, cytokine receptors fall into two basic classes: those possessing their own kinase domain (like a couch potato with a firmly grasped TV remote – well equipped to make things happen at a distance), and those lacking a kinase domain (the remote always out of reach). For both types, however, kinase activity is essential for transmitting messages downstream. Cytokine receptors that lack their own kinase domain seem to often recruit JAK kinases for this purpose. This is the type of receptor bound by the cytokines IFN- α and IFN- β . Recruited JAKs link membrane receptors to nuclear genes.

Now some details that seem relevant to the immune subterfuge wrought by HCV. In the cytoplasm, JAKs activate both STAT molecules (STAT1 and STAT2), which causes each STAT to combine with a third molecule called IRF-9. This huddle of three, scurries across the nuclear membrane to bind and activate more than 30 target genes, collectively known as Interferon α/β stimulated genes – “ISGs”. With this surfeit of acronyms the reader can be forgiven for believing that most immunologists have a military background. Some ISGs have antiviral properties – among them OAS (oligoadenylate synthase) and PKR (protein kinase R), enzymes which inhibit the generation of viral proteins. Many viruses have evolved strategies to down-regulate this system and HCV seems one of them.

Recently, de Lucas and colleagues showed that the HCV Core protein disrupts IFN- α -induced induction of ISGs (44). In these studies HCV Core seems to decrease binding of ISGF3 to an important piece of DNA called the ISRE (Interferon-stimulated response element), which controls expression of ISGs. In other studies, using different experimental conditions, the HCV

Core protein appeared to prevent importation into the cell nucleus of STAT1 and the expression of another antiviral protein, MxA (myxovirus resistance A) (45). In summary, interference by HCV with IFN- α -induced signaling through the JAK-STAT pathway seems likely; it could contribute to the resistance to IFN- α therapy observed in many treated patients and may represent a basic strategy contributing to HCV persistence. More research is needed to determine the precise details and the extent to which such mechanisms of immune evasion operate during acute HCV infection. If a compelling case can be made we can expect development of therapeutic agents designed to inhibit the viral protein inhibitors of JAK-STAT signaling.

In this section I initially stressed that while innate immunity forms the immediate line of defense against viral infections, it seamlessly integrates with and triggers the adaptive immune response. I will now devote the remainder of this chapter to the adaptive immune system, particularly those components that have been shown to engage HCV with varying degrees of success.

