GB Virus type C and its relationship with Human Immunodeficiency Virus

REVIEW

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ABSTRACT

Despite of the relative success of Highly Active Anti Retroviral Therapy (HAART), HIV/AIDS pandemic still remains as one of the major threats to world health. Due to the limitations of the current treatments and the lack of success in the development of a preventive vaccine, the discovery of novel mechanisms involved in HIV replication has become of paramount importance. GB Virus type C or Hepatitis G Virus is a recently described microorganism belonging to the *Flaviviridae* family and infecting both T and B lymphocytes. Up to now, infection with this virus has not been associated with any known pathology. Studies conducted in diverse laboratories have suggested a relationship between GBV-C infection and progression to AIDS in HIV seropositive individuals. Although these findings have not been consistently reproduced in all laboratories, broader analysis suggests the existence of a complex relationship depending on the stage of the disease. *In vitro* inhibition experiments have confirmed that one or several GBV-C proteins do interfere with HIV replication. These findings support the hypothesis that GBV-C can be indeed the cause of a slower progression to AIDS in co-infected individuals. The study of the underlying mechanisms could open new avenues in the therapy or prevention of AIDS.

Keywords: HIV, GBV-C, AIDS, inhibition, *Flaviviridae*, progression

Biotecnología Aplicada 2007;24:194-198

RESUMEN

El virus GB-C y su interacción con el virus de la inmunodeficiencia humana. A pesar del éxito relativo de la terapia antirretroviral de alta eficacia, la epidemia de VIH/SIDA sigue siendo uno de los principales problemas mundiales de salud. Ante las limitaciones de la terapia actual y la imposibilidad de desarrollar una vacuna preventiva, es imperativa la búsqueda de enfoques novedosos en la terapéutica y la prevención del VIH/SIDA. El virus GB-C, o mal llamado virus de la hepatitis G, es un microrganismo de descripción reciente. Este virus se ha clasificado como un miembro de la familia *Flaviviridae*, infecta linfocitos B y T, y no se ha podido vincular a ningún proceso patológico. Diversos laboratorios han encontrado una peculiar correlación entre la infección activa por el VGB-C y una progresión lenta hacia el SIDA. Aunque estos hallazgos no han sido reproducidos por otros grupos, los estudios más abarcadores sugieren que la relación es compleja y depende del estadio clínico en que se encuentra el sujeto. Experimentos de inhibición *in vitro* han confirmado que uno o varios elementos dentro del VGB-C interfieren con la replicación del VIH. Estos resultados respaldan la hipótesis de que tras la correlación descrita en pacientes, subyacen elementos de causalidad cuya identificación pudiera conducir a la apertura de nuevas avenidas terapéuticas o preventivas contra el VIH.

Palabras clave: VIH, VGB-C, inhibición, *Flaviviridae*, progresión

*I*ntroduction

With 38.6 millions new infections and 2.8 million deaths in 2005, HIV/AIDS pandemic is still one of the most important health problems for mankind. In spite of the positive impact of Highly Active Antiretroviral Therapy (HAART) on survival, the ultimate solution for this disease is still to be found. Current therapy do not eliminate viral reservoirs, is toxic, costly, and viral mutants displaying resistance to all drugs eventually appears [1-4]. On the other hand, more than forty vaccine candidates have been evaluated so far in clinical trials in human volunteers. Most of these candidates have been discarded after phase I trials [5-12] and only three of them have fulfilled [13] or initiated efficacy studies [14]. All the knowledge accumulated so far in this field indicates that a protective vaccine capable of putting an end to AIDS pandemia is still many years away.

Maximal priority should be given to basic research because it has a very important role to play in this situation by opening novel avenues for AIDS therapy or prevention. This is indeed the case of the association between GB virus type B (GBV-C), (also known as Hepatitis G virus), and HIV-1. GBV-C co-infection has been correlated by several research groups with a slower progression to AIDS. The present article is a concise summary of the main findings regarding the relationship between these two viruses.

