



Review

Retroviruses in Autoimmune Liver Disease: Genetic or Environmental Agents?

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Abstract. Retroviruses have been implicated in the pathogenesis of several human autoimmune conditions including Sjögren's syndrome, primary biliary cirrhosis, immune mediated diabetes, and multiple sclerosis. The human intracisternal A type particle derived from Sjögren's syndrome patients' salivary glands was the first retrovirus to be isolated from a human autoimmune disorder but the agent has yet to be cloned. In primary biliary cirrhosis patients, virus like particles have been observed by electron microscopy in biliary epithelium, endogenous retroviral sequences have been cloned from liver samples, and antibody reactivity to the human intracisternal A type particle has been observed in the majority of patients tested. However, there is no evidence to link the endogenous retroviral sequences in primary biliary cirrhosis patients to the retroviral antibody reactivity or virus like particles. In other patients with liver disease, reactivity to the human intracisternal A type particle was observed in a small but significant proportion of patients with hepatitis C virus infection. If the intracisternal A type particle is an endogenous retrovirus, it is interesting to speculate that hepatitis C virus infection may modulate the endogenous retroviral expression, as chronic hepatitis C has been linked with the development of Sjögren's syndrome. Furthermore, many patients with chronic hepatitis C virus infection have reactivity to an autoantigen of unknown significance known as GOR that has protein sequence homology with both hepatitis C virus nucleocapsid protein as well as HTLV-1 gag. This may be another example of an endogenous retroviral protein acting as an autoantigen in liver disease patients. At this time, there is little evidence to suggest that endogenous retroviruses are infectious agents that cause autoimmune disease but they may be implicated as either genetic elements or antigens. Further studies will be required to characterize the role that both exogenous and endogenous retroviruses play in the pathogenesis of autoimmune liver diseases.

Key words: autoimmune liver disease; hepatitis C virus; human endogenous retroviruses; human intracisternal A-type particle, primary biliary cirrhosis; Sjögren's syndrome; systemic lupus erythematosus.

Several recent reports have suggested a possible role for retroviruses in the pathogenesis of a variety of human autoimmune disorders including multiple sclerosis, immune mediated diabetes, Graves' disease and primary biliary cirrhosis^{7, 18, 27, 36}. An investigation into the retroviral induction of multisystem autoimmune disorders was originally prompted by the finding of indeterminate Western blot reactivity to human immuno-

deficiency virus (HIV), and human T cell leukemia virus proteins in patients with systemic lupus erythematosus (SLE) and Sjögren's syndrome^{13, 40, 41}. These observations ultimately led to the isolation of a retrovirus from the salivary glands of Sjögren's syndrome patients, known as the human intracisternal A-type particle (HIAP), named because of its morphologic resemblance to the murine endogenous A-type retroviruses¹².

Western blot studies have shown that the majority of patients with Sjögren's syndrome and SLE had marked reactivity to the characterized HIAP proteins compared to only a minority of disease controls and healthy individuals^{12, 15, 27}. Similar HIV and HIAP immunoblot studies have now been used as a surrogate marker to demonstrate that patients with primary biliary cirrhosis (PBC) and other idiopathic biliary disorders have retroviral antibodies²⁷. The significance of these findings has been enhanced by preliminary studies documenting virus-like particles in the biliary epithelium and the possibility of a transmissible factor in lymph nodes of PBC patients^{39, 49}. In this review, the evidence for retroviral induction of autoimmune diseases in general, and PBC in particular, will be discussed and we will speculate on the possible involvement of endogenous retroviruses as autoantigens in the etiology of autoimmune liver disorders.