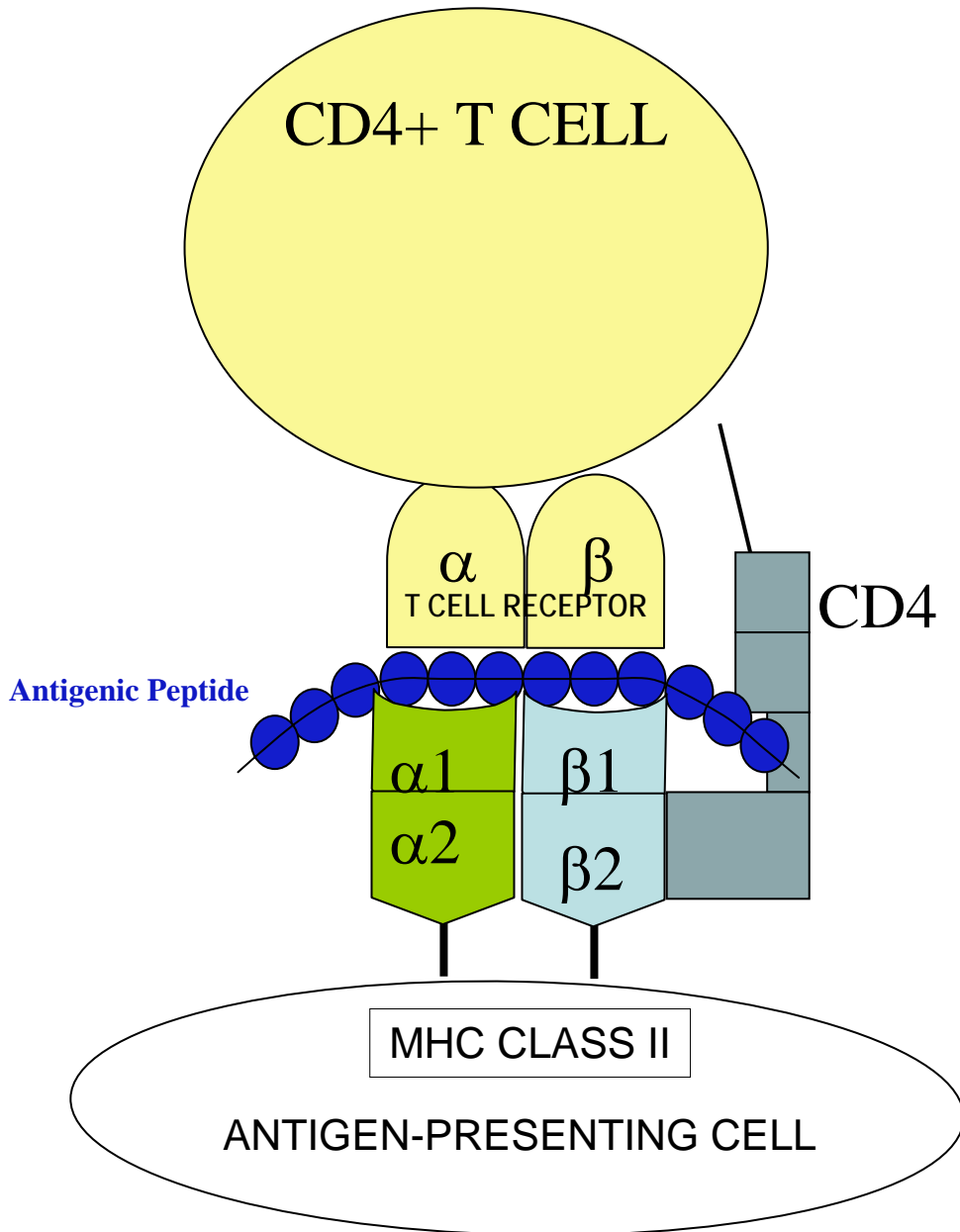
ADAPTIVE IMMUNITY

CD4⁺ T cells

When innate immune responses fail to dispatch viruses, CD4⁺ T cells emerge as the central controllers of viral immunity. CD4⁺ T cells are also called helper T cells because their activation “helps” other immune cells to combat viruses. Among the ‘other cells’ are B cells, which make antibodies and CD8⁺ T cells, which directly attack virus-infected cells. CD4⁺ T cells that promote antibody production by B cells are called T helper type 2, or “Th2” cells and those that stimulate CD8⁺ T cells are called “Th1”. Although Th1 and Th2 responses can coexist, a very strong Th1 response suppresses Th2 responses and vice versa. Thus a strong T cell response is often described as having a certain “polarity”: Th1 or Th2. A pivotal role for strong Th1-type CD4⁺ T cell activation in HCV clearance is now virtually certain.

It is important to understand that T cells only recognize tiny fragments of viral proteins (not whole viruses), and only when those fragments, called “peptides”, are presented at the infected cell surface by major histocompatibility complex (MHC) molecules. The reason for this is clever and subtle but beyond the scope of this article. CD4⁺ T cells are activated when specific receptors (called T-cell receptors, or “TCR”) engage molecular complexes of viral peptides embedded within MHC class II molecules (Figure 5). The CD4 molecule is a co-receptor that binds and provides specificity for the MHC class II molecule (Figure 5).

Figure 5. Recognition of MHC Class II-Viral Peptide Complexes by CD4⁺ T Cells



Typically, CD4⁺ T cell responses are characterized by measuring the amount of proliferation (the extent to which the T cells divide) and/or cytokine secretion when they are incubated with viral versus negative control proteins. Using these techniques, involvement of CD4⁺ T cells in successful HCV immunity was first noted by Diepolder and colleagues after comparing T cell responses in the peripheral blood of people with self-limited versus chronic infection(46). These studies suggested that prominent CD4⁺ T cell activation distinguished resolved infection. A series of subsequent experiments have fairly consistently shown that vigorous CD4⁺ T cell responses, simultaneously targeting multiple HCV proteins, correlate with HCV clearance(47-50).