*B*rief history of GBV discovery

In 1967, Deinhardt *et al* [15] inoculated tamarin monkeys (*Sanguinus Sp)* with serum from patient GB, suffering from acute non A, non B Hepatitis. The recipient monkeys also displayed biochemical and histological symptoms of acute viral hepatitis, however, either monkeys or patient GB recovered spontaneously from disease.

During the seventies and eighties sera from GB inoculated tamarins were transferred to other tamarin

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and marmoset monkeys (*Callitrix Sp*). The viral nature of the transmissible agent was demonstrated [16] and the eleventh passage was stored in the ATCC as H205 GB passage 11.

Researchers from Abbott Laboratories used GB passage 11 to inoculate other tamarin monkeys and were able to identify a Flavivirus like virus through Representational Difference Analysis (RDA) and immunoscreening of a cDNA library. The genomes of GBV-A and GBV-B were then cloned [17].

Subsequent studies lead to the identification of human sera reactive against human sera against non structural proteins of GBV-A and GBV-B, but all these samples were negative to viral RNA when assessed by Polymerase Chain Reaction (PCR).

A positive individual in West Africa could be identified by PCR using degenerated oligonucleotides from GBV-A, GBV-B and HCV helicase genes. The DNA sequence of the new virus was similar, but clearly different, from that of GBV-A and GBV-B, thus it was considered as a third virus and named GBV-C [18]. The genome of GBV-C was fully sequenced very soon [19].

Independently, Linnen *et al* [20] isolated a novel virus, denominated Hepatitis G Virus (HGV), from a patient diagnosed as Non A non B non C viral hepatitis. The DNA and amino acid sequences from this isolate were 86 and 96 percent homologous to GBV-C respectively, therefore they were considered as two different isolates from the same virus: GBV-C/HGV.

General characteristics of GBV-C/HGV

GBV-C/HGV is an enveloped, positive stranded RNA virus. It has been classified as a member of the *Flaviviridae* family, genus Hepacivirus, which causes frequent infections in humans and replicates in T and B lymphocytes but not in hepatocytes [21]. Most of the immunocompetent hosts are capable of clearing the infection some weeks after eliciting an antibody response against the external glycoprotein of the virus. However viremia can persist for decades in some individuals [22-23].

Despite being 29% homologous at the amino acid level with Hepatitis C Virus (HCV), GBV-C/HGV does not cause hepatitis, thus the name Hepatitis G Virus was clearly a mistake. In consequence, during the rest of the article it will be mentioned as GBV-C.

The GBV-C genome organization is similar to the one of HCV. It includes a single RNA positive sense strand, encoding a long Open Reading Frame (ORF), which is translated into a 3000 amino acid polyprotein. By extrapolation from HCV, the envelope glycoproteins (E1 and E2) are thought to be cleaved by a cell protease, while non structural proteins (NS) are processed by the viral proteases NS2 and NS3.

Curiously, an ORF encoding for the capsid protein has not been identified in GBV genome. Several hypotheses have been advanced to explain this peculiar omission, for example, that GBV-C use the capsid protein of HCV or that this protein is encoded by the RNA negative strand [24].

Five genotypes have been described so far which can be easily identified by Restriction Fragment Length Polymorphism (RFLP) analysis [25]. The wide distribution through all the continents and its non pathogenic nature suggest a long evolutionary history for this virus [26-27].

GBV-C prevalence

The prevalence of this virus is much lower in general population (between 1% and 9.4%) [28-32] than in HIV infected patients or risk groups, such as hemophiliacs (between 40.3% and 54.7% [33-34]); hemodialyzed patients (30.2%), men who have sex with men (30.2%) and drug addicts (74.4%) [33].

Like HIV, GBV-C is transmitted by parenteral or sexual exposure and this coincidence is clearly expressed in the high frequency of co-infection with both viruses. Prevalence data in HIV patients has shown remarkable variability among studies, fluctuating between 17.7% and 85%. This variability could be explained by the use of different methods and reagents for diagnosis, differences among viral genotypes or the immunological state of the patients [29, 30, 32, 35-46].