Retroviruses and Autoimmunity

Due to their complex replication strategy, retroviruses display a marked variety of pathogenic interactions with their host as exogenous infectious agents and endogenous "genes" integrated into the host germline. Their role in the pathogenesis of cancer, immunodeficiency states, and chronic inflammatory disorders has been well documented and there is accumulating evidence to implicate retroviruses in the etiology of autoimmune diseases as well. With regard to the latter, human endogenous retroviruses (HERV) appear to be of particular interest because of their very nature. As previous pathogens, HERV have infected germ line cells and are subsequently transmitted as a stable Mendelian genetic trait. When HERV proteins are expressed, the immune system views them as "immunologic self". HERV have co-evolved with their host and, in some instances, the relationship has been beneficial. For example, it is probable that these elements have provided the intricate mechanisms necessary for genetic rearrangement of genes essential for the formation of immunoglobulins⁴⁶. Furthermore, the long terminal repeats (LTR) of some HERV have provided functional promoters for host genes⁴⁷. However, presumably because of their potentially damaging effects, the majority of HERV cannot make functional proteins because of deletions or mutations within their open reading frames or are transcriptionally silenced by being heavily methylated⁴⁷. In some cases, however, HERV are not just molecular fossils. For example, some of the multi-copy family HERV-K can synthesize viral proteins as well as virus like particles. However,

there is little evidence to suggest that HERV may actually become infectious agents^{4, 47}.

Although an attractive proposition, the role of HERV in human autoimmune disease remains speculative at this time, even though they appear to have the right credentials to break immune tolerance. The HIAP has yet to be cloned and therefore it is not known whether this is an endogenous or exogenous retrovirus. However, the similar appearance of HIAP and the HTDV/HERV-K Western blots and the detection of an uncleaved gag product on immunoblot suggest that HIAP may be an endogenous agent, as the name suggests^{3, 12}. The multiple sclerosis retrovirus, which is probably a HERV, has been recently cloned from leptomeningeal cells from patients with multiple sclerosis³⁶. This agent has been specifically detected in the serum of patients with multiple sclerosis who have not been treated with corticosteroid therapy and in a small minority of controls³⁶. At this time, it is unknown what role this element plays in the pathogenesis of multiple sclerosis. Another HERV, referred to as IDDMK_{1,2}22, a member of the HERV-K10 family, has been linked to immune mediated diabetes. A claim was made that the onset of diabetes coincided with a demonstrable IDDMK_{1,2}22 viremia that was not observed in healthy controls⁷. However, we and others have found that similar proportions of diabetic patients and controls have evidence for IDDMK_{1,2}22 viremia as well as immunoblot reactivity to the *gag* and *env* gene products²³. Therefore, it is highly unlikely that IDDMK_{1,2}22 viremia is specific for immune-mediated diabetes. However, this agent reportedly has superantigen properties that may play a pivotal role in the pathogenesis of immune-mediated diabetes (*vide infra*).

To date, the strongest evidence suggesting a central role for both exogenous and endogenous retroviruses (ERV) in autoimmune disease comes from animal models. Several retroviral infections in horses, sheep, and rodents manifest themselves with abnormalities of immunity and disease processes not dissimilar to human disorders such as rheumatoid arthritis and multiple sclerosis, which have been well documented in a previous review¹³. There are also numerous examples describing the role of ERV in the induction of autoimmune disease by a variety of virologic, immune, genetic and regulatory mechanisms. Several reports have shown that expression of ERV particles is related to the evolution of the disease process. For example, in the NOD mouse the presence of C-type ERV particles in β islet cells directly correlates with the development of diabetes, and in the NZB lupus prone mice, the development of immune complex mediated nephritis is associated with

the production of large amounts of antibody to an endogenous mink cell focus-forming *env* protein^{13, 17}. Retrotransposition of ERV can result in an autoimmune phenotype by insertional mutagenesis and disruption of gene expression. In the MLR/lpr lupus prone mouse, the expression of *fas* is markedly diminished by a 5.3 kb insertion of the ETn transposon into the second intron of the *fas* gene, resulting in failure of apoptosis and proliferation of T lymphocytes^{5, 48}. The reader is directed towards recent reviews that have documented these examples in more detail^{13, 32}.