A characteristic of such CD4⁺ T cells is secretion of the Th1 cytokines, Interferon gamma (IFN- γ) TNF-alpha (TNF- α) and Interleukin 2 (IL-2), which stimulate CD8⁺ cytotoxic T cells (CTL). In contrast, people whose HCV progresses to chronic infection appear to display less vigorous proliferation of CD4⁺ T cells, which secrete the Th2-type cytokines, IL-4, IL-5 and IL-10.

Although an HCV-specific CD4⁺ T cell response has been shown capable of persisting for 20 years after recovery (51), its robustness, duration and potential for protecting the host from further infection are not yet clear.

CD4+CD25+ T cells

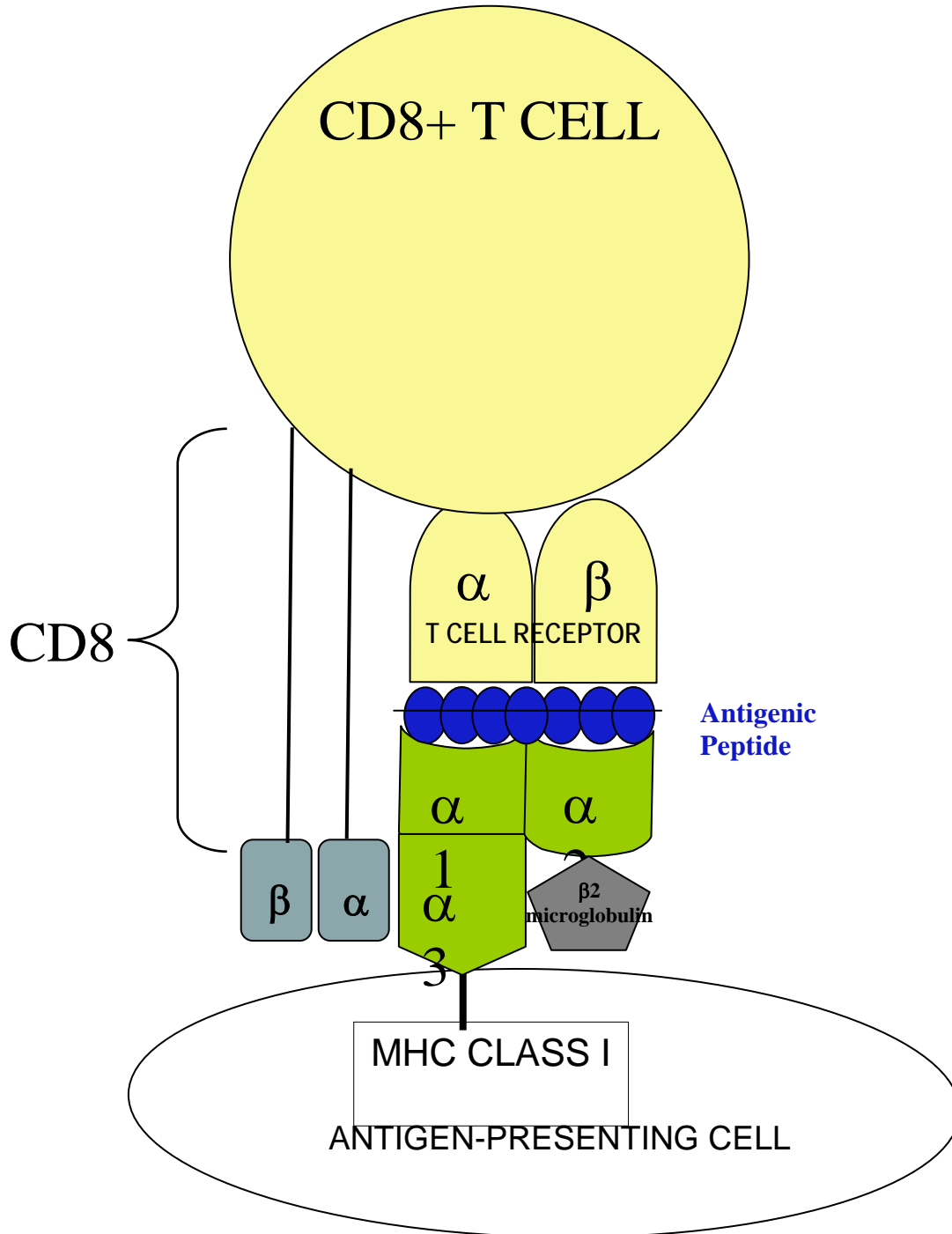
Seminal studies in the 1990s by Sakaguchi (52, 53) and Shevach (54) showed that the tendency for experimentally manipulated newborn mice to develop autoimmune disease pivots around a subset of T cells which co-express the CD4 molecule (in common with T helper cells) together with the low affinity interleukin 2 receptor (IL-2R) α chain, called CD25. The presence of CD4+CD25+ regulatory T cells (abbreviated to “Treg”) prevents, whereas depletion portends, autoimmunity. Although their precise mode of action remains uncertain, it has been shown that Treg suppress T-cell activation. Thus, Treg appear to dampen T cell responses and protect against the development of autoimmune diseases. This natural immunological braking system appears not only to protect against autoimmunity but also to benefit host immune responses to certain pathogens like Schistosomes (55) and Leishmania (56), where the immune response can cause tissue destruction. Thus, Treg are a prominent T cell population initially shown capable of mediating peripheral tolerance to self-antigens, but whose functions have now been extended to include regulation of T cell responses to viral antigens. There is, however, a potential downside of this natural dampening system. In some infectious diseases, like hepatitis C – where the immunological balance between resolution and chronicity is finely poised (1) – intervention by Treg could prematurely dampen the antiviral response and tip the balance in favor of pathogen persistence. Key in this regard may be the timing of Treg activation.

