Introduction

Viral hepatitis of man is known to be caused by a variety of agents. Since the late 1960s, multiple viruses responsible for human hepatitis have been described including Hepatitis A virus (HAV), Hepatitis B virus, Hepatitis C virus, Hepatitis delta virus (HDV) and Hepatitis E virus. The diseases caused by these viruses vary in their epidemiology, severity and pathogenesis. While advances have been made in the areas prevention and treatment of the established hepatitis viruses, several lines of evidence suggest that additional unknown agents may be transmitted enterically or parenterally and induce hepatitis in man. These observations have spurred further interest in the identification of additional viral agents of human hepatitis.

The GB agent was initially investigated at Presbyterian-St. Lukes Hospital by Dr. F. Deinhardt and colleagues during the mid-1960s, when serum from a young surgeon with acute hepatitis and the initials GB was infused into four tamarins (Saguinus labiatus) [1]. Pooled serum from these animals induced a mild hepatitis characterized by moderate elevations in serum ALT and periportal mononuclear cell infiltrates, when inoculated into additional tamarins. As work progressed on the GB agent in tamarins, it became clear that a second agent designated MS-1 was likely responsible for the majority of cases of community acquired hepatitis in man. Furthermore, work published by Parks and Melnick suggested that the GB agent represented a virus indigenous to tamarins which likely held little relevance to human disease [2,3]. Shortly thereafter, MS-1 agent became recognized as HAV and tamarins played a critical role in the investigations of disease pathogenesis and vaccine development. As a result of these events, interest in the GB agent waned such that less than a handful of publications are noted in the ensuing thirty years.

Molecular Characterization of GB Agents

In 1995 the virus discovery group at Abbott Laboratories under the direction of Dr. Isa Mushahwar revisited the GB agent. Using a technique termed representational difference analysis they were able to clone and sequence a putative viral agent from passage 11 tamarin serum stored for nearly thirty years [4]. These investigations led to the unexpected finding that the GB agent consisted of not one but two closely related viruses within the Flaviviridae family. These single stranded positive sense RNA viruses were distinct from but related to the agent of Hepatitis C and were designated GB virus A and B. Subsequently GBV-A and GBV-B were cloned and completely sequenced. The genome of these viruses, was approximately 9.4 Kb in length and encoded a single polypeptide which is cleaved by cellular and viral proteases. Genomic organization is most similar to HCV with which the viruses share 25% identity at the nucleotide level.

With degenerate oligonucleotide primers the investigators next questioned whether cases of human nonA-E hepatitis might be associated with GB-like agents [4]. They were able to detect a third virus designated GBV-C in the serum of intravenous drug users (IVDUs) and West African patients which was associated with cases of clinical hepatitis. Phylogenetic analysis of the predicted translation product of the helicase protein indicated that GBV-C was more closely related to GBV-A than to GBV-B or multiple genotypes of HCV. A second group working independently and using different technology identified and sequenced a viral agent associated with fulminant hepatitis; this agent was designated hepatitis G virus [5]. Comparison of GBV-C and hepatitis G virus revealed nearly 95% identity at the nucleotide level indicating that they were essentially the same virus. GBV-C and GBV-A appear to be unique among members of the Flaviviridae in that they lack a nucleocapsid protein near the aminoterminus of their respective polyproteins. Species specific variants of GBV-A have recently been
described in *S. labiatus*, *S. mystax*, and *Aotus trivirgatus* [6]. Thus after being nearly forgotten for almost 30 years, a second
look at the GB agent revealed a novel group of viruses within the family Flaviviridae which could infect man and a variety of
nonhuman primates.

**GB Virus C**
Following their initial recognition, epidemiologic studies have attempted to link the GB agents with human disease.
Seroprevalence studies using recombinant viral antigen and additional work to detect viral RNA in serum have failed to
verify spontaneous GBV-A or GBV-B infection of human beings. In contrast, GBV-C was identified in human serum with an
incidence of from 0.5 to 20%. GBV-C was found in asymptomatic blood donors, liver transplant patients, intravenous drug-
users (IVDUs), HCV-infected patients, dialysis patients and patients with chronic and fulminant hepatitis of unknown
etiology [7-11]. Approximately 1 - 2% of asymptomatic blood donors in the USA have GBV-C plasma viremia as detected
by RT PCR, and GBV-C (HGV) contamination of coagulation factor concentrates and intravenous immunoglobulin
preparations has been demonstrated. Marked differences in prevalence based on geographic locality have been noted and
genomic comparison from different regions of the world have indicated the existence of at least several different genotypes
[9]. Parenteral and perinatal transmission have been documented. Evidence of community acquired infections in the USA and
the high rate of seropositive children in West Africa suggests that additional routes of transmission may exist [4].

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<th>Table 1. Flaviviridae Family</th>
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<td><strong>Flaviviruses</strong></td>
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While originally identified as an agent of human hepatitis, the role GBV-C may play in producing human disease remains
unclear. Initial reports indicated an association of GBV-C infection with hepatic disease [4,12]. Although asymptomatic
blood donors in the USA have significantly higher serum ALT levels than those without GBV-C, patients infected with
GBV-C are often concurrently infected with the viruses of hepatitis C or B. The timing of transmission of GBV-C is often
difficult to determine and the etiologic relationship of GBV-C to chronic and fulminant hepatitis in man is currently
uncertain. Whether viremia may be associated with nonhepatic pathology in a subset of infected individuals is an important
consideration which has not been addressed. Recently GB virus C infection of HIV positive patients has been shown to delay
progression to AIDS. The mechanism behind this phenomenon is unknown but may relate to GB virus C alterations in
chemokine receptor expression.