Retroviral superantigen expression has been proposed as a mechanism for the induction of autoimmunity. Of interest is that this mechanism may be integral to the biology of the retrovirus, but it can also confer protection to the host if the endogenous retroviral superantigen gene is expressed. During exogenous infection, the murine mammary tumor virus (MMTV) superantigen stimulates specific T lymphocyte V β subsets and, in doing so, provides a vehicle for viral replication¹. In contrast, the endogenous expression of MMTV superantigen serves to delete specific T lymphocyte V β subsets during development and thus protects the host from infection with the exogenous strain requiring that particular subset of T lymphocytes¹. A role for a HERV superantigen has recently been proposed in the pathogenesis of immune mediated diabetes by CONRAD and colleagues^{6, 7}. This model was based on finding a selective enrichment of V β 7 T lymphocytes in the pancreas of diabetic patients, the cloning of a superantigen encoded by the envelope gene of IDDM_{1,2}22 and the demonstration that the envelope protein mediated a V β 7 superantigen effect^{6, 7}. At this time, it is unknown how the superantigen is specifically expressed in the islet cells. However, it seems unlikely that IDDM_{1,2}22 particles are specifically associated with immune mediated diabetes²³.

Retroviruses and Primary Biliary Cirrhosis

PBC is an idiopathic liver disorder characterized by the progressive granulomatous destruction of bile ducts. It is considered an autoimmune disease because 95% of patients have anti-mitochondrial antibodies (AMA) reactive to a protein on the inner mitochondrial membrane, characterized as the E2 component of pyruvate dehydrogenase (PDC)⁴⁴. The biology of this mitochondrial enzyme appears to be disturbed in PBC patients who have immunohistochemical evidence of antigens resembling PDC-E2 on the apical surface of the diseased biliary epithelium^{19, 44}. Indeed, both the detection of AMA and the demonstration of biliary antigens im-

munologically similar to PDC-E2 are highly specific for PBC and also provide a mechanism by which an immune response to a ubiquitous mitochondrial antigen may be restricted to biliary epithelium. This specific marker for disease also provides definitive evidence that PBC reoccurs following transplantation, as documented by detection of serum AMA, loss of bile ducts and immunohistochemical evidence of the antigen resembling PDC-E2 on biliary epithelium in the allograft^{34, 45}. The notion that PBC has an infectious etiology is also supported by the clustering of PBC in families and care-providers as well as the increased prevalence of disease in specific geographic location such as Sheffield, UK, within the distribution of a specific water supply^{33, 42}.

In order to isolate a putative infectious agent associated with PBC, representational difference analysis was performed using the liver and skin from a PBC patient. Previously, this methodology has been used to discover the Kaposi sarcoma virus, GBV-A and -B, as well as the transfusion transmitted virus, TTV, but it also has the utility of detecting a proportion of deletions, point mutations and rearrangements within the genome²⁶. The difference products from the PBC liver were cloned and sequenced, revealing various known endogenous retroviral sequences as well as novel retroviral sequences⁴⁹. Other investigations to identify a putative agent associated with PBC have been performed in a "blinded" fashion, using cultured biliary epithelial cells from PBC patients and controls undergoing liver transplantation. In these electron microscopy studies, several enveloped virus-like particles were observed in 3 of 4 PBC patients, whereas only a solitary particle was seen in the biliary epithelium in 1 of 4 control patients⁴⁹. The particles had a morphology and size consistent with retroviruses, but these preliminary results did not provide definitive data. In separate co-culture studies, lymph node extracts from PBC patients or liver disease controls were incubated with normal biliary epithelium and the cells were processed by immunohistochemistry to assess whether there was evidence of AMA reactivity on the cell surface, typical for PBC patients. Reactivity to PDC-E2 was up-regulated in biliary epithelial cells stimulated by PBC lymph node extracts, but not controls, indicating that PBC patients may harbor a transmissible factor with the ability to specifically alter the biliary epithelium to a PBC phenotype³⁹. Further electron microscopy and cloning studies will be required to determine the nature of the virus-like particles and transmissible agent.