GBV-C - HIV association

Positive evidences

Several studies of GBV-C RNA or seroprevalence of antibodies against GBV-C E2 in HIV/AIDS patients have found an inverse correlation between GBV-C infection and AIDS progression.

The first report on this direction came from Toyoda *et al.*, [47] who found out that progression to AIDS and death were slower in double infected patients, although the differences were not statistically significant. For this reason the authors could only conclude that GBV-C infection does not have a negative impact on the course of HIV infection.

A few months later a similar report, but including a Kaplan-Meier survival analysis, showed a significantly higher survival in GBV-C RNA positive patients than in negative ones [48]. Those findings were afterwards confirmed by Tillmann *et al.*, [49] who additionally noted that GBV-C co-infection was associated to a better quality of life in HIV/AIDS patients.

Lefrere *et al.*, [50] compared a group of GBV-C RNA positive patients with another group of people not exposed to GBV-C. After the adjustment of data by age, sex, basal HIV viral load, and CD4 lymphocyte basal count they found that progression to AIDS was faster in GBV-C RNA negative subjects. A similar, although less conclusive observation was done in HIV infected mothers [42].

An inverse correlation between GBV-C RNA and the levels of CD4 lymphocytes in West African patients was also established [51]. On the other hand, co-infected patients under HAART experienced a faster reduction of viral load (HIV RNA) and the number of cases with total virological response were higher than in GBV-C negative patients [52,53].

Two alternative explanations could be considered for the above mentioned correlation:

- The presence of GBV-C provokes, either directly or indirectly, the inhibition of HIV replication.

- GBV-C is only a marker associated with the presence of other factor which mediates an HIV

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beneficial response, and its replication is favored in those individuals conserving a high number of T lymphocytes.

In fact, results from other investigators have not been so consistent with the above mentioned findings; therefore the real relationship between these two microorganisms has been questioned.

Negative evidences

Brumme *et al*., [38] did not find any relationship between GBV-C infection and response to HAART, which is in contrast with some of the results outlined in the previous epigraph [52, 53].

In another study, carried out in Africa, significant differences between mortality and GBV-C status were not found. GBV-C infection was neither associated with HIV viral load in plasma nor with CD4 lymphocyte count [54]. It was concluded that GBV-C infection had no impact on vertical transmission of HIV [42, 55].

No association between GBV-C and progression to AIDS was found in the cohort of HIV-2 seropositive patients from French ANRS [56]. Absence of statistically significant correlation between GBV-C infection and CD4 lymphocytes count or HIV viral load was also documented in this study [57, 58].

According to Bjorkman *et al*., [59] GBV-C status was not predictive of AIDS progression in the studied cohort. These same authors observed a tendency to a reduction of viremia without appearance of anti E2 antibodies in HIV patients progressing to AIDS, which suggest that GBV-C status in HIV patients can be a secondary phenomenon instead a of an independent prognostic factor.

A similar finding was reported for the Amsterdam cohort [60]. Here the lost of viral GBV-C RNA was negatively related to AIDS progression. These authors hypothesized that GBV-C depends upon a critical number of CD4 cells for persistence; therefore a diminution of this subpopulation, associated to AIDS progression, is the cause and not the consequence of GBV-C elimination. Examined from a different perspective, slow progressors preserve better their T lymphocytes and this fact enables an optimal GBV-C replication. However, this explanation is somehow contradictory since, as it has been mentioned before, GBV-C behaves mainly as an opportunistic microorganism being cleared by immunocompetent immune systems in most of the cases, while establishing a persisting infection in the immunocompromised hosts.

Association between GBV-C and HIV depends upon the stage of HIV infection

Williams *et al*., [41] found that GBV-C viremia could be significantly associated with a prolonged survival in HIV infected patients with 5 to 6 years after seroconversion, but not in recent seroconverters (between 12 and 18 months), and that GBV-C RNA during 5 to 6 years after HIV seroconversion was associated with the worst prognosis.

Finally, a Bayesian meta-analysis of data gathered from 11 independent studies from 8 publications was unable to find a conclusive relationship between GBV-C and mortality in HIV patients during the firsts years after infection, but they do documented a lower relative hazard of death in patients with GBV-C co-infection and advanced AIDS disease [61].