Clearly more needs to be known about involvement of Treg in HCV-specific immune responses. The potential for manipulating Treg and improving the outcome of hepatitis C makes this a fertile area of research. Intriguingly, one of the Treg on/off switches appears to be tilted by compounds naturally found in the brain. Dopamine (D) has been shown to block the Treg-mediated suppression of T-effector cells, a finding with exciting potential for therapy.

CD8+T CELLS (Cytotoxic T cells or CTL)

Like CD4⁺ T cells, these cells use a specific receptor (TCR) to detect viral peptides bound by MHC molecules expressed at the surface of infected cells. The principal difference is that CTL possess the CD8 co-receptor (instead of CD4), which binds MHC class I (instead of class II) molecules on the surface of infected cells. CTL are therefore activated by cell-surface complexes of viral peptides with MHC class I molecules (Figure 6). Activation of CD8⁺ T cells by peptide/MHC complexes results in local release of chemicals that can kill and/or disinfect virus-infected cells and lead to viral eradication (57). Because CTL can directly bring about viral clearance they are often called “effector cells”.

Figure 6. Recognition of MHC Class I-Viral Peptide Complexes by CD8+ T Cells



Evidence that CD8⁺ T cells play a direct role in terminating hepatitis C was initially provided by prospective studies of acute infection in chimpanzees (58). Chimpanzees are the only non-human species in which HCV replicates efficiently. Exceptionally for an animal model of human disease, chimpanzees and humans share MHC class I and II molecules, T cell receptors and other immune response genes that are genetically indistinguishable (59-62). The chimpanzee infection

study revealed that a strong CTL response in the liver during acute infection correlated with HCV clearance.