As previous serologic studies of SLE and Sjögren's syndrome had shown that patients harbor cross reactive

antibodies to HIV gag and HIAP proteins, we conducted HIV and HIAP Western blots in individuals with various forms of liver disease. We found that none of the patients' serum had reactivity with the envelope proteins to make a Western blot diagnosis of HIV infection. Of note, approximately 35% of patients with PBC and primary sclerosing cholangitis had serologic reactivity to HIV gag protein, in contrast to only 4% of patients with toxic or metabolic liver disease and healthy volunteers²⁷. Another clinically important observation from this study concerns the significant differences in HIAP Western blot reactivity in PBC patients compared to all the other liver disease control cohorts. Over half the PBC patients studied had immunoblot reactivity to 2 or more HIAP proteins, which was found to be significantly greater than all the other liver disease comparison groups and healthy volunteers.

The biologic relevance of these findings is enhanced by the close relationship between HIAP infection and Sjögren's syndrome, as the latter multisystem autoimmune disorder shares several clinical, immunologic and pathologic features with PBC. For example, PBC patients often present with clinical manifestations of Sjögren's syndrome, and even those without evidence of sicca syndrome often have laboratory tests indicative of subclinical xerostomia and xerophthalmia^{2, 15}. In addition, patients with Sjögren's syndrome can develop antibody reactivity to PDC-E2, a finding that is highly specific for PBC patients⁴⁴. Furthermore, patients with both PBC and Sjögren's syndrome can develop aberrant expression of PDC-E2 on salivary epithelial cell surface, an immunohistochemical finding that is generally restricted to PBC biliary epithelium⁴³. As patients with Sjögren's syndrome and PBC suffer from a comparable pathophysiologic process, it is tempting to speculate that a retrovirus with antigenic similarities to HIAP plays an important role in the multi-factorial disease process of PBC.

HIV p24 Reactivity in Patients with Chronic Viral Hepatitis

As part of the comparison group of patients with chronic liver disease, serum from 48 patients with chronic viral hepatitis was used for the HIV Western blot study. About 38% of those with chronic hepatitis C virus (HCV) infection and 60% of patients with chronic hepatitis B virus (HBV) infection were found to have HIV p24 gag reactivity²⁷. This observation may be partially attributable to the shared immunodominant or conformational epitopes of both HBV and HCV with retroviral elements. Certainly, HBV shares a common

biology with retroviruses. Both viral families have similarities in genomic organization and share nucleotide homology in direct repeat, nucleocapsid and polymerase regions²⁹. Also, both viruses have an analogous replication cycle using a reverse transcription mechanism to convert the RNA pregenome into the DNA genome and *vice versa*²⁹. While a similar relationship of flaviruses and retroviruses is not immediately apparent, it is possible that HCV may share immunodominant epitopes with HIV p24 gag as well. Protein alignment studies of HBV core protein, HCV p22 and HIV p24 gag reveal that the nucleocapsid proteins of both HBV and HCV share a comparable homology with HIV p24 (Fig. 1). In fact, in this alignment the HBV core protein shares 14 conserved and 23 functionally similar amino acids with HIV p24, whereas the HCV p22 protein shares 15 conserved and 14 similar amino acids with the HIV nucleocapsid. Of note, both HBV and HCV nucleocapsid proteins share more homology with HIV p24 gag than with each other, consistent with the notion that retroviral agents may be ancestral to other viral orders.