Inhibitory effect on HIV replication *in vitro*

All these findings indicate a correlation between GBV-C infection and a favorable course of disease in HIV/AIDS patients. Nevertheless, this kind of correlation is not necessarily indicative of causality. Therefore it has been important to investigate the direct action of GBV-C and its components on HIV replication in different substrates. These experiments have demonstrated that, in fact, GBV-C infection of human peripheral blood lymphocytes reduce the replication rate of HIV [62,63]. This inhibitory effect was neither a simple function of the interferences between the replication machineries of both viruses, nor a competition established for the access to cellular materials; since defective viruses, expressing a limited number of genes, are also capable of inhibit HIV replication [64].

Molecular mechanisms involved in GBV-C mediated HIV inhibition

The studies carried out so far have pointed out to several molecular mechanisms to explain GBV-C/HIV relationship. Next, we summarize the principal hypotheses that have been advanced based on the experimental findings.

Hypothesis 1. Induction of inhibitory chemokines

Its is well known that certain chemokines such as RANTES and SDF-1 are capable of blocking HIV entry by binding to HIV co-receptors CCR5 or CXCR4, although their true role in HIV infection has been the subject of extensive debate. Xiang *et al*., [63] reported the increased production of MIP-1α, MIP-1β, RANTES and SDF-1 and reduction of CCR5 expression in GBV-C infected human lymphocyte cultures. The same authors [64] described an 85 amino acid fragment of the GBV-C protein NS5A, which was capable of inhibited HIV infection in Jurkat cells. The induction of SDF-1 production and simultaneous reduction of CXCR4 co-receptor expression could explain this inhibitory effect. However, the same group had reported earlier the lack of modulation of HIV receptors on the surface of peripheral blood mononuclear cells from double infected patients [65].

An independent study did confirm the enhanced secretion of HIV suppressive soluble factors in GBV-C infected CD4+ and CD8+ T lymphocytes, although neither SDF-1 induction, nor CXCR4 internalization was observed in this case [62].

On the contrary, Gimenez-Barcons *et al.*, [44] concluded that, in a clinical relevant context, GBV-C infection do not seems to modulate neither cytokines nor chemokines expression.

Hypothesis 2. Induction of IFN alpha associated genes

The endogenous levels of mRNA from genes associated with IFNα (2,5 oligo, MxA, AR-1 and PKR) were higher in GBV-C co-infected patients than in GBV-C negative HIV infected patients, even though these differences were statistically significant only for PKR mRNA [66]. This inhibitory effect was

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mediated by the expression of GBV-C glycoproteins and NS2/NS3 non structural proteins.

Hypothesis 3. Anti-apoptotic effect

It has been described that GBV-C can inhibit host cell apoptosis. Since apoptosis induction has been one of the mechanisms implicated in HIV mediated CD4+ T cells depletion, the abrogation of apoptotic pathways could help protecting CD4 lymphocytes [67].

Hypothesis 4. Direct inhibition of HIV replication by a viral protein

There are at least two GBV-C proteins which neutralize HIV infection *in vitro*. One of them is E2 glycoprotein [67] and the other is the 85 amino acid fragment of NS5A protein mentioned before. These two proteins could have an unknown mechanism of direct inhibition on HIV replication.

Hypothesis 5. GBV-C infection contributes to the preservation of a TH1 lymphokine pattern

Neither reduction in IL-2 and IL-12 expression nor increase in IL-4 and IL-10, characteristic of AIDS progression, could be documented in double infected GBV-C/HIV patients [40].

Conclusion

Sufficient experimental evidences to back up the hypothesis of GBV-C effect on HIV replication have been gathered, even if its true impact on AIDS pathogenesis is uncertain. More investigations on the viral and cellular elements involved in this inhibition are needed to clarify this point and it is likely that, in the next future, these efforts would lead to the development of novel alternatives to the prevention and therapy of HIV/AIDS.

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Received in march, 2007. Accepted for publication in december, 2007.