In six chimpanzees with acute hepatitis C, HCV was eradicated in the two who mounted the broadest CTL response. In contrast, both of these chimpanzee resolvers mounted extremely weak antibody responses, which quickly disappeared in one. The number and breadth of CTL that were synchronously operational in early infection seemed critical for HCV resolution. Rigorous dissection of the CTL repertoire in one chimpanzee resolver revealed at least nine distinct targets; one target defined as a specific viral peptide presented by an individual MHC class I molecule. In that individual, every class I molecule (like humans, chimpanzees can possess six) engaged CTL with peptides derived from most of the 10 HCV proteins. The plasticity of this response was striking and suggested that plasticity might indeed be key in allowing the immune response to successfully confront the spectrum of viral variants.

In chronic infection CTL seem to emerge sequentially but not in concert: a frugal property that seems unable to eradicate HCV, perhaps due – at least in part – to evolving quasispecies diversity. In chronic HCV infection, CTL select for viruses that have lost their target epitopes(63). Because viruses possessing such mutated epitopes avoid immune responses, they emerge to detectable and perhaps dominant frequency; a phenomenon that can occur in weeks. CTL, however, endure as memory cells despite epitope loss(58, 63). Therefore, the number of CTL identifiable at a given point in chronic infection might overestimate the number that are functional. Additionally, it is worth noting that when examined in bulk (not as individual clones) CTL responses tend to wane following HCV resolution but gradually expand in persistent infection (6, 33). Therefore, examination of CTL beyond the acute phase could deceive the observer – revealing either no quantitative difference between chronics and resolvers or even an apparently broader CTL response in chronically infected individuals.

Subsequent studies in human resolvers similarly supported a role for multispecific CTL responses in HCV resolution (6, 45, 46, 56, 57). For example, Grüner et al compared virus-specific CD8⁺ T cell responses in patients with self-limited versus chronic infection and found significant correlation between HCV clearance and the appearance of virus-specific CD8⁺ T cells in the first 6 months of infection (47). Taken together, these chimpanzee and human studies suggest an inverse relationship between the extent of the CTL response in early infection (which may have qualitative and quantitative components) and the likelihood of viral persistence. Factors underpinning differences in the strength and polarity of T cell responses during early HCV infection are under intense investigation. It appears that Th1-type cellular immune responses are essential for resolution of acute hepatitis C. Why so few hosts generate sufficiently potent Th1 responses is unknown and understanding this phenomenon is likely to be crucial for developing an effective vaccine.

T cell memory following HCV infection

Fundamentally, the effectiveness of a T cell vaccine will depend upon efficient induction and maintenance of an adequate repertoire of HCV-specific memory cells. Given that the principal site of HCV replication, and probably antigen presentation, is the hepatocyte, this may require (or at least benefit from) preferential memory cell induction and/or maintenance in the liver.

This could be problematic because the liver is a site where T cells can be tolerized (64) or removed (65), properties that perhaps contribute to viral persistence.

Several studies have examined multiply HCV-exposed but seemingly uninfected individuals and found apparent HCV-specific CD4⁺ and CD8⁺ T-cell responses following stimulation with HCV protein antigens (60, 61). Although this suggested the possibility that HCV-specific T cells could endure following subclinical exposure, concern existed that such T cells may have been low affinity, cross-reactive cells rather than true HCV-specific memory cells that could respond to naturally processed antigens. CTL characterized from liver biopsies of chimpanzees studied during and after acute self-limited infection confirmed that HCV-specific memory cells could endure for at least 18 months (33). The latter study hinted that memory CD8⁺ T cells might reside preferentially in liver tissue. More recently, HCV-specific memory CD8⁺ T cells have also been shown to endure in humans who clear the virus (6, 75). Persistence of HCV-specific memory CD8⁺ T cells following self-limited infection suggests that virus-specific CD4⁺ T cells similarly persist.

ANTIBODY-MEDIATED IMMUNITY

In hepatitis B virus (HBV) infection the capacity for antibodies to neutralize the virus is clear but a role for antibodies in protection against HCV infection has been difficult to prove. The first detectable antibody responses against HCV antigens usually target the NS3 and Core proteins (Figure 1) and see . Later, antibodies against NS4 and envelope proteins (E1 and E2) develop. With continued presence of virus, antibody responses typically broaden further, such that chronically infected hosts display antibodies against multiple viral epitopes (30). In contrast, viral suppression or clearance is accompanied by reduction in (or even failure to generate) specific antibodies both in humans and chimpanzees (31-33).