The detection of anti-p24 HIV gag antibodies in a variety of disease states where no HIV can be found may also be an intrinsic function of the promiscuity of antibodies reactive to the retroviral group antigens which share cross-reactive epitopes with each other. For example, it has been shown that high-affinity monoclonal anti-p24 HIV antibodies may be polyspecific despite undergoing antigen driven somatic mutation which usually ensures antigen specificity²⁰. When a monoclonal anti-p24 HIV antibody was used to screen a synthetic combinatorial library, a variety of 13 amino acid supertope motifs were encountered that competed for the antibody's paratope region. A genome search of these supermotifs revealed a number of autoantigens, suggesting that the HIV p24 gag protein is capable of activating an autoimmune response to a variety of proteins²⁰. This phenomenon has been aptly illustrated in SLE patients with antibody reactivity to HIV p24 gag and autoantibodies to the Sm small ribonuclear protein. In these studies, Sm was used to abrogate serum reactivity to HIV p24 gag in a proportion of the SLE patients, and in reciprocal studies, HIV p24 was used to inhibit the binding of an idiotypic antibody to the Sm protein⁴¹. Therefore, anti-p24 HIV antibody reactivity may either herald the presence of autoantibodies that coincidentally cross-react with HIV proteins, or it may be a surrogate marker for infection with a retrovirus antigenically related to HIV.

In fact, the two hypotheses are not necessarily mutually exclusive if the autoimmunity is precipitated by