**GB virus A**
Following the identification of GBV-A and -B, additional transmission studies were conducted in *S. labiatus* in order to
determine the natural history of disease in tamarins [13,14]. These studies used reverse transcriptase polymerase chain
reaction and enzyme linked immunosorbent assays to detect viral RNA and host immunologic response. Serial passage of
the agent indicated that GBV-B induced an antibody response and was associated with elevations in ALT when inoculated into
tamarins. In contrast no antibody response was detected to GBV-A and only minimal elevations in ALT were noted
following inoculation when this agent was present alone. These published studies used small numbers of animals and while
animals were PCR negative for GBV-A immediately prior to inoculation, previous exposure to the virus could not be ruled
out. Surveys have now revealed that GBV-A and its variants are indigenous viruses of several species of New World
primates. Disease associations and the route of transmission following natural exposure to the virus have not been explored.
The impact spontaneous GBV-A infection may have on animal health or experimental work with these species is unknown.
GB Virus B

GBV-B is more closely related to HCV than either GBV-A or C and has been proposed as an experimental surrogate model of HCV infection of man [15]. Over 100 million people worldwide are chronic carriers of HCV, with over 3.9 million in the U.S. alone (NIH, 2002). HCV infection is a significant cause of human morbidity and mortality and animals models for development of new antiviral therapies are not readily available. Until recently the only model available was the HCV-infected chimpanzee. This system has several drawbacks including expense, availability, biocontainment and ethical considerations. It is also questionable whether HCV infected chimpanzees adequately model all aspects of the human disease. Furthermore, research is impeded by the lack of appropriate infectious cell culture models for this hepatotropic pathogen. A small nonhuman primate surrogate model of HCV infection would allow the in vivo examination of host-virus interactions, disease pathogenesis and potential chemotherapeutics agents.

The natural host of GBV-B is unknown, however several New World primates develop a characteristic hepatitis following experimental inoculation. We have completed experimental studies to characterize GB virus B infection of common marmosets (Callithrix jacchus) as a surrogate animal model of HCV. Among animals inoculated with infectious serum, a rapid rise in viremia 3 - 4 weeks post inoculation, attaining titers of >10^8 GBV-B ge/ml, can be demonstrated and is associated with an increase in hepatic enzymes. Two characteristic patterns of peripheral viremia are observed and correlated with hepatic viral load. In some animals peak viremia is achieved in 4 - 6 weeks followed by rapid clearance from both plasma and the hepatic tissue. In other animals, peak viral load is delayed and such animals remained viremic for periods up to six months. Intrahepatic GB virus B is detected only during periods of peripheral viremia.

In experimentally inoculated animals hepatitis is evident as soon as 4 weeks after inoculation and recognized initially as multifocal random nonsuppurative inflammation within the hepatic parenchyma. Subsequently marked lymphocytic infiltrates develop within portal tracts. Portal lymphocytic hepatitis is associated with erosion of the limiting plate and areas of piece-meal necrosis (interface hepatitis). Immunophenotypically this hepatitis is characterized by the infiltration of large numbers of CD3 and CD8 positive lymphocytes both within the hepatic parenchyma and portal areas. CD20 positive lymphocytes are observed primarily within portal tracts and progress to form well defined lymphoid nodules in some animals. The alterations in liver enzymes correlate directly with the morphologic changes observed histologically. The increase in alkaline phosphatase coincides with a prominent portal inflammatory cell infiltration suggesting ongoing damage to the biliary epithelium. Increased numbers of CD3 positive lymphocytes are observed within septal duct epithelium and are associated with increased expression of HLA-DR an MHC II antigen on biliary epithelium.

We have developed techniques to isolate intrahepatic lymphocytes. Using this technique lymphocytes were isolated from liver tissues collected at 1 and 2 months post inoculation and the immunophenotype of the cells determined. Compared to pre-inoculation samples there was a statistically significant increase in CD3CD8 positive T-lymphocytes that correlated with changes noted histologically. This population of cells is comprised primarily of cytotoxic T-lymphocytes, an MHC class I restricted immune cell that likely plays a critical role in controlling viral infection. Concurrent with these alterations, a statistically significant increase in CD20 positive B cells was also observed. An increase in the number of CD3CD4 positive helper T-lymphocytes, an MHC class II restricted immune cell, was not evident. A slight increase in lymphocytes expressing the MHC class II antigen HLA-DR was observed on lymphocytes, but the overall population of naive T-cells, CD45RA positive cells did not reflect this trend.

GBV B infection of New World primates represents a surrogate animal model of HCV infection of man. Inoculated animals develop a characteristic hepatitis and can be followed with sequential biopsies to evaluate immunophenotypic and morphologic changes in hepatic tissue. The availability of infectious GBV-B molecular clones and an in vitro culture system should assist future studies utilizing this model to investigate aspects of disease pathogenesis and prevention.

References


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