While antibodies appear to select HCV Envelope protein variants (19, 22, 23), clear evidence that antibodies protect against natural infection remains elusive. For example, a recent report of successful antibody responses during early infection described similar responses in a substantial proportion of patients who failed to clear virus (35). And in HCV-challenged chimpanzees Bassett et al showed that viral clearance was not associated with anti-E1 or anti-E2 antibodies (32); indeed, antibody to E2 was observed only in viremic chimpanzees. Similar findings were reported in a group of Irish women who received HCV-contaminated anti-Rhesus D immunoglobulin (36). In the latter study, anti-E2 antibody was found in all viremic women but in only about 60% of women who had cleared HCV RNA.

Another approach has been to study the influence of high titer HCV antibodies induced before or co-inoculated at the time of viral challenge. Using HCV immune globulin, Krawczynski et al showed hepatitis is delayed but not prevented (37). On the other hand, Farci et al. reported the ability to prevent hepatitis C in chimpanzees if the inoculum was first incubated with hyperimmune serum (38). And at Chiron Corporation, Choo et al reported prevention of chronic infection in five out of seven chimpanzees when high titer anti-envelope antibodies were raised against homologous virus (i.e., the strain against which the antibodies were raised) (39). These experiments suggested that high titer antibodies directed against certain structural antigens,

especially envelope hypervariable regions (HVR), might at least modulate or even potentially protect against HCV infection. This was further supported by a retrospective study of 23 German women who had been infected, like their Irish counterparts, by HCV-contaminated anti-D globulin (40). In the German study, Zibert et al found that antibodies against a 16 amino acid stretch of the second Envelope protein were more frequent among twelve resolvers than among eleven women with chronic infection. The potential pitfalls of retrospective analysis notwithstanding, these data suggest that such antibodies may have played a role in resolution. Hence, finding the ‘right’ recombinant HCV protein, and the ‘best’ adjuvant for elicitation of protective antibodies, remains the Holy Grail for some vaccinologists.

There are important caveats, however: conclusive evidence that antibodies reliably afford protection still is lacking, particularly against different HCV strains, and putatively protective antibody titers may yet prove difficult to sustain. Of particular interest are studies of hepatitis C outcomes in antibody-deficient patients. Although numbers are limited, it is notable that spontaneous clearance has been reported in children who have congenital antibody deficiency (41-43), providing strong evidence that control of HCV can occur independently of antibodies. Indeed, it is notable that when data from these small studies are combined, disease termination occurred in approximately 15% of antibody-deficient children, a proportion tantalizingly similar to that anticipated in the general population. So while antibodies appear to exert selective pressure upon HCV, the ability to terminate infection remains uncertain, particularly against different strains and genotypes.

Cryoglobulins: Antibodies that can cause harm

In up to 50% of people, HCV infection is associated with the presence of antibodies, which bind to each other and precipitate out of solution when cooled below body temperature. These are called cryoglobulins. When these antibodies deposit in small blood vessels, inflammation and blockage can occur (officially termed vasculitis) and give rise to related damage and clinical syndromes. Vasculitic damage is most commonly apparent in the skin and kidney. Many people, perhaps two thirds, who have these antibodies, do not develop overt disease, however. The antibody precipitates usually contain anti-HCV antibody, HCV RNA and IgM rheumatoid factor (an anti-IgG autoantibody). In many cases successful treatment of HCV with Pegylated interferon and ribavirin clears the cryoglobulins.

Lex Parsimoniae

Seventeen years after HCV was so elegantly laid bare, details of its successful engagement by the human immune system are finally unraveling. A seamless but critical interplay between innate and adaptive cellular immune responses is emerging—connected systems that are likely to prove manipulable to host advantage. The next decade offers realistic hope for people infected or at risk, and possibly for the sleep pattern of HCV scientists.

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