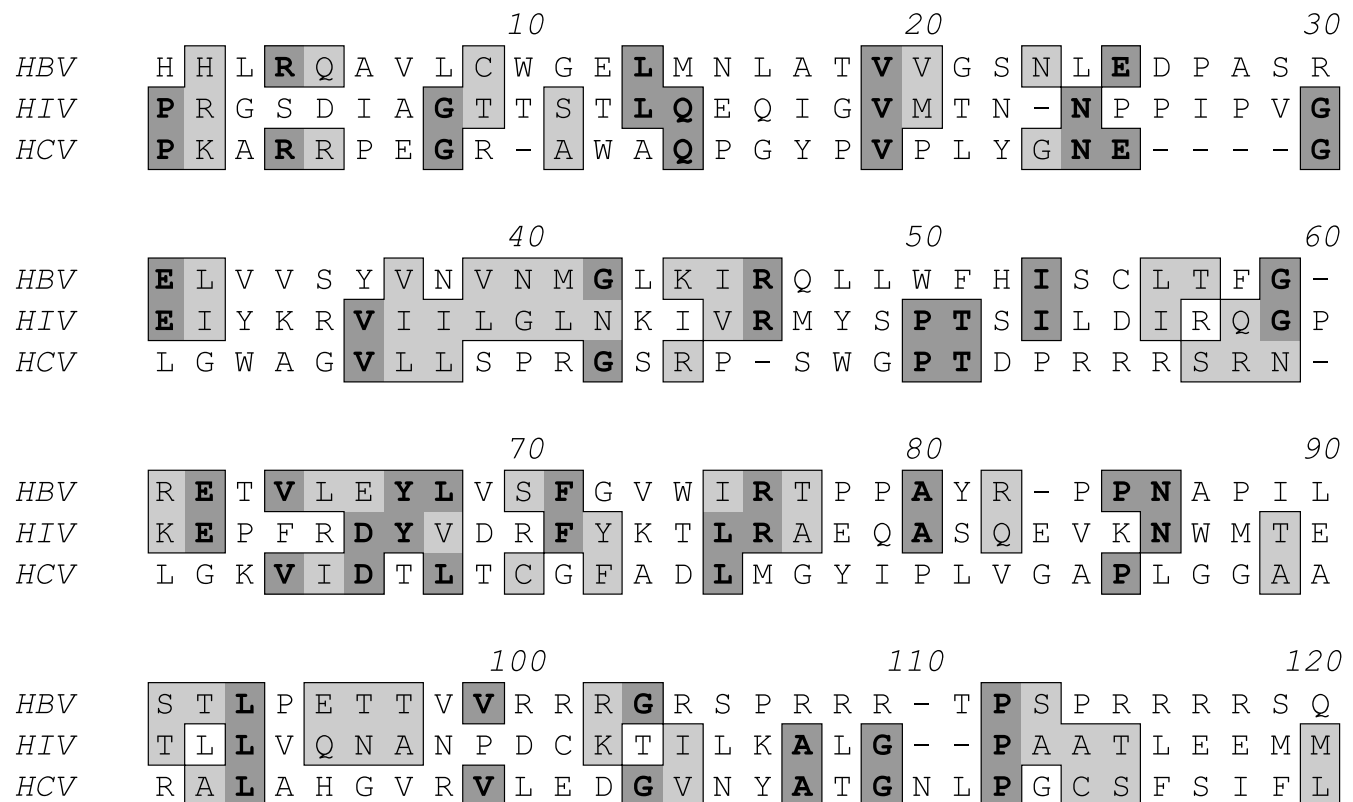


Fig. 1. Alignment studies of amino acids (aa) 56-149 of the 183 aa HBV core protein (accession number X75665), aa 66-191 of the 191 aa HCV nucleocapsid/matrix protein (accession number Q00269), and aa 99-231 of the 231 aa HIV p24 gag protein (accession number K02012), revealing shared antigenic determinants of each nucleocapsid protein. The ClustalW alignments were performed with MacVectorTM 6.5 software and the darkly shaded areas represent aa identify and the lightly shaded areas represent aa similarity between each protein

the presence of viral proteins in the first place. In our Western blot study of a chronic liver disease cohort, there was a strong correlation between reactivity to either HIV or HIAP proteins and the detection of autoantibodies associated with autoimmune liver disease, which supports both hypotheses²⁷. However, the preliminary studies of an uncharacterized retrovirus in patients with PBC clearly support the role of a retroviral mechanism for the formation of autoantibodies. Moreover, the HIAP was originally discovered using an anti-HIV gag antigen capture assay to isolate lymphoblastoid cells infected with the retrovirus¹². Subsequent HIAP Western blot studies have revealed a marked association between SLE and Sjögren's syndrome with HIAP reactivity^{12, 13, 27}. However, it is interesting to note that patients with SLE and Sjögren's syndrome react to different peptides of the HIV p24 gag protein, suggesting that either different immune responses or different agents are implicated in the pathogenesis of each disorder⁹.

HIAP Reactivity in Patients with Chronic Viral Hepatitis

Patients with chronic viral hepatitis infection may present with immune mediated syndromes such as vasculitis, glomerulonephritis and thyroiditis, and may also make a variety of autoantibodies²⁵. With regard to multisystem autoimmune disorders, sialadenitis has been observed in approximately half of the HCV-infected patients studied, even though symptoms of sicca syndrome and diagnostic autoantibodies associated with Sjögren's syndrome are rarely observed^{16, 35, 37}. Reports concerning the prevalence of HCV infection in patients with Sjögren's syndrome are contradictory and vary from 0 to 40% and some authors claim that this multi-systemic disorder only occurs in HCV-infected patients with essential mixed cryoglobulinemia^{21, 38}. However, the observation of sialadenitis in transgenic mice expressing HCV envelope genes suggests a direct role for HCV proteins in mediating salivary disease²².

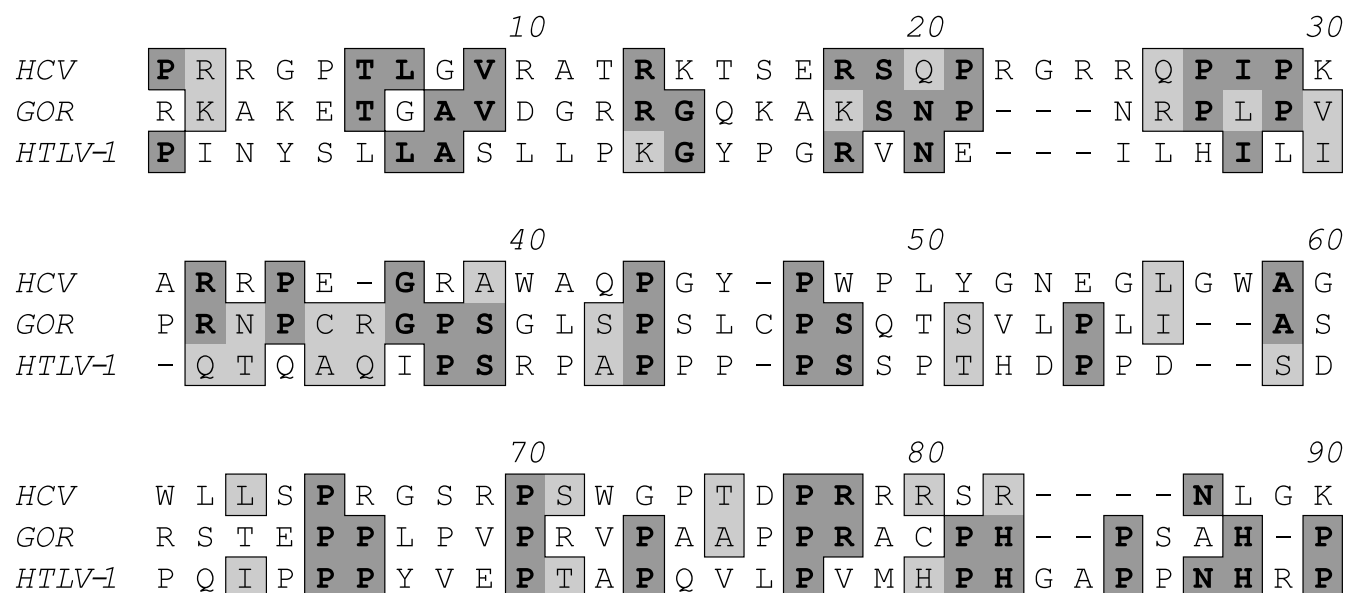


Fig. 2. ClustalW protein alignment studies of aa 62-147 of the 429 aa HTLV-1 gag protein (accession number AF033817), aa 38-125 of the 191 aa HCV nucleocapsid/matrix protein (accession number Q00269) and aa 437-522 of the 525 aa Gor antigen (accession number P48778; including the 447-461 GOR epitope), using MacVectorTM 6.5 software

The accumulated data support a role for HCV infection in the pathogenesis of sialadenitis, and possibly Sjögren's syndrome as a rare event in predisposed individuals.

Our study of patients with chronic viral hepatitis revealed interesting data with regard to HIAP Western blot reactivity and the possible role of HCV infection in mediating Sjögren's syndrome. A significant difference in frequency of reactivity to 2 or more HIAP proteins was observed between patients with chronic HBV and HCV infection (5% vs 30%)²⁷. This cannot be so easily attributed to protein homology, as limited HIAP amino acid sequence data is available for sequence comparison. However, the limited association of HCV infection with Sjögren's syndrome may provide clues to why patients with HCV infection have increased HIAP reactivity. As there is compelling data to link HIAP with Sjögren's syndrome^{12, 13}, it is tempting to speculate that HCV infection may modulate the expression of HIAP, which is presently considered an endogenously encoded retroviral element.

The notion that HCV may modulate the expression of endogenous retroviruses permits further speculation as to the nature of certain autoantigens. As reported previously, the detection of anti-mitochondrial, anti-nuclear, anti-extracted nuclear antigen, and anti-double stranded DNA antibodies correlated significantly with reactivity to the detection of HIAP antibodies²⁷. Although the nature of the mitochondrial autoantigen has

been documented, some of the nuclear autoantigens associated with autoimmune liver disease and chronic viral hepatitis have yet to be characterized in detail. This is well illustrated by the observation in HCV infected individuals of autoantibodies to a human encoded protein known as GOR, which was originally isolated as a cDNA clone from an HCV-infected Chimpanzee³⁰. Anti-GOR antibodies have been detected in 70 to 80% of HCV-infected patients and appear to be specific for HCV infection^{28, 30}. Also of interest is that these antibodies have been found to cross-react with HCV nucleocapsid protein, suggesting a role of molecular mimicry of viral and host proteins in the genesis of the antibodies³¹.

The nature of the GOR protein is not known but it appears to demonstrate sequence similarity with HTLV-1 gag as well as the HCV nucleocapsid protein (Fig. 2). In this alignment, the GOR protein shares 17 conserved and 9 similar amino acids with the HCV nucleocapsid protein, whereas there are 19 conserved and 11 similar amino acid residues with the HTLV-1 gag protein. This protein similarity provokes the notion that GOR may either be an endogenous retroviral protein or related to one. However, apart from the L1 sequence inserted at the 3' of the GOR protein coding sequence, there is insufficient nucleotide sequence data to determine other LTR, *pol*, or *env* sequences that may encode endogenous retroviral proteins³⁰. In addition, little is known about the GOR protein's function, distribution,

and expression. For example, it is not known whether HCV infection modulates the expression of the GOR protein analogous to the observation in patients with PBC, who have an abnormal distribution of the PDC-E2 autoantigen on the biliary epithelium cell surface. While the mechanisms that promote HERV expression remain unexplored at this time, immunoreactivity to these elements is observed in patients with a variety of autoimmune, infectious and neoplastic diseases¹³. Further studies will be required to investigate the possible relationship of HERV activation and HCV infection in more detail.

Prospectus

To date, we have provided preliminary evidence to suggest that retroviral agents may play a role in the induction of the autoimmune disease process of patients with PBC. We have also suggested that endogenous retroviruses may act as autoantigens in autoimmune liver disorders and discussed their possible role as genetic elements that inactivate genes, modulate gene expression, or mediate immune dysfunction in animal models of autoimmune disease. In contradistinction, it is also conceivable that endogenous retroviruses interact with exogenous retroviruses to prevent autoimmune disease. For example, it has been proposed that these genetic elements may be protective to the host through a variety of mechanisms. These include prevention of exogenous virus entering cells by competition for viral protein receptors as well as the known superantigen-mediated deletion of the T lymphocyte subsets that are required for passage of the exogenous retrovirus^{13, 47}.

There is also an argument that the lack of a protective HERV may be an important factor for patients with either PBC or autoimmune hepatitis. An association with the complement C4 gene as well as low levels of serum C4 in patients has been well described in patients with PBC and autoimmune hepatitis^{10, 24}. Both the so-called "long" C4A and C4B alleles have a full length HERV-K10 endogenous retrovirus inserted in a reverse orientation in the ninth intron of the gene; whereas this HERV is absent in the C4A and C4B "short" alleles⁸. As carriers of the long alleles make 2 antisense HERV genomes when the C4 haplotype is transcribed in the liver, it has been suggested that these HERVs may act as an antisense mechanism to abrogate exogenous retrovirus infection⁸. Of note, patients with PBC and those with autoimmune hepatitis have a greater frequency of C4 deletions in one of the two C4 genes as well as a short gene in the remaining C4 allele^{10, 24}. Therefore, patients with autoimmune liver disease often

lack the hepatocyte protection afforded by this putative antisense mechanism.

This hypothesis would be in keeping with the observation that a transmissible element from PBC lymph nodes can cause the PBC phenotype in any biliary epithelial cells in culture that are derived from healthy subjects³⁹. Family studies suggest that the development of PBC has a strong genetic component³³ and, if all biliary epithelial cells are susceptible to the transmissible agent, then the genetic predisposition to disease is probably at the level of prevention of infection and the immune response to the agent. It is likely that family studies will ultimately be required to gain a deeper understanding of the predisposing genetic factors associated with PBC that are outside the HLA region.

At this time, little is known about the aberrant expression of the PDC-E2 on the biliary epithelium cell surface, which is the likely cause of breaking tolerance to this autoantigen in PBC patients. Moreover, the levels of anti-mitochondrial antibodies observed in patients with PBC do not correlate with the clinical disease and therefore it is unknown whether the destruction of bile ducts is mediated through an autoimmune disease process³³. While it is tempting to speculate that both endogenous and exogenous retroviruses play a role in the generation of the biliary disease and the associated autoimmune features, further molecular and immunologic studies will be required to implicate a viral etiology to help decipher the complex interaction of these agents in mediating PBC.

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